JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Galactoglucomannan Oligosaccharides (GGMO) from a Molasses Byproduct of Pine (*Pinus taeda*) Fiberboard Production

Neil P. J. Price,*[,†] Trina M. Hartman,[†] Trevor A. Faber,[‡] Karl E. Vermillion,[†] and George C. Fahey, Jr.[‡]

[†]National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Peoria, Illinois 61604, United States

[‡]Department of Animal Sciences, 132 Animal Sciences Laboratory, University of Illinois, 1207 West Gregory Drive, Urbana, Illinois 61801, United States

Supporting Information

ABSTRACT: "Temulose" is the trade name for a water-soluble molasses produced on a large scale (300−400 tonnes per year) as a byproduct of the fiberboard industry. The feedstock for Temulose is predominantly a single species of pine (*Pinus taeda*) grown and harvested in stands in southeastern Texas. Because of the method of production, the molasses was predicted to consist of water-soluble hemicelluloses, mainly arabinoxylan-type and galactoglucomannan-type oligosaccharides, plus minor components of lignin, but no detailed structural study had been reported. The structure and composition of the molasses has now been deduced by a combination of MALDI-TOF mass spectrometry, size exclusion chromatography, proton and ^{13}C NMR techniques, and classic carbohydrate analysis. Limited acid hydrolysis released a series of galactoglucomannan oligosaccharides (GGMO) that were selectively recovered from the acid-labile arabinogalactan by precipitation with ethanol. The precipitate was named "Temulose brown sugar" because of its appearance, and is shown to consist of GGMO with a degree of polymerization (DP) from 4 to 13, with the major component being DP 5−8. The structure of these oligosaccharides is a β-1,4-linked backbone of Man and Glc residues, with occasional α-1,6 branching by single galactosyl units.

KEYWORDS: Oligosaccharide, galactoglucomannose, carbohydrate analysis

INTRODUCTION

Temulose molasses is a commercial byproduct in the production of medium-density fiberboard. The water-soluble molasses is produced on a large scale (typically 0.9 tonne per day) and has a brown-colored, viscous appearance. The feedstock for Temulose is loblolly pine (*Pinus taeda*), predominantly from natural stands in eastern and central Texas. Loblolly pine is often referred to as southern yellow pine, which is not a single tree species but rather a variety of species, such as longleaf pine (*Pinus palustris* Mill.), shortleaf pine (*Pinus echinata* Mill.), loblolly pine (*P. taeda* L.), and slash pine *Pinus elliottii* Engelm.), that grow well in the acidic red clay soil found in most of the southern United States. Previous studies have shown that pine wood is typically composed of lignocellulosics, arabinoxylan hemicelluloses, and galactoglucomannan (GGM).

Pine wood hemicelluloses are typically two-thirds GGM and about one-third arabino-4-*O*-methylglucuronoxylan. The GGM backbone generally consists of β-(1→4)-D-mannopyranosyl and β-(1→4)-D-glucopyranosyl residues, with occasional α-(1→6)-D-galactopyranosyl branching[1,2] with a degree of polymerization (DP) between 100 and 150 residues (16−24 kDa). Mannose, glucose, and galactose ratios are typically in a molar ratio of 3:1:1.[3−8] The majority of the mannosyl units (for Norway spruce, about 65%) are partially substituted by *O*-acetyl groups at either the C-2 or C-3 position in a ratio of 2.2:1.[4] Another study of the spruce (*Picea abies*) GGM structure showed a DP from 11 to 20, with the Man/Glc/Gal molar ratio of 4:1:0.1.[8,9] About one-third of D-mannosyl units were *O*-acetylated, almost equally distributed between C-2 and C-3. The acetyl content varied between 5.9 and 8.8%.[3] The ratio between mannose and glucose residues reported for other softwood glucomannans also varies, such as Norway spruce between 3.5 and 4:1[9−14], red cedar 2.5:1,[15] western hemlock 3:1,[16,17] loblolly pine 2.7:1,[18] Scots pine 3.1−3.7:1,[12] and Sitka spruce 2.5:1.[19] Other reports of softwood GGM have included those from red pine,[20] southern pine,[21] white spruce,[22] and poplar.[23]

To be consistent with these reported data, we expected that the loblolly pine-derived Temulose molasses may consist of predominantly water-soluble arabinoxylan and GGM type hemicelluloses, plus minor components of lignin-derived phenolic esters. The material has a notable but pleasant odor that GC-MS analysis showed to be predominantly due to complex vanillin esters (data not shown). Here we report the structural composition of the Temulose carbohydrate components as determined by a combination of limited hydrolysis, monosaccharide composition and linkage analysis, size exclusion fractionation and MALDI-TOF/MS analysis of component GGM oligosaccharides, and 1D and 2D NMR techniques.

