Dextrin characterization by high-performance anion-exchange chromatography–pulsed amperometric detection and size-exclusion chromatography–multi-angle light scattering–refractive index detection

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Abstract

Starch hydrolysis products, or dextrins, are widely used throughout the food industry for their functional properties. Dextrins are saccharide polymers linked primarily by α-(1→4) D-glucose units and are prepared by partial hydrolysis of starch. Hydrolysis can be accomplished by the use of acid, enzymes, or by a combination of both. The hydrolysis products are typically characterized by the ''dextrose equivalent'' (DE), which refers to the total reducing power of all sugars present relative to glucose. While the DE gives the supplier and buyer a rough guide to the bulk properties of the material, the physiochemical properties of dextrins are dependent on the overall oligosaccharide profile. High-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection and size-exclusion chromatography (SEC) with multi-angle light-scattering and refractive index detection were used to characterize dextrins from commercial sources. HPAEC was used to acquire the oligosaccharide profile, and SEC to obtain an overall molar mass distribution. These methods in combination extended our understanding of the relationship between oligosaccharide profile, DE, and the hydrolysis process. Data from the two techniques enabled a method for estimating the DE that gave results in reasonable agreement with the accepted titration method.
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1. Introduction

Dextrins are starch hydrolysis products produced by acid hydrolysis, enzyme hydrolysis, or a combination [1,2]. The extent of hydrolysis is normally expressed in terms of the ''dextrose equivalent'' (DE), a quantity usually determined by titration and a measure of the total reducing power of the sugars present relative to dextrose (D-glucose) standard, on a dry mass basis. A higher DE reflects a greater degree of hydrolysis and thus a smaller average molecular mass of the resulting oligomers. DE can be expressed as $100/DP_{avg}$, where DP_{avg} is the average degree of polymerization of the material. Thus, glucose has a DE value of 100, while intact starch would have a DE of effectively zero [3]. Starch hydrolyzates with DE values below 20 are

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referred to as maltodextrins, while those with DE greater than 20 are typically referred to as glucose syrups, glucose solids, or corn syrup solids. Importantly, DE is an average value and as such gives only a very broad picture of the extent of hydrolysis.

Dextrins with the same DE can have different properties and molecular compositions depending on the starch and how it is digested [4]. Properties include hygroscopicity, fermentability, viscosity, sweetness, stability, gelation, solubility, and bioavailability. Starch contains both linear α-(1→4) amylose and branched α-(1→6) amylopectin, the relative amounts depending on the type of starch, i.e., whether derived from corn, potato, rice, wheat, etc. Enzyme hydrolysis with α-amylase efficiently hydrolyzes the α-(1→4) linkages, but not the α-(1→6) linkages, leaving behind a small amount of high-molecular-mass residues. On the other hand, hydrolysis with a α-(1→6) specific enzyme (e.g., pullulanase) will render a higher proportion of linear α-(1→4) oligosaccharides [2], which are more susceptible to retrogradation and gelling. Generally, acid hydrolyzates will contain larger amounts of residual high molar mass oligomers than their enzyme-hydrolysis counterparts [1]. All starch hydrolyzates will typically contain an assortment of branched and linear oligosaccharides [4].

Higher DE materials generally exhibit more browning, hygroscopicity, sweetness, and solubility, while lower DE materials are used for bulk, viscosity control, cohesiveness, or film-forming. Maltodextrins are low-converted, nutritive starch polymers consisting of D-glucose units linked mostly by α-(1→4) bonds. They are non-sweet, soluble, and have found widespread use as bodying agents, coatings, spray-drying aids, fat replacers, adsorbents, adhesives, film formers, freeze-control agents, crystallization inhibitors, nutritive supplements, and flavor carriers [3,4]. Practical applications have been found in pharmaceutics, cosmetics, foods, beverages, detergents, textiles, paper and paint.

As has been stated, dextrins of the same DE can have different properties and hence different functionality in the desired application. Thus, a complete oligosaccharide profile, in addition to DE, is desired in order to better understand a dextrin's physical and biological functionality. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD) and size-exclusion chromatography with multi-angle light-scattering and refractive index detection (SEC–MALS–RI) were used to characterize dextrins from commercial sources. HPAEC was used to obtain a well-resolved oligosaccharide profile, while SEC gave an overall molar mass distribution. Together, the two techniques provided increased understanding of the hydrolysis process and enabled an estimation of DE from chromatographic data that is in reasonable agreement with an accepted titration method [5].

