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Preparation and characterization of isolated low degree of polymerization food-grade maltooligosaccharides

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ABSTRACT

Research involving human responses to the consumption of starch and its hydrolysis products would benefit from convenient sources of well defined, low cost, food-grade maltooligosaccharides (MOS). This report addresses such need by presenting an approach to obtain aforementioned MOS. A chromatography-ready MOS sample containing proportionately high amounts of low degree of polymerization (DP) MOS is initially prepared from commercially-available maltodextrins (MD) by taking advantage of the DP-dependent differential solubility of MOS in aqueous-ethanol solutions. The low DP-enriched MOS preparation is subsequently fractionated via preparative column chromatography using cellulose-based stationary phases and step-gradient aqueous-ethanol mobile phases. The resulting fractions yielded isolated food-grade MOS ranging in DP from 3 to 7. NMR spectra of isolated MOS indicated minimal amounts of branched saccharides. Typical yields from a single fractionation protocol (2 g MD starting material), including solvent partitioning through preparative chromatography, ranged from ~40 mg for DP 4, 5, and 7 to ~100 mg for DP 3 and 6.

1. Introduction

Hydrolysis of native starch yields a variety of shorter-chain starch hydrolysis products (SHP) that are widely used throughout the food industry (Brooks & Griffin, 1987). Of these, maltodextrins (MD) are primarily composed of glucose oligomers and polymers, i.e., maltooligosaccharides (MOS) and maltopolysaccharides (MPS), with an overall average degree of polymerization (DP) greater than 5 (Damodaran, Parkin, & Fennema, 2007). Throughout this paper, we will define MOS and MPS as SHP with a DP of 3–10 and DP > 10, respectively. MD are typically used in foods to improve texture, volume, and stability (Takeiti, Kieckbusch, & Collares-Queiroz, 2010; Damodaran et al., 2007; Hofman, van Buul, & Brouns, 2016). These attributes have led researchers to explore their use as replacements for sugar (Chen, Zhao, Pang, & Li, 2015), fat (Hadnađev, Dokić, Hadnađev, Pajin, & Krstonošić, 2011), artificial preservatives (Barreteau, Delattre, & Michaud, 2006), antistaling agents (Durán, León, Barber, & Benedito de Barber, 2001), and as encapsulating food ingredients (Kenyon, 1995). The common use of MOS in foods has generated interest in their structural/functional relationships. The properties of these saccharides are of direct relevance to starch, because when consumed, native starch is typically hydrolyzed to generate MPS, MOS, maltose, and glucose (Zhang, Hasek, Lee, & Hamaker, 2015)

The extent to which a given MD preparation influences the different functional and organoleptic properties of a food is highly dependent on the product's DP profile, which itself is determined by the component saccharides. Commercial MD products are typically polydisperse; i.e., the component saccharides have a wide DP distribution, and are sold based on their average DP. However, the DP profile of an MD product, as compared to the product's average DP, is a more adequate determinant of the product's functionality (Marchal, 1999; Kearsley & Dziedzic, 1995). Commercial MD products with high dispersity are often not appropriate for studies aimed at determining the role of DP in dictating the characteristics of MOS/MPS-preparations. In such cases it is better to have MOS/MPS preparations that cover a relatively narrow range of DP.

Preparative fractionation of MOS- and lower DP MPS-containing MD preparations has been accomplished using a variety of techniques. Whistler and Durso (1950) first reported the use of charcoal/celite stationary phases, in conjunction with water/ethanol mobile phases, to remove mono-, di-, and trisaccharides from MOS. This technique was the basis for several subsequent studies on MOS separation with