Received: September 24, 2010
Accepted: January 13, 2011
Revised: January 7, 2011
Published: February 02, 2011

MATERIALS AND METHODS

Materials and supplies were obtained commercially from Sigma-Aldrich Chemicals, St. Louis MO. Temulose molasses was kindly provided by Temple Inland Inc., Diboll, TX.

Compositional and Linkage Analysis by Gas Chromatography−Mass Spectrometry (GC-MS). Duplicate samples were hydrolyzed on a reaction block using aqueous trifluoroacetic acid (TFA, 2.0 M, 110 °C, 1 h). After cooling, the solvent was removed by evaporation, and aldononitrile acetate or alditol acetate derivatives were prepared as described previously.[24,25] Permethylation linkage analysis was performed as described.[26] GC-MS analysis was performed on an Agilent (Santa Clara, CA) 6890N gas chromatograph interfaced with an Agilent 5973N mass-selective detector configured in EI mode and with a Hewlett-Packard (Santa Clara, CA) 7683 series autoinjector. A Hewlett-Packard DB-5 ms column (30 m; 0.2 mm i.d.) was used in split injector mode with helium as the carrier and a linear gradient from 150 to 300 at 10 °C/min. Mass spectra were recorded in positive-ion mode over the range m/z of 50−550. Injector and detector/interface temperatures were 275 and 300 °C, respectively. Data analysis was done off-line using HP Chemstation software.

Size Exclusion Chromatography (SEC). The Temulose "brown sugar" was recovered by ethanol precipitation from acid-hydrolyzed (0.2 M TFA, reflux, 1 h) Temulose and fractionated by SEC on a 1.27 m Bio-Gel P2 column. A typical hydrolysis used 20 mL of 1.0 M aqueous TFA plus 100 mL of Temulose molasses and was precipitated with an equal volume of ethanol. The brown sugar precipitate was recovered by filtration. The SEC column was eluted with deionized water, and fractions (5 mL) were collected using an automated fraction collector. The fractions were analyzed by MALDI-TOF/MS, and those containing oligosaccharides of equivalent molecular mass were pooled and concentrated by rotary evaporation.

MALDI-TOF/MS Analysis. MALDI-TOF mass spectra were recorded on a Bruker Daltonic Omniflex instrument (Bruker Daltonics, Billerica, MA) operating in reflectron mode. Samples were typically dissolved in acetonitrile, and the matrix used was 2,5-dihydrobenzoic acid. Ion source 1 was set to 19.0 kV and source 2 to 14.0 kV, with lens and reflector voltages of 9.20 and 20.00 kV, respectively. A 200 ns pulsed ion extraction was used with matrix suppression up to 200 Da. The instrument was calibrated externally on a DP series of malto-oligosaccharides. Excitation was at 337.1 nm, typically at 60% of 150 μJ maximum output, and 80 shots were accumulated. The linear mass resolution (fwhm) for m/z 2465 (ACTH 18−39) was >3500.

NMR Spectroscopy. All NMR experiments were performed on a Bruker Avance spectrometer (Bruker BioSpin Corp., Billerica, MA) operating at 500.11 MHz using a standard 5 mm z-gradient BBI probe at 27 °C. Chemical shifts are reported as parts per million from tetramethylsilane calculated from the lock solvent. The deuterated solvents used were obtained from Cambridge Isotope Laboratories (Andover, MA). The pulse sequences used were those supplied by Bruker, and processing was done with the Bruker TOPSPIN software package (version 1.3).

RESULTS AND DISCUSSION

Monosaccharide Compositional Analysis and MALDI-TOF/MS. The total monosaccharide composition of the Temulose was determined by GC-MS analysis of peracetylated aldononitrile acetate derivatives. The nonhydrolyzed (free) monosaccharides in the Temulose were predominantly arabinose (52.2%) and xylose (17.6%), with smaller quantities of free mannose, glucose, and galactose. The free mannose content of the nonhydrolyzed Temulose was 6.6%. Following mild acid hydrolysis with aqueous trifluoroacetic acid, the predominant monosaccharides present were mannose (56.5%), glucose (13.5%), and xylose (10.7%). Comparable results were obtained on filtered Temulose (Table 1). To investigate the mass distribution of these sugars, the Temulose was diluted and dialyzed against a 3500 Da cutoff membrane. GC-MS compositional analysis of the eluent retentate showed that the Man-Glc-Gal component was evenly distributed, whereas the arabinoxylan was predominantly retained (Figure 1).

