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On the use of differential solubility in aqueous ethanol solutions to narrow the DP range of food-grade starch hydrolysis products

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ABSTRACT

Considerable research is focused on understanding the functionality of starch hydrolysis products (SHP) consisting of glucose, maltose, maltooligosaccharides (MOS), and maltopolysaccharides (MPS). A confounding factor in this research is the high molecular dispersity of commercially available SHP. The study presented herein characterizes a flexible fractionation approach for lowering the dispersity of such products. This was accomplished by fractionating a corn syrup solids (CSS) preparation based on the differential solubility of its component saccharides in aqueous-ethanol solutions. Products obtained from selected fractionations were characterized with respect to degree of polymerization (DP; liquid chromatography), dextrose equivalency (reducing sugar assays), and prevalence of branching (NMR). Glucose and maltose were preferentially removed from CSS using high (⩾90%) ethanol extractants. Preparations with relatively narrow ranges of MOS, lower DP MPS, and higher DP MPS were obtained through repetitive 70%-ethanol extractions. Linear, as opposed to branched, MOS and MPS were preferentially extracted under all conditions tested.

1. Introduction

Starch hydrolysis products, including maltodextrins, corn syrup solids, high fructose corn syrups, glucose syrups, and cyclodextrins, have been commercially available for use as food ingredients for many years. Of these, maltodextrins (MD) and corn syrup solids (CSS) are primarily composed of glucose and glucose polymers (*i.e.*, disaccharides, maltooligosaccharides (MOS), and maltopolysaccharides (MPS)) (Damodaran, Parkin, & Fennema, 2008). MOS and MPS are typically classified based on two factors, (a) their chain length expressed as degree of polymerization (DP) and (b) whether or not the molecules contain α-1, 6 linkages (Whistler & BeMiller, 1997). The IUPAC suggestion with respect to the nomenclature of polymers with repeating units, as is the case with MOS and MPS, is to use the term "oligo" for those polymers with DP 3–10. Therefore, in this paper MOS and MPS are defined as having DP 3–10 and DP > 10, respectively. Commercial MD and CSS are differentiated based on their dextrose equivalency (DE), where DE is the reducing power of the product as a percentage of the reducing power of an equivalent weight of glucose. CSS have DE values equal to or greater than 20; MD have DE values less than 20. DE values of products are inversely related to the number-average DP of the component glucose polymers.

The structural, functional, and nutritional properties of commercial CSS/MD preparations have been studied extensively (for general reviews see Chronakis, 1998; Hofman, Van Buul, & Brouns, 2015; Marchal, Beeftink, & Tramper, 1999). This includes studies pertaining to their use in fat replacement formulations (Hadnađev et al., 2014), thickener applications (Avaltroni, Bouquerand, & Normand, 2004; Wang & Wang, 2000), bulking agent applications (Shah, Jones, & Vasiljevic, 2010), emulsion stabilization (Dokic-Baucal, Dokic, & Jakovljevic, 2004), gelation (Loret, Meunier, Frith, & Fryer, 2004), flavor encapsulation (Madene, Jacquot, Scher, & Desobry, 2006), applications as drying aids (Werner, Fanshawe, Paterson, Jones, & Pearce, 2007), their use in infant and clinical nutrition (Braquehais & Cava, 2011), and as a starting material for the production of novel dietary fibers (Leemhuis et al., 2014). The vast majority of such studies compare the performance of commercially available CSS/MD preparations based solely on their DE values. This is bothersome as CSS/MD preparations of equivalent DE may have significantly different DP profiles and it is the DP profile that is likely to dictate functionality

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(White, Hudson, & Adamson, 2003). An approach to improving the interpretability of such studies is to use CSS/MD preparations having relatively narrow, well-defined DP profiles. This approach would also be beneficial in sensory studies investigating the taste properties of CSS/MD preparations. In such cases, it would be particularly important to remove the simple sugars from CSS/MD preparations since they, in particular, evoke sweet taste (Hettinger, Frank, & Myers, 1996; Lapis, Penner, & Lim, 2014; Turner, Byblow, Stinear, & Gant, 2014).

A number of fractionation techniques can be applied to the task of narrowing the DP range of CSS/MD preparations. These separation techniques, which are based on differences in molecular size, ion interactions, hydrophobicity, solubility, etc., are analogous to those used in carbohydrate analyses (Sanz & Martínez-Castro, 2007). The techniques best suited for CSS/MD fractionation are expected to be dependent, at least to some extent, on how the fractionated CSS/MD are to be used. For example, product end use may dictate the permissible DP range, DP profile, and food-grade nature of the CSS/MD fractions. CSS/MD-based studies in the food sciences often attempt to correlate the physicochemical properties of food-grade CSS/MD-containing products with the sensory attributes of those products. With this in mind, the fractionation technique used to modify the DP profile of a CSS/MD preparation (1) should be capable of producing relatively large amounts of material such that functional and sensory tests can be performed (*i.e.*, tens to hundreds of grams of refined CSS/MD preparations are likely to be required), (2) the resulting final products must be food grade, and (3) the methods used cannot be prohibitively expensive when working at the scale necessary for functional/sensory studies. Furthermore, it would be beneficial if the glucose and maltose content of the fractionated MOS/MPS preparations was minimized since the sweetness associated with these sugars may confound the preparations' other sensory properties (Blanchard & Katz, 2006; Feigin, Sclafani, & Sunday, 1987). Fractionation schemes based on the differential solubility of MOS/MPS in ethanol/water mixtures are capable of meeting all of the above criteria. A further benefit of such schemes is the antimicrobial nature of ethanol.