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charcoal (Andrews, Hough, & Powell, 1956; Hoover, Nelson, Milner, & Wei, 1965; Moon & Cho, 1997; Whelan, Bailey, & Roberts, 1953; French, Robyt, Weintraub, & Knock, 1966). Charcoal has also been used in batch mode to separate low versus high molecular weight saccharides in natural products (Morales, Sanz, Olano, & Corzo, 2006). Cellulose adsorbents have also been used for MOS separation; this being done using both paper (Commerford, VanDuzee, & Scallet, 1963; Thoma & French, 1957) and column (Thoma, Wright, & French, 1959) chromatography. Notably, Thoma et al. (1959) were successful in isolating MOS/MPS up to DP 18 using a cellulose-based column in conjunction with a butanol-based mobile phase; however, the dependence on butanol makes this method prohibitively expensive for most food-grade applications. Alternative sorbents for preparative MOS fractionation, including other polymer-based materials, have also been explored (Lee, Kwon, & Moon, 2003; Kondo, Nakatani, & Hiromi, 1981). Balto et al. (2016) developed a food-safe preparative fractionation method based on differential solubility in aqueous-ethanol solutions to significantly narrow the DP range of MOS/MPS-containing preparations. That approach is attractive in the sense that the resulting products are food-grade and recovered in relatively high yields (hundreds of grams), but is limited in that the resulting preparations do not provide isolated MOS. The aforementioned fractionation approaches were designed either to acquire relatively small amounts of isolated MOS for chemical analyses and/or enzyme specificity studies or to obtain MOS preparations with narrow DP profiles. Thus, all of these techniques suffer from one or more of the following: 1) the resulting MOS preparation is not food-grade, 2) the MOS preparations are prohibitively polydisperse, or 3) the approach is excessively expensive and/or tedious on the scale necessary to produce sufficient refined MOS for metabolic and sensory work.

Studies that would benefit from refined food-grade MOS preparations include those that attempt to understand the role DP plays in dictating physiological responses to MOS consumption (for related review, see Hofman et al., 2016). Three active areas of research falling within this general subject area are (a) taste perception of MOS (Lapis, Penner, & Lim, 2014, 2016; Pullicin, Penner, & Lim, 2017) (b) impact of oral carbohydrate/MOS sensing on physical performance (Jeukendrup & Chambers, 2010; Rollo & Williams, 2011), and (c) metabolic responses to MOS (Glendinning et al., 2017). These physiological phenomena undoubtedly involve enzyme, receptor and/or transporter systems that are at least somewhat DP-specific. Studies within these fields of research sometimes involve human subjects (as opposed to animal, cell culture, or cell-free systems). In such cases the MOS preparations must not only be well defined; they must also be food-grade and in sufficient quantity for human testing.

The objective of this study was to develop a separation method that would (1) yield isolated MOS from SHP in quantities sufficient for human testing, (2) use only food-safe materials and thus result in food-grade MOS preparations, (3) use relatively inexpensive materials and equipment, and (4) be highly reproducible. The work focused on optimizing a relatively simple fractionation approach based on the DP-dependent differential solubility of MOS/MPS in aqueous-ethanol solutions and microcrystalline cellulose-based preparative chromatography to generate isolated MOS preparations. Various aqueous-ethanol washes were examined and compared for use in the solubility step, and multiple stationary phases and solvent systems were explored for use in flash chromatography-based fractionation.

2. Materials and methods

2.1. Materials

Maltodextrin (MD) was kindly provided by Tate & Lyle Ingredients Americas, Decatur, IL (Corn syrup solids: STARDRI® DE20). Solvents included ACS/USP-grade 100% ethanol (Pharmco Aaper, Shelbyville, KT); and deionized (DI) water (18.2Ω), produced using a Millipore Direct-Q® 5 UV-R water purification system. Microcrystalline cellulose (Avicel PH 101) was from FMC Corp. (Philadelphia, PA). TLC silica gel 60 plates were purchased from EMD Millipore (Billerica, MA). Carbohydrate standards included glucose and maltose from Sigma Aldrich Corporation (St. Louis, MO); maltotriose and maltotetraose from Carbosynth Limited, (UK); maltopentaose, maltohexaose, and maltoheptaose from TCI America (Portland, OR). 1-napthol (ReagentPlus® ≥99%) and butyl alcohol (*n*-butanol, ≥99%, FCC, FG) were from Sigma-Aldrich (St. Louis, MO); sulfuric acid (ACS, 95–98%) and anthrone (ACS) were from Alfa Aesar (Ward Hill, MA); bicinchoninic acid sodium salt (BCA) from Pierce Chemical Co. (Rockford, IL); and deuterium oxide (99.96%) from Cambridge Isotope Laboratories (Tewksbury, MA).