2. Experimental

2.1. Materials

Saccharide standards, glucose through maltoheptaose, were obtained from Sigma (St. Louis, MO, USA). Seven commercial dextrin samples were used in this study ranging from DE 4 to DE 25, with two suppliers' DE 18 for comparison. The powders were dried for 2 h at 70 °C under vacuum prior to preparing solutions.

2.2. Chromatography

HPAEC–PAD was performed with a Dionex (Sunnyvale, CA, USA) DX500 chromatograph equipped with an ED40 pulsed-amperometric detector. The waveform used was as follows: $E_1 = +50$ mV ($t_1 = 400$ ms, sampling period = 200 ms), $E_2 = +750$ mV ($t_2 = 200$ ms), $E_3 = -150$ mV ($t_3 = 400$ ms). The column used was a Dionex CarboPAC PA1 (250×4 mm I.D.) equipped with a guard column (50 mm×4 mm I.D.) of the same material. The mobile phase consisted of 100 mM sodium hydroxide (eluent A) and 100 mM sodium hydroxide containing 600 mM sodium acetate (eluent B). The gradient was a simple linear program: 20% eluent B at 0 min, 90% at 50 min. The flow-rate was 1 ml/min and the injection volume was 25 μl. Peaknet (Dionex) version 6.2 was used for data analysis.

SEC–MALS–RI was performed with a Waters (Milford, MA, USA) 2690 Separation Module and Wyatt Technology (Santa Barbara, CA, USA) Minidawn MALS and Optilab DSP differential RI detectors. The columns were a Waters Ultrahydrogel

250 (300×7.8 mm I.D., 10 μm, 250 Å, M_w exclusion limit: 100 000) and Tosoh (Montgomeryville, PA, USA) TSK-GEL G-Oligo-PW (300×7.8 mm I.D., 6 μm, 125 Å, M_w exclusion limit: 2000) columns in series. A Tosoh guard (40×6 mm I.D.) column of the same packing material as the G-Oligo-PW was used. The column temperature was 40 °C and the RI detector was held at 35 °C. The mobile phase consisted of 25 mM each disodium hydrogenphosphate and potassium dihydrogenphosphate (pH 7) with 50 mM sodium chloride added. The flow-rate was 0.5 ml/min and the injection volume was 100 μl. Astra (Wyatt Technology) version 4.72 software was used for calculation of the molar mass from the MALS and RI signals. A refractive index increment (dn/dc) of 0.140 ml/g was used in the calculations.

2.3. Preparation of solutions

Saccharide standards and commercial dextrin samples were dried for 2 h at 70 °C under vacuum prior to use. For HPAEC, the PAD response was determined for seven saccharides, glucose through maltoheptaose. A standard mixture was prepared by weighing approximately 0.1 g of each into a 100-ml volumetric flask and diluting to volume with deionized water. This solution was then made into 100, 50, 25, 12.5 and 6.25 ppm by appropriate dilutions. For the dextrin samples, approximately 1000 ppm solutions were prepared by weighing 0.1 g of each dry material into a 100-ml volumetric flask and diluting to volume with deionized water. Samples were filtered through 0.45-μm syringe-tip filters prior to injection. For SEC, approximately 0.2 g of each dextrin was weighed, transferred to 10-ml volumetric flasks and dissolved in phosphate buffer. Samples were filtered through 5-μm syringe-tip filters prior to injection. The DE 7, 10, 18 and 25 maltodextrins were titrated according to a modified Lane–Eynon procedure [5] for comparison of the dextrose equivalent.

3. Results and discussion

There have been numerous studies of starch hydrolyzates by chromatographic methods, including high-performance liquid chromatography (HPLC) [6–11], SEC [12,13] and HPAEC–PAD [14–17]. Kiser and Hagy [6] showed that a DE calculation from HPLC data was possible by assuming that the RI detector response was similar for all of the components and by normalizing the total chromatographic peak area to yield the area percent of each component. The limitations to their procedure were that the oligomer peaks were not baseline resolved, the RI detector responses were probably not equivalent, and the real DE for each oligomer was likely different from the theoretical one due to incomplete reaction stoichiometry [2]. Mariller et al. [7] attempted to overcome these limitations by developing a semi-empirical transformation to get a better fit between theoretically calculated DEs and the titrimetric ones.