The general relationship between the DP of MOS/MPS and their relative solubility in ethanol/water mixtures is well established. In general, MOS/MPS decrease in solubility with increasing ethanol concentrations and for any given ethanol concentration the higher the DP of the MOS/MPS the lower its solubility (Bouchard, Hofland, & Witkamp, 2007; Defloor, Vandenreyken, Grobet, & Delcour, 1998). Low molecular weight sugars are generally quite soluble in water and alcohol, presumably due to their many hydroxyl groups and the associated polar character. Based on this rationale, their solubility is expected to decrease as the polarity of the solvent decreases, as with the addition of ethanol. The lower solubility of the higher molecular weight MOS/MPS relative to low molecular weight MOS/simple sugars has been attributed to the latter having more free hydroxyls per sugar unit (Wrolstad, 2012).These relationships have been exploited in cases where the DP of MOS/MPS is relevant to data interpretation. For example, Robyt and French (1967) used ethanol precipitation (final concentration 66% w/v) to separate larger MPS (average DP $\geqslant$ 20) from smaller MOS/MPS (DP $\leqslant$ 12) while studying the action-pattern of amylase-catalyzed amylose hydrolysis. Frigård, Andersson, and Åman (2002) used a similar approach, precipitating MOS/MPS with sequential additions of ethanol (ethanol concentrations from 20% to 80% w/v), to study the enzymatic digestion of amylopectins. Gelders, Bijnens, Loosveld, Vidts, and Delcour (2003) also used stepwise increases in ethanol content (10% w/v increments) to obtain MOS/MPS of similar DP for subsequent chromatographic analyses. The amounts of MOS/MPS produced in each of these studies were on the analytical scale, typically milligrams. Fractional precipitation with ethanol has also been used on the preparative scale, such as for the separation of amylose and amylopectin from starch dispersions/solutions (Patil, Somvanshi, Gupte, & Kale, 1974) and for the partial fractionation of MD preparations in an investigation of their role in bread firming (Defloor et al., 1998).

The present paper describes an ethanol-based fractionation approach for use with commercially available CSS/MD products that results in food-grade MOS/MPS preparations having relatively narrow DP profiles. The approach is an extension of that presented by Defloor et al. (1998) in which they used single ethanol precipitations/extractions to narrow the DP profile of commercial MD preparations. Their approach was successful in that the average DP of the MOS/MPS preparations shifted relative to that of the starting material; the associated standard deviations describing DP dispersity decreased but the actual DP-ranges of the different MOS/MPS preparations remained large. This result is undoubtedly due to the use of single ethanol extractions for fractionations. Equilibrium considerations based on component saccharide solubilities suggest that multiple precipitations/extractions will significantly improve the DP character of the resulting MOS/MPS preparations. That is the approach outlined in this work to obtain relatively large amounts of food-grade solvent-free MOS/MPS preparations of relatively narrow DP range containing minimal amounts of glucose and maltose.

2. Materials and method

2.1. Materials

Corn syrup solids (CSS): STARDRI® DE20, kindly provided by Tate & Lyle Ingredients Americas (Decatur, IL).

Carbohydrate standards: glucose and maltose (Sigma Aldrich Corporation, St. Louis, MO); maltotriose, maltotetraose, and maltooctaose (Carbosynth Limited, UK); maltopentaose, maltohexaose, and maltoheptaose (TCI America, Portland, OR).

Reagents: ACS-grade anthrone (99%, Alfa Aesar, Ward Hill, MA); bicinchoninic acid sodium salt (BCA; Pierce Chemical Co., Rockford, IL); cupric sulfate pentahydrate (Sigma Aldrich Corporation, St. Louis, MO)

Solvents: ACS/USP-grade ethanol (100%, Pharmco Aaper, Shelbyville, KT); deuterium oxide (99.96%, Cambridge Isotope Laboratories, Tewksbury, MA); deionized (DI) water for aqueous solutions and HPLC analyses (18.2 Ω, produced using a Millipore Direct-Q® 5 UV-R water purification system).

2.2. Methods

MOS/MPS sample preparation procedure: In the following text the term "washed" is used in reference to components recovered from the solid phase following centrifugation of a liquid/solid two phase system; the term "extracted" is used in reference to components recovered from the liquid phase following centrifugation of a liquid/solid two phase system.

A scheme illustrating the following fractionation steps is presented in Fig. 1.

2.2.1. Fractionation step 1

A 50% (w/v) CSS in water mixture was prepared by adding 75 g CSS to a 150 mL volumetric flask to which DI water was added to volume; stirring was continued until a translucent solution was obtained. The solution was then split into thirds, 50 mL each, in three separate beakers. To each beaker was added 450 mL 100% ethanol; ethanol addition resulted in immediate formation of a white opaque suspension and visible precipitate. The opaque suspension was stirred for 5 min at 300 rpm (using a magnetic stirrer), the liquid phase was then transferred to 250 mL high-density poly-

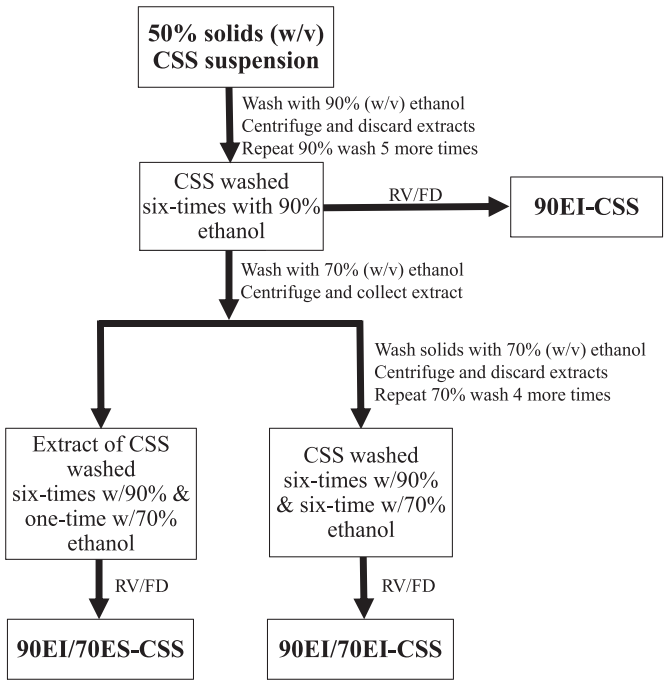

Fig. 1. A diagram for aqueous-ethanol solubility-based fractionation of corn syrup solids (CSS). Acronyms used in figure: "RV/FD", rotary evaporation with repeated solvent exchanges (3× water) followed by freeze-drying; "90EI-CSS", 90% ethanol-insoluble solids-enriched CSS; "90EI/70ES-CSS", 70% ethanol-soluble solids-enriched 90EI-CSS; "90EI/70EI-CSS", 70% ethanol-insoluble-enriched 90EI-CSS.

ethylene bottles and centrifuged for 15 min at 10,000 rpm. The resulting clear supernatant was decanted and saved for analysis. The washed white pellet was returned to the original beaker containing remnant precipitated solids; the combined solids in the beaker, at this point, had thus been washed once with 90% ethanol. To the once-washed solids in each beaker was added 50 mL of DI water, with stirring, to again produce a clear solution. Ethanol, 450 mL, was again added to the solution, followed by mixing, centrifugation, and decantation as previously described. The solids at this point were twice-washed with 90% ethanol. This overall process was repeated four more times. The recovered solids had thus been six-times washed with 90% ethanol, all done at ambient temperature, *i.e.*, 18–21 °C. Each wash consisted of first dissolution of the solids in water, then precipitation by the addition of ethanol. The six-time 90% ethanol-washed solids, contained in the three beakers, were either dried for use directly or further processed as described below (see Section 2.2.2). When drying directly, the solids in each beaker were first dissolved in 50 ml DI water and then combined in one 1000 ml round bottom flask. Residual solvent was removed by repeated solvent-exchanges; the resulting solvent-free viscous aqueous solution was then freeze-dried as described below (see Section 2.2.3). The resulting solid preparation is hereafter referred to as 90% ethanol-insoluble corn syrup solids (90EI-CSS).