2.2. Methods

2.2.1. Chromatography-ready maltooligosaccharides (CMOS) preparation

Two grams MD was dissolved in 3 mL deionized water at 50–55° with stirring. Following dissolution, 3 mL of 100% ethanol was added and stirring/heating continued until a clear solution was obtained. Additional 1 mL increments of ethanol were then added up to a total of 9.5 mL ethanol, giving a 76% ethanol/water mixture (all solvents described herein are on a v/v basis). Up to 2 min, with stirring/heating, was given between each incremental ethanol addition to ensure sufficient mixing and allow higher DP MOS precipitation. After making the sample 76% ethanol, the mixture was stirred for an additional five minutes, after which the top liquid phase, which appeared as a translucent white suspension, was pipetted off for immediate loading onto the column or freeze dried for subsequent analyses.

2.2.2. CMOS fractionation using microcrystalline cellulose (MCC) columns

Column chromatography was performed using a 73 mm I.D. × 305 mm long column with 1 L reservoir and fritted disc. The microcrystalline cellulose stationary phase was prepared by making a 140 g slurry in 70% ethanol and then carefully funneling the slurry down the walls of the previously wetted column. The packed column was then rinsed with 70% ethanol, aided by compressed air (~1 psi), until the eluate went from deep yellow to colorless and a final column height of roughly 10 cm was reached. Note that the cellulose bed should not be disturbed during or after this step, as this was found to negatively impact flow rates. The column was then equilibrated with 100 mL 90% ethanol and allowed to drain until the packing surface was infinitesimally submerged. CMOS sample (~12 mL) was then added by pipetting it slowly down the walls of the column; the sample was allowed to percolate into the packed bed of the column. A step gradient, in percent ethanol, was then run as follows: 1.25 L of 90%, 1.25 L of 85%, 1.50 L of 80%, 1.0 L of 75%, and 1.0 L of 70%, totaling 6.0 L of mobile phase. The first 1700 mL of eluate contained only glucose and maltose and were discarded. Collection began immediately thereafter in 100 mL increments. Fractions were analyzed during the chromatography run by thin layer chromatography (2.2.3).

2.2.3. Thin layer chromatography (TLC)

TLC was used to verify contents of collected fractions. A capillary spotter was used to introduce 1 µL of each fraction 6 times to a single spot, totaling 6 µL of each fraction on a given plate. Plates were developed using an ethanol/water/butanol solvent (69/17/14, respectively) until a solvent front of ≥5.5 cm was achieved. Plates were removed from the solvent, dried completely with a heat gun, and then dipped in the staining solution described by Robyt and Mukerjea (1994). Spots were visualized by evenly waving a heat gun across the plate for 20–30 s. Upon heating, plates became light gold and carbohydrates develop a deep purple color. Spots were immediately marked with pencil and recorded; fractions with the same MOS composition were combined in sealed containers. For routine semi-quantitative analyses, test samples were developed on plates alongside known

concentrations of MOS standards and compared visually; where more precise quantitative results were desired (*e.g.*, for developing chromatograms), plates containing samples and known concentration standards were digitally scanned and then quantified *in silico* using ImageJ or similar software. Representative TLC chromatograms are included in the Supplementary materials (Fig. S3).