PAD works on the principle of oxidation of active hydroxyl sites on the carbohydrate oligomers under alkaline conditions. It has previously been reported [14,17] that the detector response per unit mass decreases as the size of the oligomer increases for DP 1 to 7. This has been partly attributed to the lower proportion of the latent aldehyde group and the lower diffusion coefficients of larger molecules. Others have reported [14–16] that the relative detector response per HCOH group is nearly constant to DP 13, after which it decreases, possibly due to lack of access to reactive hydroxyl sites as the larger oligomers assume a random coil shape. We found that the molar detector response increased for DP 1 through 5 and appeared to level off at DP 6–7 (Fig. 1). The leveling of the detector response above DP 7 was used to advantage in later calculations.

For all of the dextrins analyzed by HPAEC–PAD, the individual members of the homologous series of oligosaccharides were baseline resolved on the CarboPAC PA1 column under the conditions specified. Smaller peaks barely observable and occurring between peaks of DP>4, presumably due to branching, were judged to be insignificant and were not integrated. Figs. 2 and 3 illustrate the differences in HPAEC–PAD profiles between a DE 10 and a DE 20 maltodextrin. The extent of hydrolysis is reflected in these profiles. Namely, a higher proportion of longer chain oligomers are observed in the DE 10 material than in the DE 20, where the extent of hydrolysis to shorter chains is greater. Fig. 4 illustrates the results
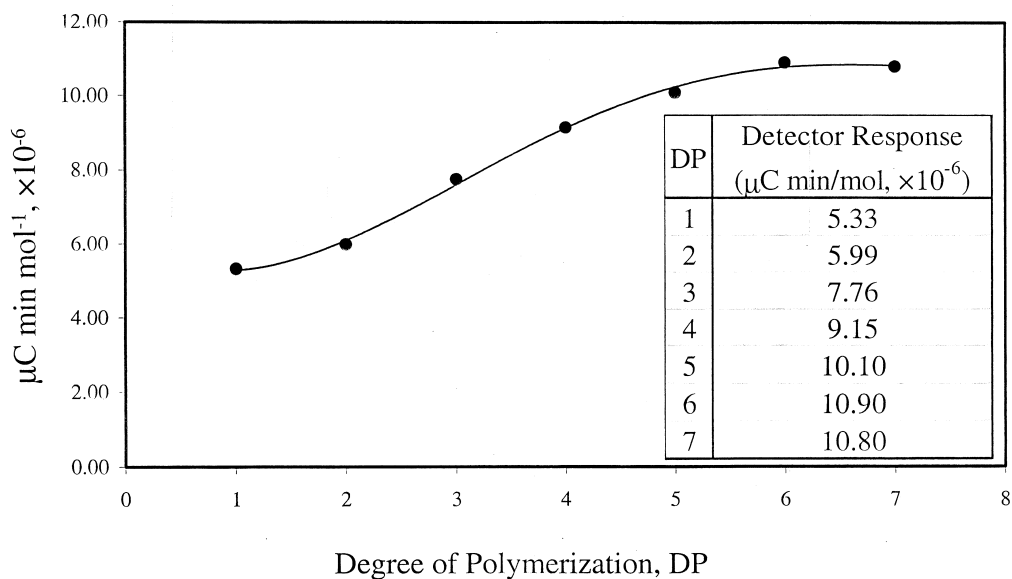
Fig. 1. PAD molar detector response for glucose (DP 1) through maltoheptaose (DP 7). Chromatographic conditions: DX 500 HPLC: column, CarboPAC PA1 (250×4 mm I.D.): eluent A, 100 mM sodium hydroxide; eluent B, 100 mM sodium hydroxide containing 600 mM sodium acetate: gradient program, 20% eluent B at 0 min, 90% at 50 min: flow-rate, 1 ml/min: detector, ED40 PAD (waveform: $E_1 = +50$ mV, $t_1 = 400$ ms, sampling period $= 200$ ms, $E_2 = +750$ mV, $t_2 = 200$ ms, $E_3 = -150$ mV, $t_3 = 400$ ms): temperature, ambient.