2.2.2. Fractionation step 2

The six-time 90% ethanol-washed solids (90EI-CSS; contained in three beakers as a result of Section 2.2.1) were again dissolved in 50 ml water, then 117 mL 100% ethanol was added to give a 70% ethanol suspension (in such cases the 117 ml ethanol was measured by weight, taking into account the density of the ethanol preparation). After stirring at 200 rpm for 5 min the readily-decanted off-white 70% ethanol suspension was centrifuged at 10,000 rpm for 15 min. The clear supernatant was decanted into a round bottom flask, ethanol was removed as described below, and the resulting aqueous solution was freeze-dried resulting in a preparation hereafter referred to as 90% ethanol-insoluble/70% ethanol-soluble corn syrup solids (90EI/70ES-CSS). The insoluble solids resulting from the first 70% ethanol wash formed a translucent gel at the bottom of the beaker, as did subsequent washes. The insoluble solids (*i.e.*, the gel) were subsequently washed five more times by first dissolving the solids in 50 mL DI water, adding ethanol to a final concentration of 70%, stirring, centrifugation, and decantation of the liquid phase in a manner analogous to that described for Section 2.2.1. The five 70% ethanol extracts were discarded; *i.e.*, only the initial 70% ethanol extract was used to make 90EI/70ES-CSS. The resulting six-times 70% ethanol-washed solids were processed to remove ethanol and freeze dried as described below; this preparation is hereafter referred to as 90% ethanol-insoluble/70% ethanol-insoluble corn syrup solids (90EI/70EI-CSS).

The entire fractionation scheme used to prepare the three MOS/MPS preparations (*i.e.*, 90EI-CSS, 90EI/70ES-CSS and 90EI/70EI-CSS) is depicted in Fig. 1.

2.2.3. Solvent removal and drying

Ethanol was removed from all preparations using a rotary evaporator (Büchi Rotovapor R-205, Büchi Labortechnik AG) equipped with a 60 °C water bath (Buchi B-490) and a high vacuum pump (Chemglass Scientific Apparatus/10 Torr). Complete ethanol removal required several solvent-displacement steps as follows; initial solvent removal was done by rotary evaporation for 10 min at 120 rpm (at this point samples were highly viscous liquids), 100 mL of DI water was then added to the sample with mixing, rotary evaporation was again done for approximately 10 min. This solvent-displacement process, *i.e.*, adding DI water followed by evaporation, was repeated twice more (*i.e.*, three solvent exchanges following initial solvent removal). 90EI/70ES-CSS, due to its greater solvent content, required rotary evaporation for 20 min for initial solvent removal. In all cases, complete ethanol removal was assessed using proton NMR (see below). Final ethanol-free samples, as viscous aqueous solutions, were then frozen at −12 °C (−10 °F) and subsequently dried by lyophilization in a VirTis CONSOL 4.5 freeze dryer.

2.2.4. Total carbohydrate assay

The total carbohydrate content of each MOS/MPS preparation was determined by the spectrophotometric anthrone/sulfuric acid assay as described by Brooks and Griffin (1987). In the standard protocol, 3 ml anthrone reagent (0.1% (w/v) in 12.4 M sulfuric acid) was added to 25 µL of an aqueous carbohydrate-containing solution (prepared from the dried carbohydrate preparations) in appropriately sized test tubes; tubes were immediately topped with glass marbles to prevent evaporation and immersed in boiling water for 5 min. Tubes were then removed and quickly submerged in ice water for 15 min, after which absorbance was read at 630 nm. Calibration curves were prepared using solutions containing 0–3.0 mg/mL glucose (0–75 µg glucose per assay mixture). All calibration curve-derived total carbohydrate values for MOS/MPS samples were multiplied by 0.90 to adjust for the water of hydrolysis. Reported carbohydrate values are on a dry-weight basis; moisture contents having been determined by oven drying at 105 °C for 24 h. Assays were done in triplicate.

2.2.5. Reducing sugar assay

Reducing ends were quantified using the BCA/copper-based assay as described by Kongruang, Joo Han, Breton, and Penner (2004). One-milliliter of aqueous carbohydrate-containing solution was mixed with 1 mL BCA working reagent (prepared as in Garcia, Johnston, Whitaker, & Shoemaker, 1993) in glass tubes which were then capped with glass marbles and incubated at 80 °C for 30 min. Tubes were then cooled to room temperature and the absorbance was measured at 560 nm. Calibration curves were prepared with

solutions containing maltose (0, 5, 15, 30, 45, 60, 75 μM). Assays were done in triplicate.

2.2.6. Calculation of dextrose equivalency (DE) and number average degree of polymerization (DP)

DE and DP values were determined based on the reducing sugar content of an accurately weighed amount of CSS/MOS/MPS preparation. DE was calculated as DE = (moles reducing ends/100 g preparation) * 180. DP was calculated as DP = 111/DE. Reducing ends were determined as described above.

2.2.7. High performance liquid chromatography–evaporative light scattering detector (HPLC–ELSD)

Saccharide profiles were determined using a Prominence UFLC-HPLC system (Shimadzu, Columbia, MD) equipped with a system controller (CMB-20A), degasser (DGU-20A), solvent delivery module (LC-20AD), autosampler (SIL-10A), column oven (CT20-A), and evaporative light scattering detector (ELSD-LT II). Samples and standards were dissolved in DI water prior to chromatography. Samples were separated on combined Ag^{2+} polystyrene ion-exchange guard and analytical columns (Supelcogel, Hercules, CA) using DI water as the mobile phase. The mobile phase flow rate was 0.20 mL per minute; the column temperature was kept at 80 °C. The ELSD was kept at 60 °C and had a nitrogen gas pressure of >350 kPa. Simple sugar (*i.e.*, DP1–2) and MOS concentrations (*i.e.*, DP3–8) were calculated from external standard curves prepared using commercially available standards for MOS DP 1–8. Integration was done using LCsolution computer software (Shimadzu, Kyoto, Japan). The limit of detection (LOD) for each standard was calculated by taking the minimum detectable signal as 3 standard deviations above baseline and then calculating the corresponding concentration from the respective calibration curves (Skoog & Leary, 1992). Measured LOD values for the standards are: DP1 = 0.006 mg/ml, DP2 = 0.003 mg/ml, DP3 = 0.004 mg/ml, DP4 = 0.001 mg/ml, DP5 = 0.001 mg/ml, DP6 = 0.002 mg/ml, DP7 = 0.003 mg/ml, DP8 = 0.001 mg/ml. Calibration standards for MOS/MPS DP > 8 were not commercially available and the resolution of these saccharides was not sufficient for quantification.