2.2.4. Solvent removal and lyophilization

Ethanol removal from MOS-containing chromatography fractions was achieved using a rotary evaporator (Büchi Rotovapor R-205, Büchi Labortechnik AG) equipped with a 55 °C water bath (Buchi B-490) and vacuum pump (Chemglass Scientific Apparatus/10 Torr). Fractions were reduced to a minimal volume and rinsed using two subsequent 50 mL DI water additions to completely remove traces of ethanol. Concentrated samples were stored at −23 °C until drying by lyophilization in a VirTis CONSOL 4.5 freeze dryer. Complete ethanol removal was confirmed using NMR (see 2.2.6).

2.2.5. High performance liquid chromatography (HPLC)

Lyophilized samples were re-solvated in DI water and percent saccharide compositions 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) on a combined Ag^{2+} polystyrene ion-exchange guard and analytical columns run at 80 °C (Supelcogel, Hercules, CA). DI water was used as the mobile phase at a rate of 0.20 mL per minute. The ELSD was kept at 60 °C with a nitrogen gas pressure of 350 kPa. Standard curves prepared with commercially available MOS DP 1–7 standards were used to determine MOS concentrations; LOD values for DP 1–7 standards were found to be ≤0.006 mg/mL (Balto et al., 2016). Integration was done using LCsolution computer software (Shimadzu, Kyoto, Japan).

2.2.6. Nuclear magnetic resonance (NMR)

NMR analyses were carried out to verify the absence of ethanol from the preparations and also to determine relative amounts of α-1,4 and -1,6 linkages (Nilsson, Gorton, Bergquist, & Nilsson, 1996). A Bruker AVIII 700 MHz 2-channel spectrometer with a 5 mm dual carbon (DCH) cryoprobe with a 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. Prevalence of bond linkages were determined by integration of peak areas for α-1,4 (5.305–5.395 ppm) and α-1,6 (4.881–4.924 ppm) signals.

2.2.7. Total carbohydrate assay

Total carbohydrate content of the MD preparation, CMOS, and final MOS products was determined using the spectrophotometric anthrone/sulfuric acid assay described by Brooks and Griffin (1987). Three mL of anthrone reagent (0.1% (w/v) in 12.4 M sulfuric acid) was added to test tubes containing 25 µL of aqueous carbohydrate solution. Tubes were topped with glass marbles and immersed in a boiling water bath for 5 min, followed by placement in an ice water bath for 15 min. Solutions were distributed into cuvettes and absorbance was read at 630 nm. A calibration curve was produced using aqueous glucose samples at 0–2 mg/mL. These values were multiplied by 0.90 to adjust for water of hydrolysis. Assays were done in triplicate.

2.2.8. Reducing sugar assay

Reducing ends of MD preparation and CMOS were quantified using the BCA/copper-based assay described by Kongruang, Han, Breton, and Penner (2004). One mL of BCA working reagent, as prepared in Garcia, Johnston, Whitaker, and Shoemaker (1993), was added to test tubes containing 1 mL of aqueous carbohydrate solution. Tubes were immediately capped and immersed in an 80 °C water bath for 30 min., followed by immersion in an ice water bath for 1 min and then brought room temperature. The solutions were distributed into cuvettes and absorbance was read at 560 nm. A calibration curve was produced using aqueous maltose samples of 0–75 µM. Assays were done in triplicate.

3. Results

The following results describe the combined use of differential solubility and preparative chromatography to prepare gram quantities of individual, low-DP, food-grade MOS from commercially-available MD products. The DP-dependent differential solubility of MOS allows preparation of a "chromatography-ready MOS" (CMOS) sample enriched in DP 1–7 MOS. Preparative chromatography with a cellulose-based stationary phase and a 5% step-gradient aqueous-ethanol mobile phase (90 → 70% ethanol) was used to isolate MOS ranging from DP 3 to 7.