Table 1. Temulose Compositional Analysis by GC-MS of Sugar Aldononitrile Acetate Derivatives

sugar	free sugar (%)	after hydrolysis (%)	filtered, then hydrolyzed (%)
Ara	52.2	5.5	5.2
Xyl	17.6	13.7	13.2
Man	6.6	56.5	59.1
Glc	3.7	13.5	12.5
Gal	14.7	10.7	10.0
total	94.8	99.9	100.0

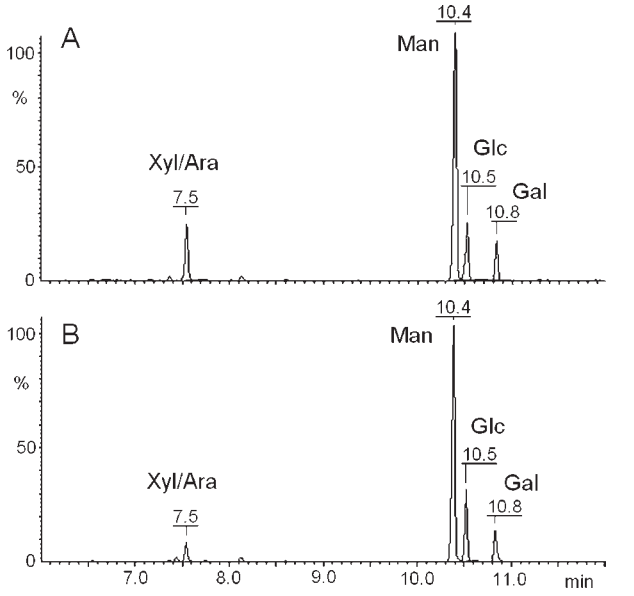

Figure 1. GC-MS monosaccharide compositional analysis of dialyzed Temulose carbohydrates (3500 Da cutoff): (A) eluted; (B) retained. Total carbohydrates were acid hydrolyzed and analyzed as aldononitrile acetates.

To investigate the molecular mass distribution (degree of polymerization, DP) further, the Temulose molasses was analyzed by matrix-assisted laser dissociation/ionization time-of-flight mass spectrometry (MALDI-TOF/MS). The molasses consisted of a complex series of oligosaccharides predominantly in the mass range of 400−1500 Da (Figure 2). These were assigned by the mass of the observed $[M + Na]^+$ sodium adduct ions and were composed of hexosyl oligosaccharides (Man, DP 3−8), both mono- and diacetylated hexosyl oligosaccharides (ManAc, DP 4−8, and $ManAc_2$, DP 3−8), plus pentosyl oligosaccharides (Xyl, DP 3−10), and two methyl-glucuronate-containing pentosyl oligosaccharides (Xyl_8MeGA and Xyl_9MeGA). Biphasic fractionation of the diluted molasses with butanol, ethyl acetate, or diethyl ether was undertaken, and the phases were analyzed by MALDI-TOF/MS. Ether was observed to preferentially extract