of SEC–MALS–RI for the same materials. SEC separates according to size with the larger components eluting first. It is readily seen from the RI signal that the DE 10 contains a higher proportion of high molecular mass material than does the DE 20. The light scattering signal is proportional to both molecular size and concentration, while the RI signal is concentration dependent only. A combination of
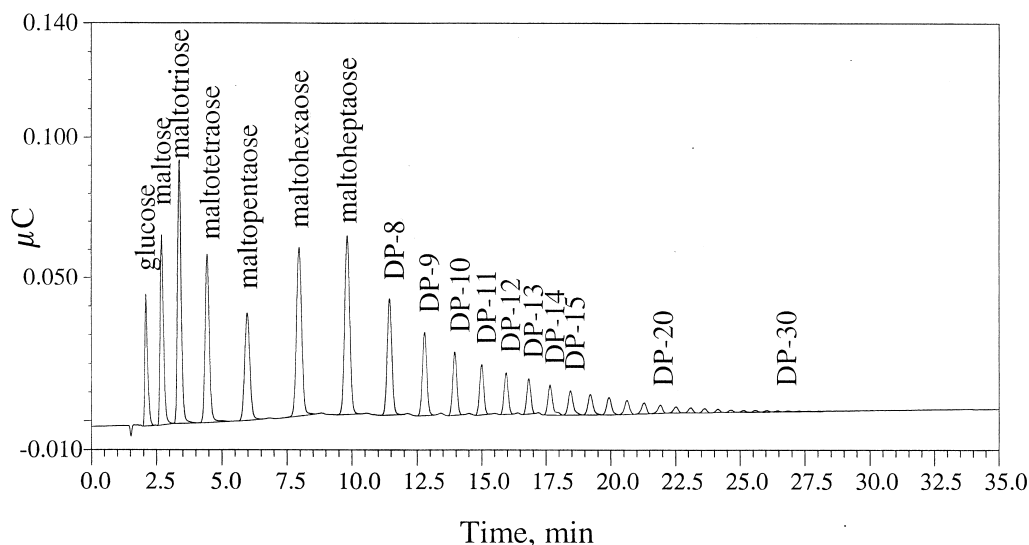

Fig. 2. HPAEC–PAD oligosaccharide profile of a DE 10 maltodextrin. Chromatographic conditions as in Fig. 1.
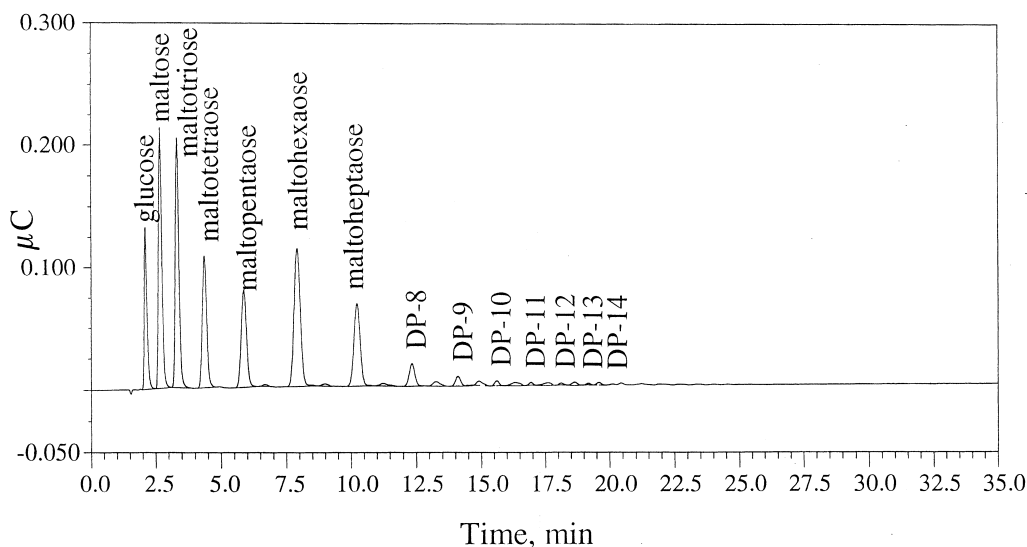
Fig. 3. HPAEC–PAD oligosaccharide profile of a DE 20 maltodextrin. Chromatographic conditions as in Fig. 1.