2.2.8. High performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

Saccharide profiles were also determined using HPAEC-PAD in order to better understand higher MOS/MPS profiles (i.e. DP > 8). HPAEC-PAD analyses were done using a Dionex modular chromatograph system (Dionex, Sunnyvale, CA, USA) equipped with a gradient pump (GP50), autosampler (AS3500), column container (LC30) kept at 25 °C, and a pulsed amperometric detector (electrochemical detector; ED40) using the quad potential and a disposable Au electrode. Samples were dissolved in 100 mM NaOH prior to their injection (10 μL) into the chromatograph for separation using a linear gradient elution with a CarboPac PA-200 column (4 × 250 mm)/CarboPac PA-200 guard column (3 × 50 mm). The mobile phase, at a flow rate of 0.4 mL/min, was developed from eluent A (100 mM sodium hydroxide) and eluent B (100 mM sodium hydroxide containing 500 mM sodium acetate) such that the composition of the mobile phase at times 0, 30, 40, and 45 min were (%A–%B) 98–2, 60–40, 0–100, and 98–2, respectively. Dionex Peaknet software version 5.21 was used for data analysis.

2.2.9. Nuclear magnetic resonance (NMR)

NMR analyses were used to verify the absence of ethanol in the MOS/MPS preparations (Gottlieb, Kotlyar, & Nudelman, 1997) and to determine the relative amounts of (1 → 4) and (1 → 6) linkages (Nilsson, Bergquist, Nilsson, & Gorton, 1996). All MOS/MPS preparations were dissolved in D_2O prior to analyses. Prevalence of bond linkages were determined by integration of the peak areas for the α-(1 → 4) (5.305–5.395 ppm) and α-(1 → 6) (4.881–4.924 ppm) signals. The α-(1 → 4)/(1 → 6) ratios were calculated and are tabulated in Table 1. A Bruker AVIII 700 MHz 2-channel spectrometer with a 5 mm dual carbon (DCH) cryoprobe with a *z*-axis gradient and a Bruker AVI 400 MHz 2-channel spectrometer with a 5 mm Broad Band Observe with Fluorine (BBO-F) probe with *z*-axis gradient was used to analyze samples at room temperature dissolved in D_2O. Topspin 2.1 computer software was used to acquire spectra (data presented in "Supplemental materials," see Fig. S1).

3. Results and discussion

The aim of the presented work was to develop a simple approach to obtain food-grade MOS/MPS preparations containing minimal amounts of glucose and maltose and having relatively narrow DP ranges. The approach was to be relatively inexpensive and applicable to the preparation of tens-to-hundreds of grams of material, as is often needed for structure/function/sensory studies in the food and nutritional sciences. The outcome of this work is a rather simple procedure to fractionate CSS/MD preparations based on the differential solubility of the saccharide components in aqueous-ethanol solutions, as presented in Fig. 1. The following text provides the details underlying this fractionation approach along with commentary on modifications for altering the nature of the resulting MOS/MPS preparations; characterizations of representative fractionated MOS/MPS preparations are included.

3.1. Qualitative studies of MOS/MPS solubility in aqueous ethanol solutions

Initial work focused on qualitative estimates of the relative solubility of glucose, maltose, MOS and MPS in aqueous ethanol solutions containing ⩾50% ethanol. This was done by chromatographic analyses of the composition of the extracts obtained from liquid–solid extractions of the CSS starting material with aqueous-ethanol mixtures differing in ethanol content. Each experiment required first dissolving the CSS preparation in water followed by the addition of the appropriate amount of ethanol; the initial dissolution in water was required due to the clumping of CSS solids when directly exposed to ⩾50% ethanol solutions. Differences in the nature of the precipitates formed in different ethanol concentrations were obvious. The 90% ethanol extract of CSS formed a white opaque colloidal suspension immediately upon addition of ethanol; whereas addition of 70% ethanol to the 90% ethanol-washed CSS rapidly formed a translucent gel at the bottom of the aqueous-ethanol liquid phase. HPLC analyses of the different extracts (i.e., liquid phases) provided information on the relative extractability of the different CSS components. As anticipated, the solubility of all components decreased with increasing ethanol content and, in general, the size of the components was inversely related to their extent of extraction into the different aqueous-ethanol solutions. Relatively simple break points were observed with regard to the extraction of MOS and MPS: (a) extracts containing ⩾90% ethanol contained appreciable amounts of glucose, maltose and MOS of DP 3–7 (MOS_{DP3-7}), *i.e.*, MOS/MPS with DP ⩾ 8 were not detected in chromatographic analyses of these extracts and (b) extracts containing ⩽70% ethanol contained, along with the lower DP components, appreciable amounts of higher DP MOS and some MPS. With respect to glucose, maltose, and MOS_{DP3-7}, amounts recovered in aqueous ethanol extracts containing ⩾70% ethanol decreased as ethanol concentrations increased from 70% to 95%.

Table 1
Chemical characterization of CSS and MOS/MPS preparations[a].

Preparation[b]	Percent carbohydrate[c,d] (%)	mmoles reducing ends per gram[c,e]	Number-average DP[f]	Dextrose equivalent (DE)[g]	Linkage prevalence $(1 \rightarrow 4)/(1 \rightarrow 6)$[h]
CSS	92.8 ± 0.52	1.09 ± 0.08	5.6	19.6	15.2:1
90EI-CSS	95.5 ± 1.28	0.44 ± 0.03	14.0	7.9	7.7:1
90EI/70ES-CSS	95.7 ± 0.94	0.94 ± 0.06	6.6	16.9	24.9:1
90EI/70EI-CSS	99.0 ± 0.81	0.14 ± 0.01	44.4	2.5	5.9:1

[a] CSS = corn syrup solids, MOS = maltooligosaccharides, MPS = maltopolysaccharides.
[b] Acronyms denoting sample preparations are as defined in Fig. 1.
[c] Values are means ± SD (where applicable) expressed on a dry weight basis.
[d] Determined as "total carbohydrate" using the anthrone/H_2SO_4-assay with glucose as standard.
[e] Determined using Cu/bicinchoninic acid-assay with maltose as standard.
[f] DP = degree of polymerization; calculated as DP = 111/DE.
[g] DE = dextrose equivalency; calculated as DE = (moles reducing ends/100 g preparation) * 180.
[h] Determined from NMR spectra.