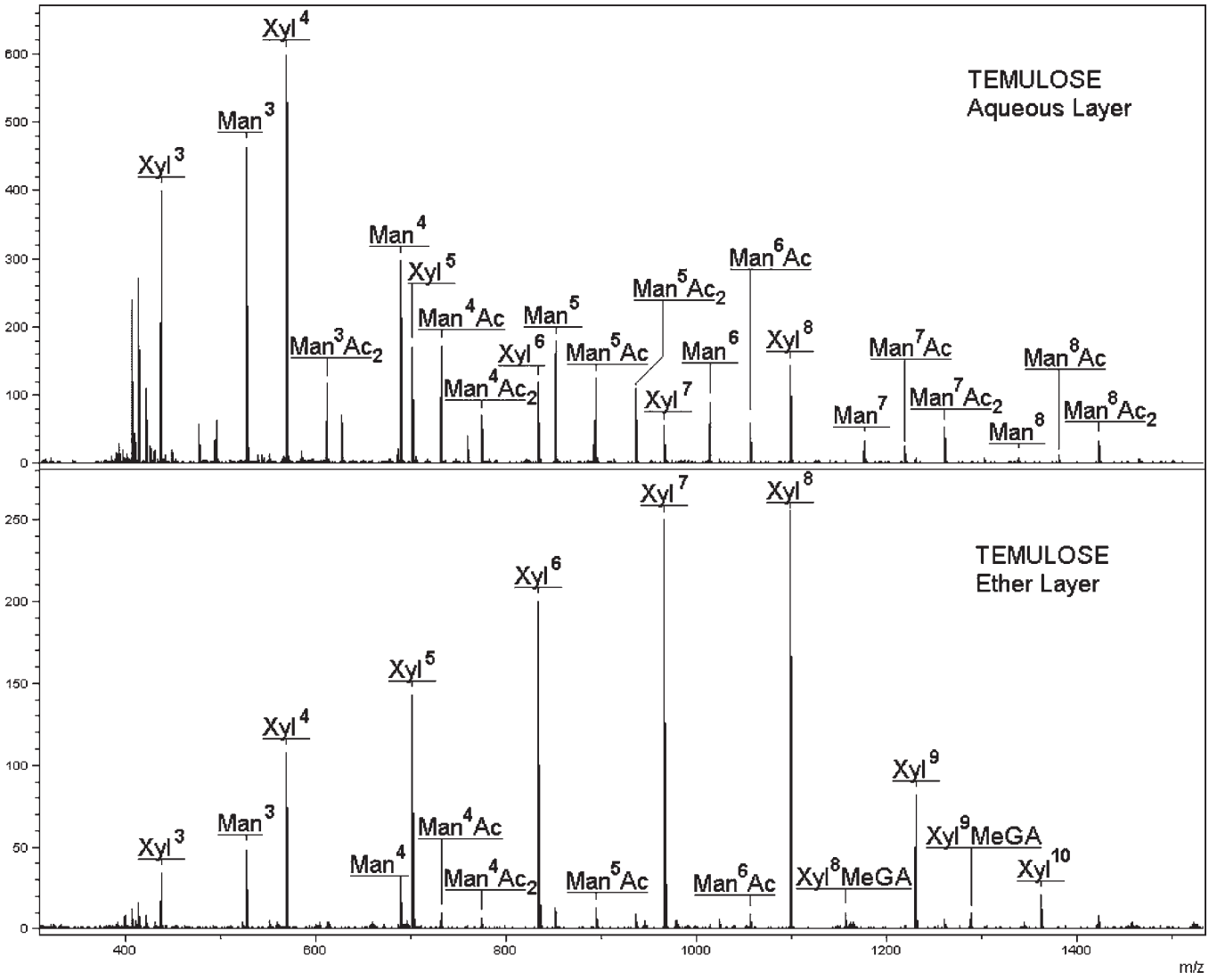

Figure 2. MALDI-TOF mass spectrometric analysis of Temulose molasses following ether partitioning. The nomenclature refers to hexosyl oligosaccharides (Man) and pentosyl oligosaccharides (Xyl), with the superscript referring to the degree of polymerization (DP). Monoacetylated (Ac) and diacetylated (Ac_2) hexosyl oligosaccharides and methyl-glucuronylated (MeGA) pentosyl oligosaccharides are also assigned.

the partially acetylated hexosyl oligosaccharides and, to a great extent, the series of pentosyl oligosaccharides (Figure 2).

A more detailed oligosaccharide compositional analysis by MALDI-TOF/MS for the mass range of 600−1000 Da is shown in Figure 3. Cellulo-oligosaccharides (Cel, DP 3−5) were used as controls to assign hexosyl oligosaccharide masses and xylo-oligosaccharides (Xyl, DP 5−7) as pentosyl oligosaccharide controls. The isobaric oligosaccharidic ions from Temulose were assigned at m/z 689, 851, and 1013 (Man, DP 4, 5, and 6, respectively) and m/z 701, 833, and 965 (Xyl, DP 5, 6, and 7, respectively). Sodium adduct ions at m/z 731 and 893 correspond to 689 + 42 and 851 + 42 Da, respectively, where 42 Da is the mass of a single acetyl group (ManAc). Similarly, ions at m/z 773 and 935 arise from diacetylated oligosaccharides ($ManNAc_2$), with masses 689 + 42 + 42 and 851 + 42 + 42 Da (Figure 3). These data are consistent with the untreated Temulose molasses containing eight xylan or arabinoxylan oligosaccharides plus two methylglucuronyl-containing arabinoxylans/xylans, most probably derived from the wood hemicelluloses. The pentose-containing components were not acetylated. In addition, five free GGMO were detected in this mass range, together with five monoacetylated and five diacetylated GGMO. Hence, a total of 26 free oligosaccharides is reported for the untreated Temulose material.

Susceptibility to Mild Acid Hydrolysis. The Temulose molasses was subjected to a regimen of acid hydrolysis using a variety of acidic conditions (see the Supporting Information). Strong acid hydrolysis, with either 0.5 M hydrochloric acid or 2.0 M trifluoroacetic acid, resulted in predominantly monosaccharides and small DP oligosaccharides as determined by MALDI-TOF/MS. Milder acid hydrolysis (0.2 M TFA, refux, 1 h) resulted in a mass series typical of hexose-containing oligosaccharides of DP 2−12. The observed $[M + Na]^+$ pseudomolecular ions differed by 162 Da, indicative of the mass difference of one hexose unit. The lowest mass ion m/z 365 corresponds to $[M + Na]^+$ for a DP 2 hexosyl disaccharide and the highest recorded mass ion m/z 2149 for a DP 13-mer. This series was assigned as mild acid-resistant GGMO oligosaccharides. No partially acetylated GGMO were observed, indicating that all of the acetyl groups were removed by the mild acid treatment. Moreover,