the two signals allowed calculation of the molar mass (M_w) at any point on the chromatogram [13]. Calculated values of log(M_w) vs. elution volume (V_e, ml) for successive chromatographic slices can be

seen to decrease in a linear fashion (Fig. 4). Scatter in the low-M_w region of the plot is due to inadequate light-scattering signal. Nonetheless, the linear behavior was approximated by the equation, log(M_w)=
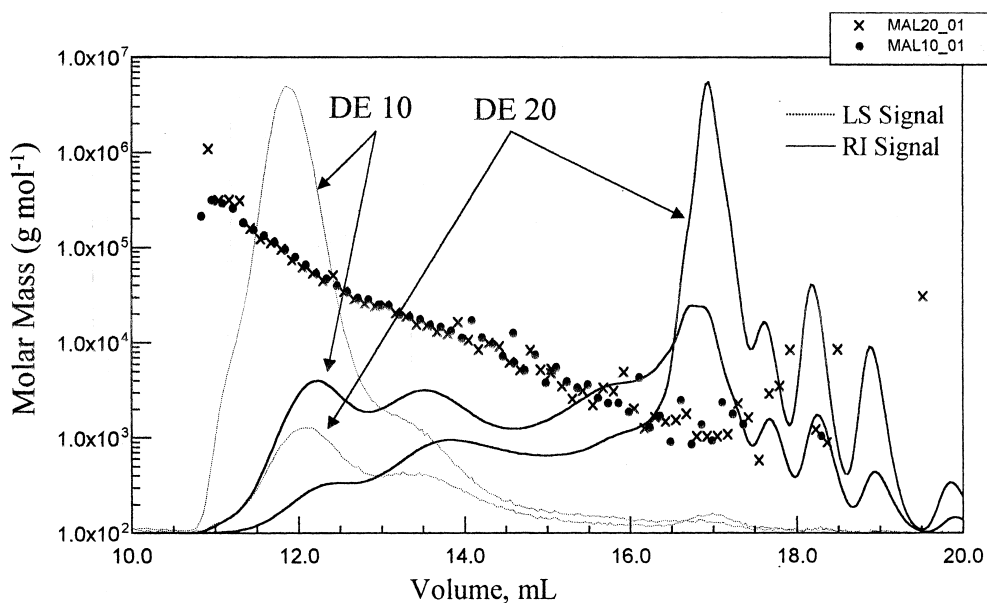

Fig. 4. SEC–MALS–RI chromatogram of DE 10 and DE 20 maltodextrin with overlay of calculated log(M_w) values vs. elution volume. Chromatographic conditions: Waters 2690 HPLC: columns, Ultrahydrogel 250 (300×7.8 mm I.D.), TSK-GEL G-Oligo-PW (300×7.8 mm I.D.): temperature, 40 °C: mobile phase, 25 mM disodium hydrogenphosphate, 25 mM potassium dihydrogenphosphate, (pH 7) and 50 mM sodium chloride: flow-rate, 0.5 ml/min: detectors, Minidawn MALS, Optilab RI.

$-0.375V_e + 9.37$ ($R^2 = 0.95$) and this best-fit line was used to determine the SEC cutoff volume, and hence mass percent of PAD-detectable oligosaccharides relative to the whole. With the DE 10 and DE 20 materials as examples, 33 peaks were quantitated for the DE 10 material, whereas only 14 peaks were detected for the latter. Table 1 summarizes the results with the number of peaks quantitated for each dextrin sample, along with the M_r of the highest DP oligomer detected. Plugging M_r into the $\log(M_w) = f(V_e)$ equation above enabled the calculation of the SEC cutoff volume, or the V_e that corresponds to the highest molar mass oligomer detectable by PAD. It was assumed that material eluting the SEC columns prior to this volume was of sufficiently high molar mass that it did not contribute to the DE. On the other hand, material eluting after the cutoff, detectable by PAD, was likely to contribute to the overall DE of the dextrin material. Hence, the mass percent of quantifiable oligomers was determined by integrating the RI signal and determining the ratio of the area collected after the cutoff volume to the total area. From Table 1 it can be seen that as DE increases the number of higher-DP oligomers detected by HPAEC–PAD decreases, with the exception of the DE 25 material, likely to have been acid hydrolyzed. Correspondingly, the mass fraction of low-molar-mass components as determined by SEC increases.