3.2. CSS fractionation with aqueous ethanol solutions and characterization of extracted solids

The two-step fractionation scheme depicted in Fig. 1 is based on the observations noted in the preceding paragraph. Throughout this section the analytical focus is on the extracted solids; analytical characterization of the preparations resulting from this fractionation are presented in the following Section 3.3. An initial 90% ethanol fractionation step was chosen to remove glucose and maltose from the original CSS preparation based on the noted insolubility of MOS/MPS with DP $\geqslant$ 8, the sufficiently low solubility of MOS_{DP3-7}, and the reasonable solubility of glucose and maltose at this ethanol concentration. An alternative initial fractionation step using 95% ethanol was considered because it would likely improve the recovery of MOS and MPS in subsequent steps, but the lower solubility of glucose and maltose in 95% ethanol meant additional extractions were required for their removal and this, in turn, increased both reagent cost and time of preparation. Thus, the first fractionation step, the principle aim of which was to remove glucose and maltose from the CSS starting material, was achieved through sequential extractions with 90% ethanol. The number of extractions required for glucose and maltose removal was determined from HPLC analyses of successive extracts. Fig. 2("a" and "b") depicts chromatograms characterizing the extracts from the first and sixth 90% ethanol extractions. The absence of glucose and the trace remaining maltose in the sixth extract points to the sufficiency of six extractions; the presence of MOS_{DP3-7} in the sixth extract demonstrates the detrimental effect of further unnecessary extractions on MOS_{DP3-7} yields. The result of the first fractionation step, which consists of six 90% ethanol extractions of the CSS starting material, is an MOS/MPS preparation effectively free of glucose and maltose and containing substantially reduced amounts of the lower DP MOS. The descriptor "effectively free" or "free" is used herein to indicate that a component cannot be detected using the HPLC system employed for these analyses (estimated detection limits for standards of DP 1–8 were all <0.01 mg per mL extractant; see Section 2.2.7 for details). Instrumentation with lower detection limits are likely to show the presence of these components (see discussion of HPAEC-PAD data below). As noted in the "Section 2.2", the preparation resulting from the first fractionation is herein referred to as 90% ethanol-insoluble CSS (90EI-CSS). The name is appropriate from the standpoint that the preparation is the insoluble phase remaining after six 90% ethanol washes, but it is a misnomer in the sense that some of the lower DP MOS contained in that preparation would partition into the liquid phase if yet another 90% ethanol wash were done (as depicted in Fig. 2b).

The second fractionation step was designed to enrich the MOS/MPS preparation resulting from the first fractionation (90EI-CSS) with respect to MOS and to prepare a higher DP fraction that was essentially free of the lower DP MOS. This was accomplished by doing sequential 70% ethanol extractions/washes (see "Section 2.2"). The liquid phase resulting from the first 70% ethanol extraction provided the MOS-enriched sample (referred to as 90% ethanol-insoluble/70% ethanol-soluble CSS; 90EI/70ES-CSS). Only the first extract was used to obtain 90EI/70ES-CSS because the extent of MOS enrichment decreased with each subsequent extraction (as depicted in Fig. 2c). Thus, maximum enrichment of MOS is achieved by using only the first extract of 90EI/70ES-CSS; the tradeoff in using only the first extract is a reduced yield. The second goal of the 70% ethanol fractionation step was to prepare an MPS-enriched preparation having minimal amounts of the lower DP MOS. This was achieved by doing six successive 70% ethanol extractions of 90EI-CSS (the first extract is used to prepare 90EI/70ES-CSS as just discussed, the following five extracts containing lower DP MOS were discarded). The efficacy of using six washes to reduce MOS content and enrich MPS content is illustrated in Fig. 2("c" and "d"); the second extract is shown to contain considerable MOS_{DP5-8}, whereas there are only minimal amounts of MOS_{DP7-8} in the sixth extract. The six-time 70% ethanol-washed solids (referred to as 90% ethanol-insoluble/70% ethanol-insoluble CSS; 90EI/70EI-CSS) is thus highly enriched in MPS.

The complete process for the fractionation of commercially available CSS/MD preparations, as depicted in Fig. 1 and described above, results in three MOS/MPS preparations: 90EI-CSS, 90EI/70ES-CSS, and 90EI/70EI-CSS. Average yields for each of the preparations, reported as weight percent of the amount of CSS starting material, were as follows: 90EI-CSS, 51.2 ± 1.3%; 90EI/70ES-CSS, 8.0 ± 0.37%; 90EI/70EI-CSS is 25.9 ± 2.1%. A yield of 50% indicates that 50 g of that preparation was obtained from 100 g of CSS starting material. Errors associated with yield values reflect the reproducibility of the entire fractionation process, including multiple washings, centrifugations, and decantations. Yield data are based on four replicates from two different experiments. Relative yields are as expected, with the highest being associated with the 90EI-CSS preparation and the lowest with the 90EI/70ES-CSS preparation. Some of the yields are relatively low (*i.e.*, that for 90EI/70ES-CSS being only 8.0 ± 0.37%), but that is the nature of the chosen fractionation method. The method requires multiple extractions; the bulk of the extracted solids are discarded in order to obtain preparations having relatively narrow DP ranges. It is important to recognize that even the lowest yield does not nullify the applicability of the overall method since both the starting material and the fractionating solvent are relatively inexpensive.

3.3. Characterization of MOS/MPS preparations resulting from CSS fractionation

The general characteristics of the three preparations, 90EI-CSS, 90EI/70ES-CSS, and 90EI/70EI-CSS, are summarized in Table 1.