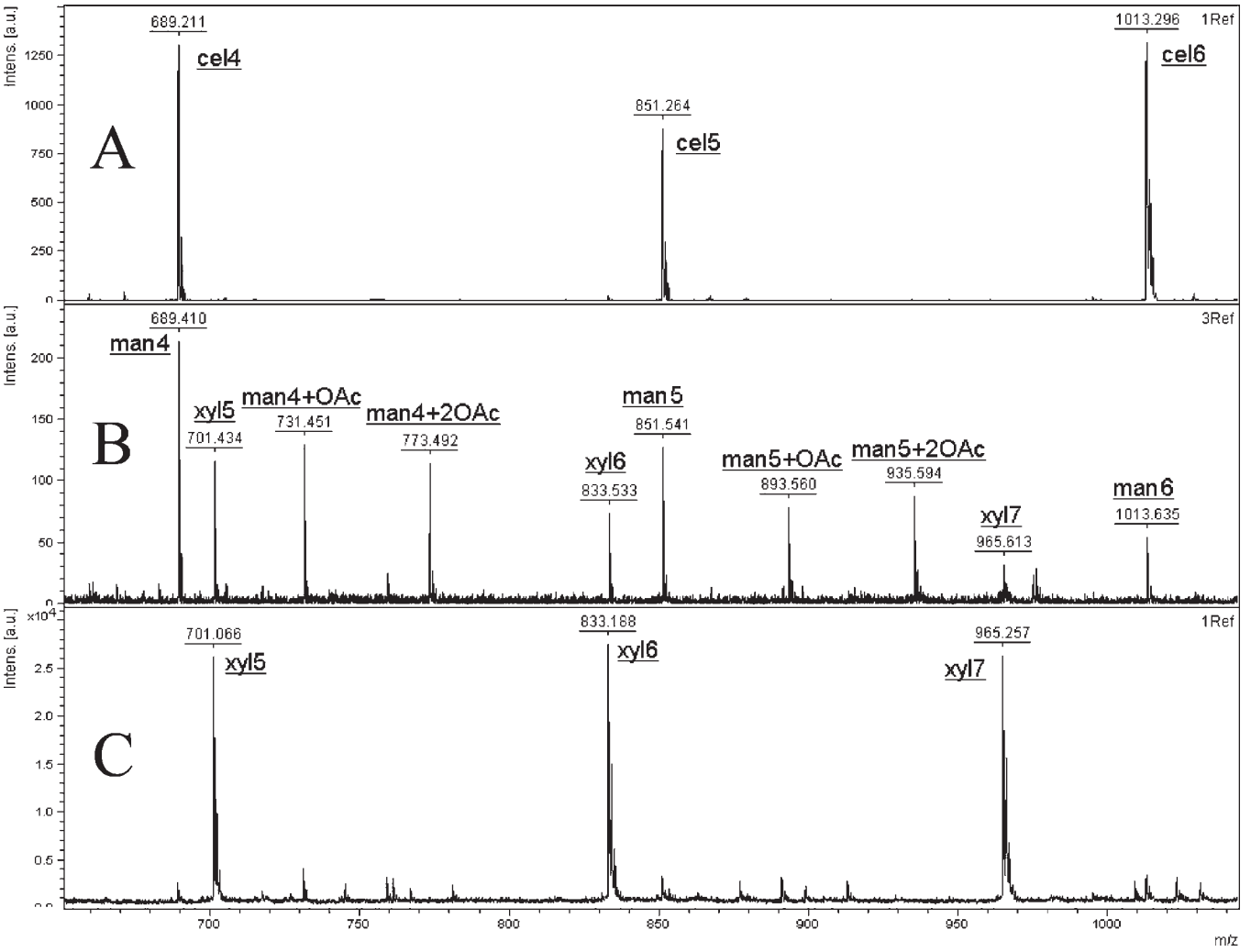

Figure 3. MALDI-TOF mass spectra of oligosaccharides in Temulose molasses, shown in the mass range of 600−1000 Da; (A) cellulo-oligosaccharide mass controls; (B) Temulose galactoglucomannans, monoacetyl-galactoglucomannans, diacetyl-galactoglucomannans, and arabinoxylan hemicellulosic oligosaccharides; (C) xylo-oligosaccharide mass controls.

no hemicellulosic arabinoxylan or xylan pentosyl oligosaccharides were seen by MALDI-TOF/MS after the mild acid hydrolysis, indicating that these are susceptible to degradation by the weaker acidic conditions.

To confirm this apparent selective susceptibility to acid hydrolysis,[27] the Temulose was treated with mild acid (aqueous trifluoroacetic acid, 0.2 M) and reduced with sodium borohydride. Hence, monosaccharides released by the mild acid treatment were converted to alditols, whereas the acid-resistant GGMOs remained intact except for their reducing residues. Continued treatment with strong acid (aqueous trifluoroacetic acid, 2.0 M) resulted in hydrolysis of the remaining GGMOs to component monosaccharides, which were subsequently converted to aldononitriles. Following peracetylation, the weakly acid-labile monosaccharide alditols formed alditol acetates, and the strongly acid-labile components formed aldononitrile acetates (PAANs). This mixture of derivatives was analyzed by GC-MS and compared to the PAAN profile from fully hydrolyzed Temulose (Figure 4). Noticeably, the major alditol acetates observed were arabinitol acetate and xylitol acetate, indicating that the arabinoxylan hemicellulosics were hydrolyzed by the mild acid treatment. Less mannitol acetate and glucitol acetate were produced and almost no galactitol acetate. Conversely, the major aldononitrile acetates seen were mannose-PAAN, glucose-PAAN, and galactose-PAAN, resulting from the strong acid hydrolysis of the GGMOs. Peaks for the arabinose-PAAN and xylose-PAAN were small, because these two weakly acid labile monosaccharides were converted to alditols by the borohydride treatment. Hence, this experiment confirmed that the arabinoxylan was degraded by mild acid and that the GGMOs were more resistant to this treatment.