3.1. Preparation and characterization of CMOS

Appropriate aqueous-ethanol solutions for the preparation of CMOS from starting MD were determined by guided trial-and-error (Balto et al., 2016). A single wash of 76% ethanol was found to be optimum based on two criteria: recovery of lower-DP MOS (DP 3–7) and removal of the higher DP MOS (DP ≥ 8). The percent of the individual MOS recovered in the CMOS preparation under the recommended conditions ranged from 33% (DP 7) to 93% (DP 1); this percent recovery was correlated with the DP of the individual MOS (see Supplemental Material, Table S1). Overlaid chromatograms depicting the DP profiles of the starting MD material and CMOS (Fig. 1) illustrate the notable extent to which high DP MOS were eliminated from the CMOS preparation. Quantitative values for individual DP MOS in the MD and CMOS preparations are presented in Table 1; further characterizations of each preparation is presented in Table 2. Values for MOS/MPS with DP ≥ 8 are combined in Tables 1 and S1 due to the limited resolution of the analytical system and lack of commercially available standards for higher DP MOS/MPS. Roughly half of the original MD preparation was comprised of MOS/MPS with DP ≥ 8; the preparation of CMOS successfully dropped this fraction to ~4% while maintaining, at a minimum, at least one-third of each of the targeted MOS (DP 3–7). This compositional change is reflected in the average DP values for the two preparations: MD DP_{avg} = 6.6, CMOS DP_{avg} = 3.3 (see Table 2). Removal of DP ≥ 8 MOS/MPS from the MD preparation also significantly increased the ratio of α-1,4 to α-1,6 linkages in the resulting CMOS preparation (see Table 2), this being indicative of a higher ratio of branched saccharides in the higher DP MOS/MPS (see Supplemental

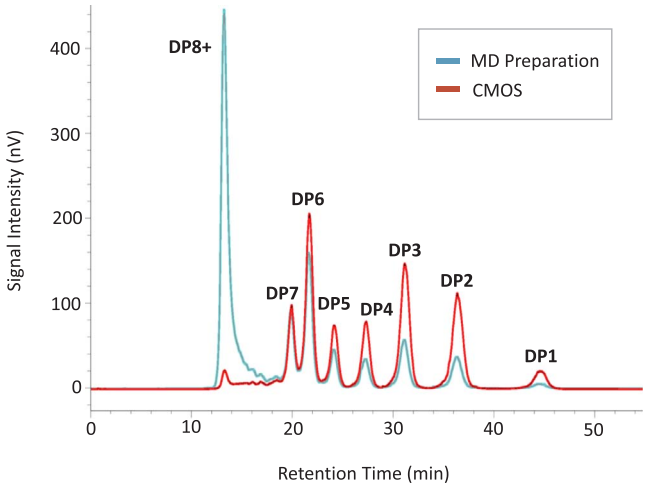

Fig. 1. Overlaid chromatograms from HPLC-ELSD depicting the shift in degree of polymerization (DP) profiles of the starting maltodextrin (MD) preparation and the chromatography-ready maltooligosaccharides (CMOS), produced through a single wash of the MD preparation with 76% aqueous ethanol.

Table 1
Percent saccharide composition of starting maltodextrin (MD) preparation and chromatography-ready maltooligosaccharide (CMOS) preparation.

	MD Preparation[b]	CMOS[b]
DP[a] 1	1.7 ± 0.1[b]	5.59 ± 0.03[b]
DP 2	5.1 ± 0.1	17.96 ± 0.03
DP 3	7.6 ± 0.1	20.1 ± 0.1
DP 4	4.79 ± 0.04	9.7 ± 0.1
DP 5	5.2 ± 0.1	9.10 ± 0.04
DP 6	16.16 ± 0.04	23.1 ± 0.1
DP 7	8.0 ± 0.1	10.47 ± 0.03
Total DP 1–7	48.4 ± 0.1	96.0 ± 0.2
Total DP 8+	51.56 ± 0.03	4.0 ± 0.2

[a] DP = degree of polymerization.
[b] Values are means ± SD of three replicates determined by HPLC-ELSD analyses (see 2.2.5).