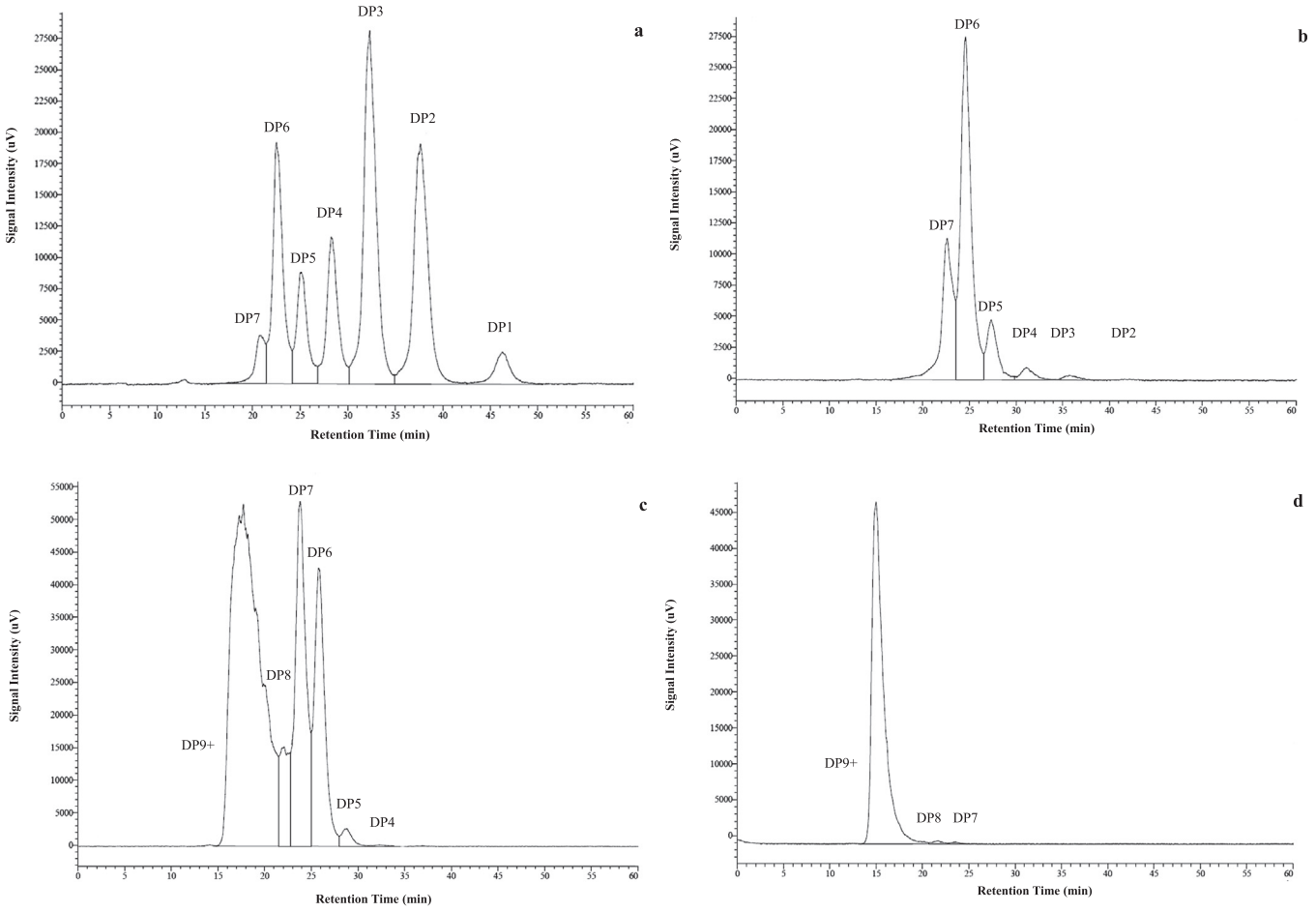

Fig. 2. Representative chromatograms from HPLC–ELSD depicting the saccharide character of extracts noted in Fig. 1: (a) first 90% ethanol extract of CSS; (b) sixth 90% ethanol extract of CSS; (c) second 70% ethanol extract of 90EI-CSS, (d) sixth 70% ethanol extract of 90EI-CSS. Acronyms are as defined in Fig. 1; DP = degree of polymerization.

The anthrone/H_2SO_4 assay-based carbohydrate content of each preparation was greater than 95%; which is an increase relative to the CSS starting material. Relative numbers of reducing ends per unit mass trended as expected based on the solubility of the preparations in aqueous ethanol. The least soluble preparation (90EI/70EI-CSS) had the lowest number of reducing ends per unit mass and correspondingly, its MOS/MPS composition has the highest number-average DP (calculated as number of reducing ends per unit mass); this also dictates that 90EI/70EI-CSS has the lowest DE value. All preparations had lower DE values than the starting material, which was expected based on the first fractionation step removing the lowest molecular weight components. The prevalence of branching for the different preparations is reflected in the $(1 \rightarrow 4)/(1 \rightarrow 6)$ ratios obtained from NMR spectra (data Table 1, Fig. S2 in "Supplemental materials"). The extent of branching in the different preparations bracket that found for corn starch ($(1 \rightarrow 4)/(1 \rightarrow 6)$ of ~20; Li et al., 2014). The ratio of linear to branched MOS/MPS is likely to be important in processes involving molecular recognition, such as enzyme-substrate and receptor-ligand interactions. The higher $(1 \rightarrow 4)/(1 \rightarrow 6)$ ratio for CSS compared to 90EI-CSS and of 90EI/70ES-CSS compared to 90EI/70EI-CSS reflects the preferential extraction of linear $(1 \rightarrow 4)$ MOS components into the aqueous ethanol phase. This result is taken to indicate that the higher DP MOS/MPS have higher percentages of branched linkages since the presence of $(1 \rightarrow 6)$ branching per se, in oligosaccharides/polysaccharides of equivalent mass, is expected to favor dissolution based on steric compatibility and the additional degree of freedom associated with the $(1 \rightarrow 6)$ linkage (Whistler, 1972).

Quantitative values for the glucose, maltose and MOS_{DP3-8} content of each preparation are given in Table 2; the corresponding chromatograms from HPLC–ELSD are depicted in Fig. 3. Values for MOS/MPS having DP $\geqslant$ 9 are combined due to the extent of resolution of this analytical system and because standards for MOS/MPS having DP $\geqslant$ 9 are not commercially available. Amounts of glucose and maltose in each preparation were below the detection limit of the system (corresponds to levels <0.1%); note from Table 2 that the CSS starting material is ~7.5% in combined glucose and maltose. The removal of glucose and maltose from each preparation is important with respect to keeping these primary "sweet saccharides" at negligible levels in studies with sensory/taste applications. Relative to the CSS starting material: 90EI-CSS was enriched in the higher DP MOS and MPS (due to the higher solubility of lower DP MOS in 90% ethanol), 90EI/70ES-CSS was enriched with respect to MOS and was effectively devoid of the highest MPS (the latter conclusion is based on data of Fig. 4, see below; enrichment was due to the higher solubility of the MOS relative to the MPS in 70% ethanol), and 90EI/70EI-CSS was enriched in MPS and effectively free of the lower DP MOS (due to the lower DP MOS having been removed through sequential 90% and 70% ethanol washes).