The resistance of the GGMOs to mild acid hydrolysis provided a straightforward way to purify the GGMOs from the arabinoxylan. A large-scale mild acid treatment of Temulose with 0.2 M trifluoroacetic acid followed by precipitation with ethanol resulted in a light-colored brown sugar in good yield (Figure 5). Analysis of the brown sugar by MALDI-TOF/MS showed it to consist of hexose-containing oligosaccharides from DP 2 to 13, and mainly DP 4, 5, and 6 (Figure 6). These medium-sized GGMOs were entirely free of arabinoxyan or xylan oligosaccharides, and GC-MS monosaccharide analysis of the ethanol-soluble phase showed that it contained predominantly free xylose and arabinose (data not shown).

The selective acid hydrolysis of GGMOs has been studied previously, when it was noted that 50% of southern pine

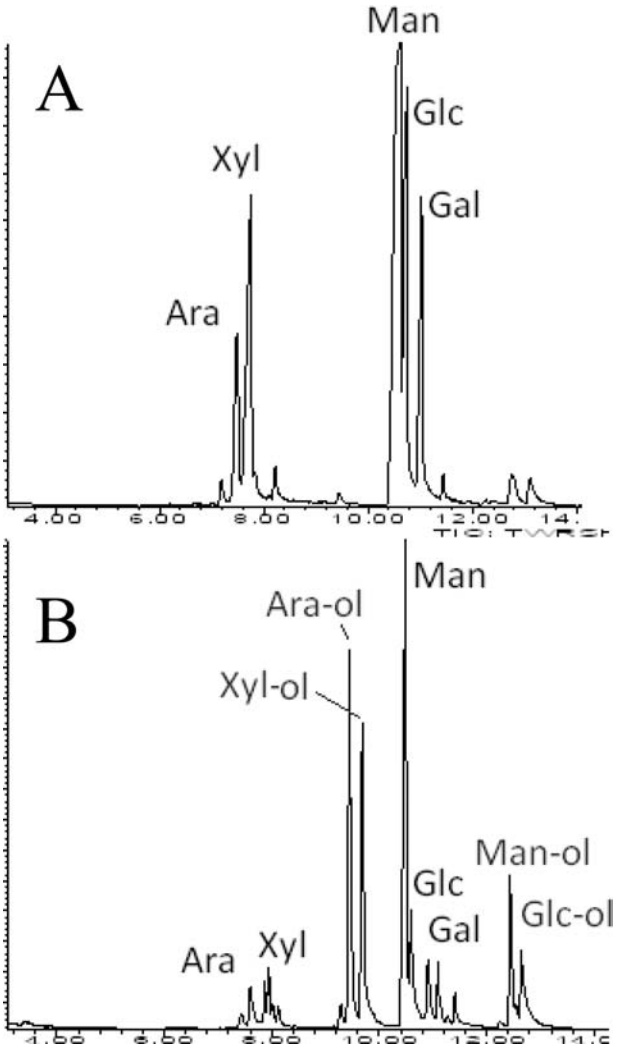

Figure 4. Susceptibility of Temulose sugars to acid hydrolysis: GC-MS profiles of strong (2 M TFA; panel A) and weak (0.2 M TFA, panel B) acid hydrosylates. Weak acid-labile sugars are predominantly arabinose (Ara-ol) and xylose (Xyl-ol) derived from arabinoxylan (B). Stronger acid also releases the GGMO monosaccharides, to give predominantly Man, Glc, and Gal (A).

galactoglucomannan was converted to monosaccharides by refluxing in a 0.05 N oxalic acid solution for 6 h.[21] Under these conditions,[21] the galactose side chain was shown to be more labile than the glucomannan backbone. We tested the acid susceptibility of the Temulose molasses to several food-grade organic acids. Aqueous oxalic acid (1.0 M) results were comparable to those obtained with dilute trifluoroacetic acid. The MALDI MS spectrum of the ethanol-precipitated pellet was a simple hexose-containing series, free from pentosyl oligosaccharides. This series was from DP 1 (minor amount) to DP 11 (minor amount) with DP 6 and 7 being most abundant when peak intensities were compared. The other acids tested (acetic, malonic, and citric) all contained a pentosyl oligosaccharide series and were at various states of hydrolysis with many intermediates after 1 h. To summarize, food grade oxalic acid can be substituted for the more toxic and expensive trifluoroacetic acid for the selective degradation of the arabinoxylan component of Temulose molasses. Following this treatment, the GGMO are readily recovered by a straightforward precipitation with ethanol. The typical