Dextrose equivalent was calculated as follows. From PAD calibration data, it was possible to accurately quantitate oligomers up to DP 7. Beyond DP 7, it was assumed that the molar detector response remained constant and its value was used to calculate the amounts of higher DP oligomers from peak areas. Table 1 contains the total moles calculated for each dextrin from HPAEC–PAD data. An average degree of polymerization (DP_{avg}) for each dextrin was calculated as, $DP_{avg} = \Sigma[DP \cdot mol]_i \div \Sigma mol_i$, where the mole quantity of each oligomer, i, is weighted by its DP and the sum over i is divided by the total moles found. An apparent DE was then calculated as $DE = 100/DP_{avg}$ which, after multiplying by the mass correction as determined by SEC, yielded DE per gram of material on a dry mass basis. The last column in Table 1 gives the titration values for four of the dextrins as determined in our laboratory, for comparison purposes. Multiple titrations of each DE sample were run and the results are averages with a standard deviation of less than 0.1 unit.

4. Conclusions

We have applied HPAEC–PAD and SEC–MALS–RI to characterize commercial dextrins. HPAEC–PAD enabled the quantitation of oligosaccharides to DP 7. Assumption of a constant molar detector response above DP 7 enabled quantitation for the purpose of estimating dextrose equivalent from chromatographic data. We have shown that DE can be estimated with relatively good accuracy from a combination of oligosaccharide profiling by HPAEC–PAD and an understanding of molar mass distribution obtained by SEC–MALS–RI. This was

Table 1
Summary of results

DE (nominal)	Maximum DP detectable	Molar mass, M_r (g/mol)	SEC cutoff volume (ml)	Σmol_i ($\cdot 10^8$)	$\Sigma[DP \cdot mol]_i$ ($\cdot 10^8$)	DP_{avg}	$100/DP_{avg}$	Mass correction	Calculated DE	Titration DE
4	40	6503.8	14.8	0.647	6.05	9.4	10.7	0.315	3.4	–
7	36	5855.3	14.9	0.615	4.16	6.8	14.8	0.342	4.8	6.7
10	33	5368.8	15.0	2.06	12.1	5.9	17.0	0.598	10.3	12.3
18 (supplier A)	20	3260.9	15.6	1.81	8.17	4.5	22.2	0.679	15.2	–
18 (supplier B)	20	3260.9	15.6	2.07	8.74	4.2	23.7	0.707	16.9	20.1
20	14	2288.0	16.0	2.23	8.25	3.7	27.0	0.752	20.5	–
25	25	4071.7	15.4	2.42	8.33	3.4	29.1	0.858	24.8	25.8

SEC cutoff volume (column 4) = [log ($1/M_r$) + 9.37]/0.375, where M_r is molar mass of highest DP detected (column 3). DP_{avg} (column 7) = $\Sigma[DP \cdot mol]_i \div \Sigma mol_i$, where the summation is taken over all detectable oligosaccharides, i. Calculated DE = $DP_{avg} \cdot$ mass correction (see text).

accomplished by quantitating oligosaccharides, determining an average degree of polymerization, and correcting for the mass of ''inert'' dextrin not likely to contribute to the reducing power. Potential sources of inconsistency between calculated DE and that obtained by titration might include: (1) incomplete SEC resolution and errors in the estimate of cutoff volume, (2) all PAD-detectable oligosaccharides may not necessarily reduce Fehling's solution, (3) nonstoichiometry of the titration method, (4) the molar PAD response may not be strictly constant above DP 7. Nevertheless, the combination of methods provides a thorough characterization of starch hydrolysis products that can aid product developers and formulators to better understand the relationship between composition and functionality.

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