Qualitative profiles of the saccharide component content of each preparation were obtained using HPAEC-PAD (Fig. 4). The resolution allows visualization of higher DP MOS and MPS components through DP 25; following this is a broad peak (retention time ~35–43 min) for the unresolved higher DP MPS. The four chromatograms nicely illustrate the disparity in DP content for

Table 2
Percent saccharide composition of CSS and MOS/MPS preparations based on HPLC–ELSD analyses.[a,b]

	CSS	90EI-CSS[c]	90EI/70ES-CSS[c]	90EI/70EI-CSS[c]
DP1[d]	1.9 ± 0.0	ND[e]	ND	ND
DP2	5.6 ± 0.2	ND	ND	ND
DP3	8.6 ± 0.4	1.4 ± 0.2	4.0 ± 0.3	ND
DP4	5.1 ± 0.1	1.4 ± 0.1	4.4 ± 0.3	ND
DP5	5.7 ± 0.2	2.2 ± 0.2	7.6 ± 0.3	ND
DP6	15.6 ± 0.4	9.0 ± 0.8	28.2 ± 1.5	ND
DP7	7.5 ± 0.2	8.0 ± 0.2	20.5 ± 1.1	ND
DP8	4.2 ± 0.2	4.3 ± 0.2	10.3 ± 0.5	ND
DP1–2	7.5 ± 0.2	ND	ND	ND
DP3-8	46.6 ± 1.6	26.4 ± 1.5	75.5 ± 1.8	ND
DP9+	45.9 ± 1.8	73.6 ± 1.5	24.5 ± 1.8	100

[a] CSS = corn syrup solids, MOS = maltooligosaccharides, MPS = maltopolysaccharides; all values are average ± SD of four replicates.
[b] Values for DP 1–8 are based on integrated peak areas from HPLC–ELSD analyses; the value for DP9+ was obtained by taking the difference between the summed values for DP 1–8 and the mass of CSS or MOS/MPS used in the analyses.
[c] Acronyms denoting sample preparations are as defined in Fig. 1.
[d] DP = degree of polymerization; # = number of glucose units, "9+" indicates glucose polymers with ⩾9 glucose units.
[e] ND = Not Detected.

the different preparations. Chromatograms "a" and "b" illustrate the preferential extraction of glucose, maltose, maltotriose, and maltotetraose through the initial 90% ethanol fractionation step. Chromatograms "c" and "d" illustrate the impact of the subsequent 70% ethanol fractionation step; preparation 90EI/70ES-CSS is shown to be devoid of the higher DP MPS which, due to their low solubility in 70% ethanol, have been concentrated in 90EI/70EI-CSS. Chromatograms from anion-exchange liquid chromatography were not used for MOS quantification due to difficulties in obtaining reliable detector response factors for all MOS (Koch, Andersson, & Aman, 1998).

The fractionation approach used to obtain 90EI-CSS, 90EI/70ES-CSS and 90EI/70EI-CSS is similar to that used recently by Sen, Gosling, and Stevens (2011) to selectively enrich galactosyl oligosaccharide preparations. The starting materials in the Sen et al. study and the present one differ considerably in that starch hydrolysis products contain primarily (1 → 4) linked α-D-glucopyranosyl units with some (1 → 6) branching while the galactosyl oligosaccharides of Sen et al.'s study are known to have much greater heterogeneity (Gosling, Stevens, Barber, Kentish, & Gras, 2010). The trends established in the two studies are similar, although in the present work both the initial and final monosaccharide/disaccharide content of the preparations was significantly lower.

4. Concluding comments

The work presented herein outlines a rather simple approach to obtain food-grade MOS/MPS preparations having relatively narrow DP ranges. The approach is expected to be generally transferable with respect to the aqueous-ethanol solubility of components common to commercially available CSS and MD preparations.