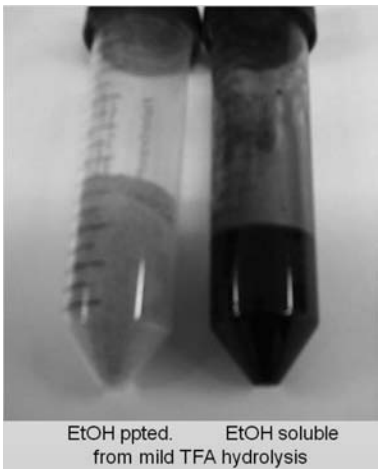

Figure 5. Ethanol-precipitated galactoglucomannan oligosaccharide (GGMO) "brown sugar" (tube on the left) from mild acid hydrolyzed Temulose molasses.

yield of the GGMO was 25% w/w, equivalent to about 100 tonnes per year of oligosaccharide material. This also represents a significant potential source of mannose, perhaps as much as 50−70 tonnes per year.

SEC, Permethylation Linkage Analysis, and NMR Analysis. The Temulose molasses was partially hydrolyzed with mild acid (0.2 M TFA, 100 °C, 30 min). This hydrolyzed the arabinoxylan present to arabinose and xylose monosaccharides, but retained the hexosyl GGMO. The latter were precipitated from the hydrolysate by adding ethanol. Free Ara and Xyl remained in the ethanol solution, whereas the GGMO are selectively precipitated. These were redissolved in water and reprecipiated with ethanol before lyophilizing, resulting in a clean white product. MALDI-TOF/MS and GC-MS compositional analysis indicated a series of GGMO from DP 2 to 16, with the major products around DP 6/7. This material was permethylated with NaOH/DMSO/methyl iodide as described.[26] The completeness of methylation was monitored by MALDI-TOF/MS. The permethylated GGMO then was hydrolyzed with 2.0 M TFA and dried. Permethylated aldononitrile acetate (Me-PAAN) derivatives were prepared and analyzed by GC-MS linkage analysis.

Three GC peaks were apparent from the permethylation analysis and were assigned by reference to the electron impact ionization MS spectrum. The major peak (retention time of 7.17 min, 79.12%) was assigned as 2,3,6-MeMan PAAN and is indicative of 1,4-linked mannose residues. A smaller peak at 7.39 min (2,3,6-MeGlc, 16.90%) was assigned as due to a 1,4-linked glucosyl residues, suggesting that the 1,4-mannosyl backbone is interspersed with 1,4-glucosyl groups at an average frequency of five mannose to one glucose units. This conforms reasonably well to the compositional analysis. A third peak at 6.21 min was assigned as a 2,3,4,6-MeHexose PAAN and is most probably due to the branched-chain galactosyl residues. This is in a ratio of ~1:20 with the larger peak at 7.17 min, that is, about 5% Gal branching of the β-1,4-linked glucomannan oligosaccharide backbones. However, the permethylation analysis did not provide information on the linkage or frequency of occurrence of the galactosyl residues observed in the compositional analysis. GGMO fractions from the size exclusion column were also analyzed by permethylation, with essentially the same result. This suggested that structural heterogeneity in the linkage composition remained after SEC, and as confirmed by NMR analysis.