Materials for NMR spectra).

3.2. Chromatographic preparation and characterization of MOS

A representative chromatogram depicting an entire chromatographic run is given in Fig. 2. The data is taken from digitized density readings of TLC plates developed for each collected fraction (see 2.2.2 and 2.2.3); the chromatogram thus allows visualization of each independent MOS. The chromatogram illustrates that the resolution is sufficient to allow recovery of the majority of each individual MOS simply by avoiding leading and tailing peak edges. Typically, independent chromatographic runs were reproducible in that elution volumes for each of the isolated MOS varied by less than 20 mL. HPLC-ELSD chromatograms of individual MOS collected in this way are overlaid in Fig. 3; these chromatograms illustrate that the approach outlined in this work allows isolation of individual MOS. Table 3 gives further information on the isolated MOS. The percent carbohydrate values obtained using the anthrone/sulfuric acid method, which are all ≥98%, are included for comparison with the values in Table 2. HPLC-based quantification of the different purified MOS, calculated relative to commercial standards, indicated 100% purity. The linkage prevalence values in Table 3 reflect that the NMR signal resulting from the presence of α-1,6 linkages was not detected in any of the purified MOS preparations. This indicates that each of the isolated MOS are > 98% linear saccharides (based on the NMR-determined 50:1 linkage prevalence in CMOS; see Table 2). Further NMR data, verifying the nature of a representative isolated MOS (DP4), is presented in the "Supplemental Materials" associated with this paper. Recoveries of individual MOS, based on amount recovered as isolated MOS from the column relative to the amount in the CMOS preparation, were all above 90% (Table 3).

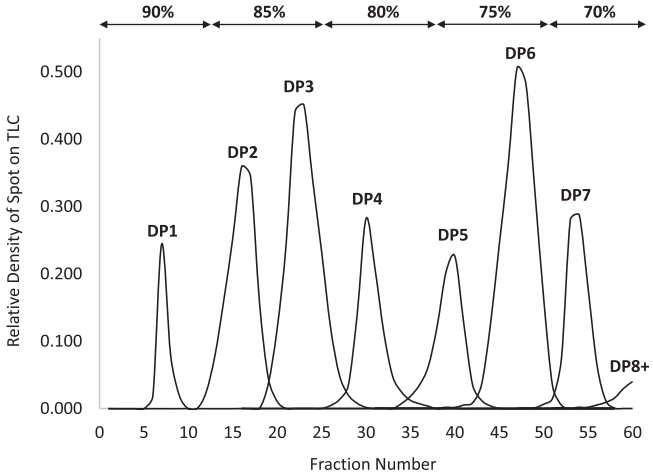

Fig. 2. Representative elution pattern of chromatography-ready maltooligosaccharides (CMOS) on cellulose column described herein. Chromatogram developed by measuring relative spot densities of each collected fraction on TLC plates. Elution began with 1.25 L of 90% (v/v) ethanol, followed by 1.25 L of 85%, 1.5 L 80%, and 1.0 L of each 75% and 70%. (The values across the top of the chromatogram represent % ethanol values.) Fractions of 100 mL were collected. Purified MOS were obtained from collected fractions by pooling those fractions that contained only the sought-after MOS; fractions containing multiple MOS, as is the case for those fractions corresponding to the leading and tailing peak edges, were avoided/discarded. Only isolated material was kept for further analyses.