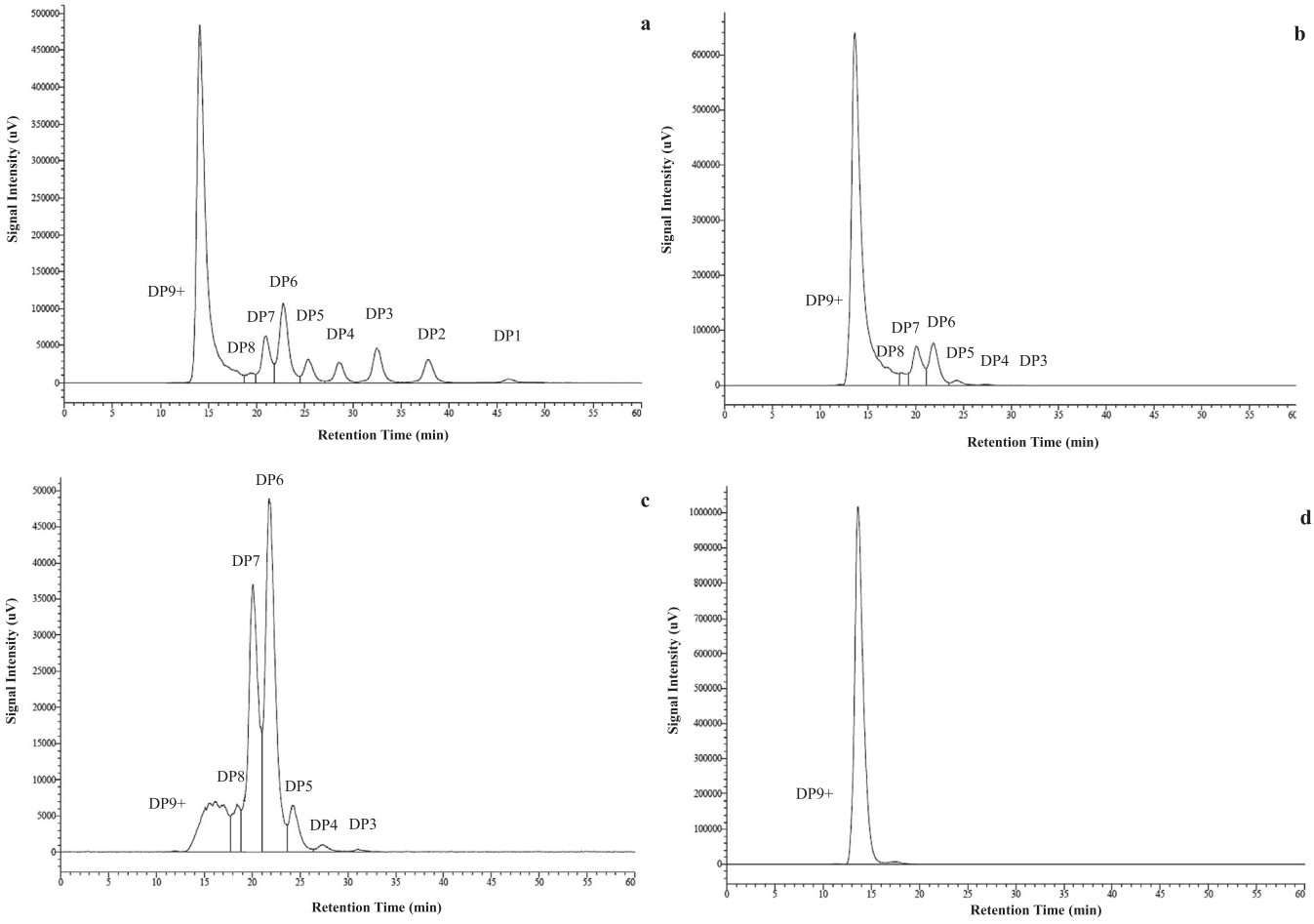

Fig. 3. Representative chromatograms from HPLC–ELSD depicting the saccharide character of (a) CSS, (b) 90EI-CSS, (c) 90EI/70ES-CSS, and (d) 90EI/70EI-CSS. Acronyms are as defined in Fig. 1; DP = degree of polymerization.

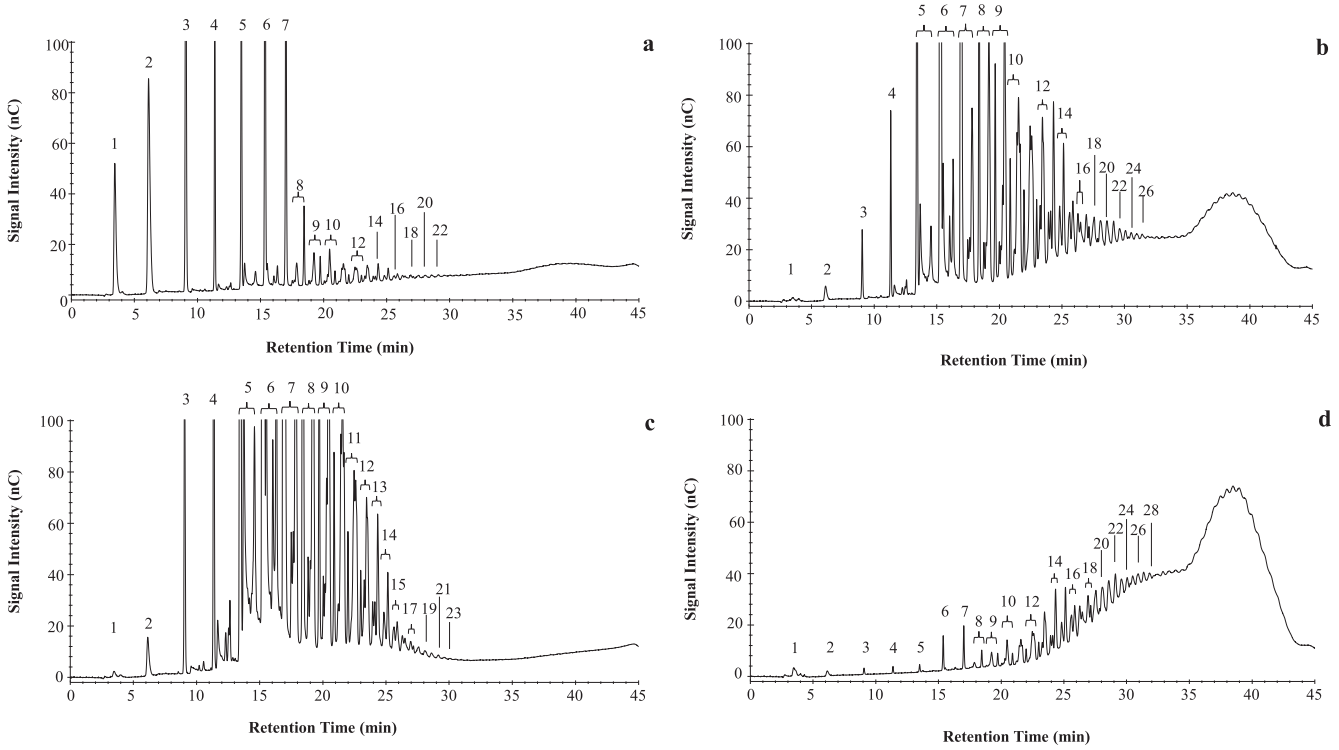

Fig. 4. Representative chromatograms from HPAEC-PAD depicting the saccharide character of (a) CSS, (b) 90EI-CSS, (c) 90EI/70ES-CSS, and (d) 90EI/70EI-CSS. Acronyms are as defined in Fig. 1; peak integers correspond to DP values.

The presented work was based on a CSS starting material; the relative yields and DP profiles obtained in this work reflect that starting material. If one were to use a low-DE MD preparation as the starting material, for example, then yields from the presented ethanol fractionation scheme are expected to be higher for the higher DP MPS-containing preparations and lower for the predominantly MOS-containing preparations. This is because a low-DE MD starting material would have a higher percentage of high DP MOS and MPS, relative to low DP MOS, than the CSS starting material used in the present study. Altering ethanol concentrations and/or using different food-grade solvents for the fractionation steps will likewise change yields and DP profiles of the resulting preparations. Additional processing steps may also be included in order to adapt the method to different needs. For example, selective hydrolysis of the α-(1 → 6) linkages (Koch et al., 1998; Wang & Wang, 2000) may be used to enhance the linear (1 → 4) MOS/MPS content of preparations. Clearly, the approach characterized in this work can be readily adapted to meet different objectives; the data provided herein is expected to provide a fundamental basis upon which to make such adaptations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.10.120.

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