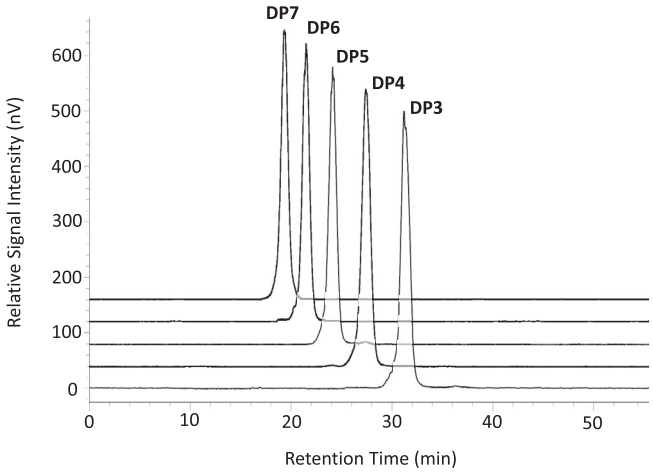

Fig. 3. Overlaid chromatograms depicting individual, isolated MOS (i.e., those from fractions showing a single MOS) collected from chromatographic fractionation of chromatography-ready maltooligosaccharides (CMOS) as depicted in Fig. 2. DP = degree of polymerization.

4. Discussion

A commercial MD product with a relatively low average DP (~6.6, based on reducing sugar equivalents per gram product) was chosen as the starting material due to the target MOS being those with DP ≤ 7. The initial step in MOS production is solvent extraction of higher DP

Table 2
Chemical characterization of starting maltodextrin (MD) preparation and chromatography-ready maltooligosaccharides (CMOS).

Sample	Percent Carbohydrate[ab]	Moles RE per 100 g[ac]	DE[d]	Average DP[e]	Linkage prevalence[f] (1,4-):(1,6-)
MD Preparation	92.5 ± 0.8	0.09 ± 0.09	16.7	6.6	16.6:1
CMOS	94.1 ± 0.1	0.19 ± 0.04	33.3	3.3	50:1

[a] Values are means ± SD in triplicate, expressed on a dry weight basis.
[b] Determined using anthrone/H_2SO_4-assay with glucose as a standard.
[c] Determined using Cu/bicinchoninic acid-assay with maltose as a standard.
[d] DE = dextrose equivalent; a quantitative measure of the average reducing power of starch hydrolysis products as a percentage of the reducing power of an equivalent dry-weight of D-glucose. Calculated as moles of reducing ends per 100 g × 180.
[e] DP calculated as 111/DE.
[f] Determined from NMR spectra.

Table 3
Chemical characterization and column recovery for isolated maltooligosaccharides.

Sample	Percent Carbohydrate[a,b]	Linkage prevalence[c] (1,4-):(1,6-)	Mass Recovery[d] (mg)	Percent Recovery[e]
DP[f] 3	98.19 ± 0.02	1,6 ND[g]	94.5 ± 0.7	94.5 ± 0.7
DP 4	98.24 ± 0.01	1,6 ND	45.0 ± 1.4	93.8 ± 2.9
DP 5	99.27 ± 0.01	1,6 ND	42.5 ± 2.1	94.4 ± 4.7
DP 6	99.28 ± 0.01	1,6 ND	106.0 ± 2.8	93.0 ± 2.5
DP 7	99.50 ± 0.01	1,6 ND	48.5 ± 2.1	93.3 ± 4.1

[a] Values are means ± SD from triplicate measurements, expressed on a dry weight basis.
[b] Determined using anthrone/H_2SO_4-assay with glucose as a standard, performed in triplicate on isolated MOS preparations.
[c] Determined from NMR spectra.
[d] Indicates average mass ± SD collected off two columns.
[e] Percent recovery based off expected mass; these values include isolated material only.
[f] DP = degree of polymerization.
[g] 1,6 ND = α-1,6 linkage not detected in NMR spectrum.

MOS and MPS from this starting material. A single aqueous 76%-ethanol extract proved adequate for this purpose (see Table 1). The removal of the higher DP MOS and MPS, and thus enrichment of MOS with DP ≤ 7, was important for two reasons: (1) the higher DP MOS and MPS are only sparsely soluble at the high ethanol concentrations corresponding to optimum column performance and (2) the enrichment step allowed greater amounts of DP 1–7 MOS to be chromatographed in each run.

The choices of suitable mobile and stationary phases for MOS chromatography were limited due to the overall goal of obtaining relatively inexpensive food-grade MOS. Water, ethanol, and butanol were considered as the mobile phases of choice due to their history of use in MOS chromatography (dating back to Thoma & French, 1957; Thoma et al., 1959) as well as their availability as food grade solvents. MOS chromatography done with a three-component mobile phase (butanol/ethanol/water) gave somewhat better resolution than the corresponding system with a two-component mobile phase (ethanol/water) (data not shown). However, the protocol described herein uses a two-component ethanol/water mobile phase for two reasons: 1) the improvement in resolution was rather small and 2) food-grade butanol is relatively costly. A side-benefit of using either of the alcohol/water mobile phases is that they are antimicrobial.

Several stationary phases were considered for the chromatographic separation of MOS (including silica, potato starch, microcrystalline cellulose, and paper). Cellulose was found to be the most productive when coupled with the food-grade mobile phases. All others failed to give isolated products in the quantities desired. Specifically, excessive tailing was observed with analogous silica columns. Starch-based columns approached the resolution of cellulose, but suffered from extremely slow flow rates. Attempts using paper chromatography were limited by the inability to chromatograph sufficient quantities of CMOS and the amount of work required to identify and extract MOS from the paper. A step gradient/flash chromatography technique was used in the present work. This was deemed adequate based on the relatively high recoveries of isolated MOS (> 93%). It is plausible that even higher recoveries could be achieved using linear gradients in a conventional liquid chromatography system; and higher yields may be important for some applications. The "optimum" chromatographic system for the production of isolated MOS is likely to depend on many factors, including the cost and DP profile of starting materials, equipment available, time constraints and the ultimate application of the isolated MOS.

The chromatographic isolation protocol described herein fractionates CMOS preparations which are themselves derived from commercial MD products by taking advantage of the DP-dependent solubility of MOS/MPS (Balto et al., 2016). The MOS products resulting from the application of this protocol are isolated, essentially linear, food-grade MOS ranging in DP from 3 to 7. These products, in addition to the novelty of being food-grade, are produced at relatively low cost. The linearity of the products obtained in this study appears to be a result of the MD starting material having minimal amounts of branched low DP MOS. If this had not been the case, and the resulting MOS were a mixture of linear and branched MOS, then treatment of the CMOS preparation with a debranching enzyme prior to chromatography would have been necessary. Yields of individual MOS per g starting material will be highly dependent on the DP-profile of the starting material. The major factor limiting the recovery of all MOS is the loss that occurs during the preparation of CMOS as a result of the low resolution of the solvent extractions. MOS yields were not the sole determinant in developing the presented protocol since the MD starting materials are relatively inexpensive. The protocol presented herein was deemed optimum based on reagent/material costs and labor input as well as MOS yield. The presented protocol is flexible; *e.g.*, if isolated MOS are not required then one can obtain MOS preparations with relatively narrow DP ranges by increasing mobile phase polarity which, in turn, would shorten run times and decrease solvent usage.

5. Conclusions

A straightforward method to fractionate commercial MD preparations to obtain isolated, low-DP (3–7), food-grade MOS has been presented. Such MOS are anticipated to be of most direct importance in studies investigating human responses to starch and starch hydrolysis product consumption. This includes, but is not limited to, research dealing with human sensory perception. The method is distinct from previous work concerning MOS isolation in that the process is performed with relatively simple, low cost, food-appropriate materials (*i.e.*, food-grade cellulose, ethanol, and MD products). Single runs result in ~40 mg of DP 4, 5, and 7, and ~100 mg of DP 3 and 6. The preparative approach described herein is robust in that it can be modified based on the resolution required for the final MOS products. The nature of the commercial starting material, the extraction scheme used to prepare CMOS, and the chromatographic conditions are all expected to have an impact on final yields of individual MOS.

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Conflict of interest statement

The authors declare no competing financial interests.

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.2017.10.039.

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