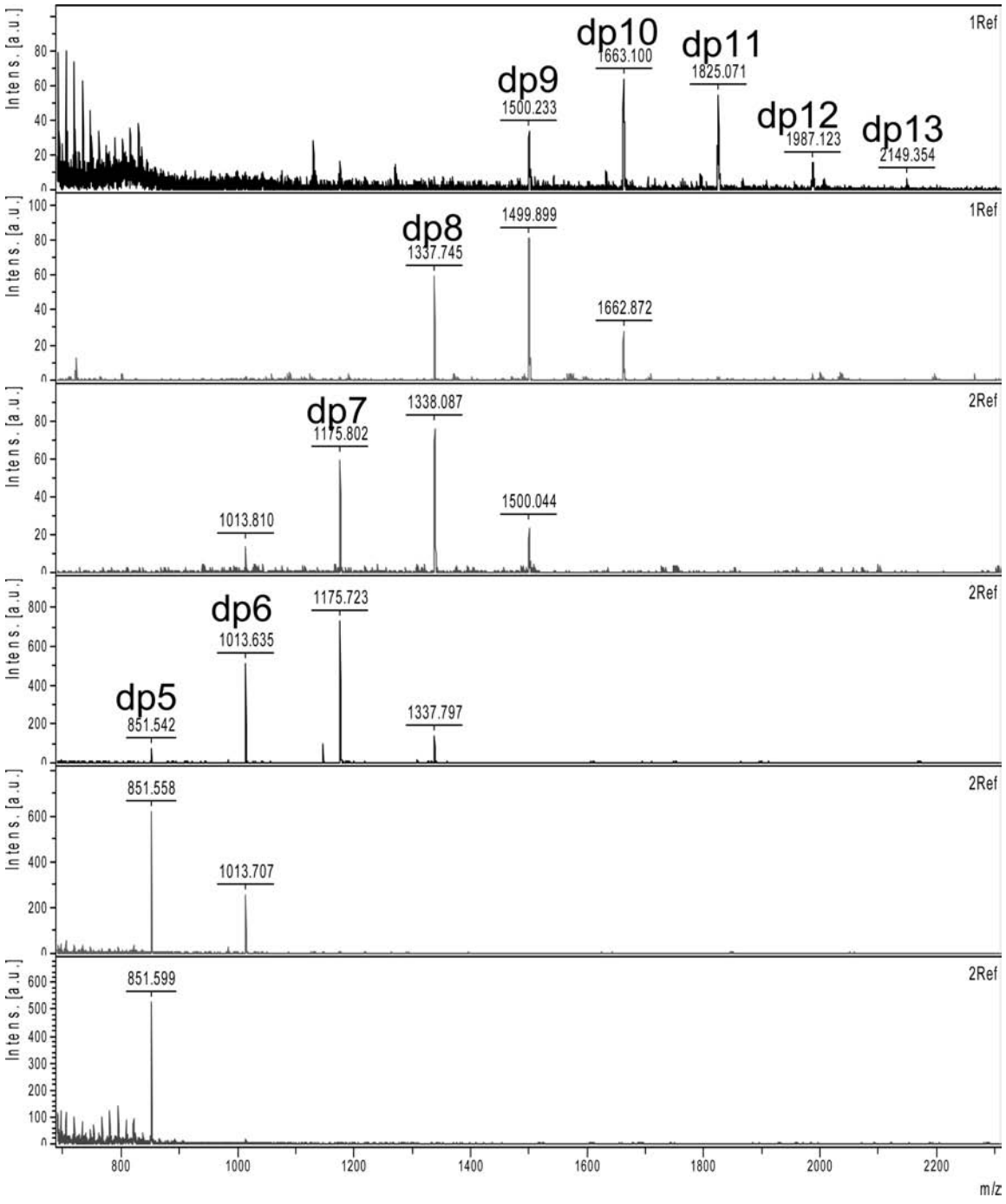

Figure 6. Large-scale size exclusion fractionation of galactoglucomannan oligosaccharides: MALDI-TOF mass spectra of galactoglucomannan oligosaccharides (GGMOs) from ethanol-precipitated Temulose brown sugar. Sufficient quantities (several grams) of GGMOs of various lengths (DP 5−13) were produced by size exclusion separation on a 1.27 m Bio-Gel P2 column. The MS spectra (range m/z 700−2300) are shown in pileup.

Temulose NMR Analysis. Large quantities (10−15 g) of individual size-fractionated GGMOs were required to enable biological testing (see accompanying paper[33]) and to enable the linkage and NMR analysis. All of the fractions were analyzed my MALDI-TOF/MS, and those containing equivalent molecular masses (DP 5−13) were pooled for further analysis (Figure 6).

The ethanol-precipitated mild acid (0.2 M TFA, 100 °C 20 min) hydrolysate of Temulose was deuterium-exchanged by lyophilization from D_2O (99.9 atom % D), and was redissolved in D_2O (99.96 atom % D) for analysis by NMR. Similarly, NMR data were obtained on the fractions purified by gel filtration. Spectra were obtained on a Bruker 500 at 27 and 40 °C and displayed signals typical of nonacetylated oligosaccharides (Table 2). The major anomeric H-1 proton signal was observed at 4.69 ppm with a J-coupling constant of 2 Hz, as determined from a J-resolved spectrum. The coupled H-2 proton was apparent in the COSY spectra at 4.07 ppm. Heteronuclear coupling, observed from a HSQC spectrum, identified the coupled ^{13}C nuclei C-1 and C-2 at 100.0 and 70.0 ppm, respectively. A second β-1,4-linked mannose residue was characterized by a smaller anomeric NMR signal at

Table 2. ^{1}H and ^{13}C NMR Assignments

position	chemical shifts[a] (ppm) ^{1}H	^{13}C
β-D-1,4-Man		
1	4.69 (2 Hz)	99.5
2	4.07 (2 Hz)	70.0
3	3.74	71.5
4	3.75	77.0
5	3.50	75.0
6a	3.65	60.5
6b	3.80	60.5
α-D-Man reducing termini		
1	5.12 (2 Hz)	94.5
2	3.90 (2 Hz)	NA
β-D-Man reducing termini		
1	4.85 (1 Hz)	94.0
2	3.80 (1 Hz)	NA

[a] The *J* coupling constants are in parentheses. NA, not assigned.

4.67 ppm, coupled to a H-2 proton at 4.01 ppm. The coupled anomeric ^{13}C nucleus was also observed at 100.0 ppm, where it overlapped the other β-1,4-Man carbon-1. The presence of two distinct β-1,4-linked mannosyl residues is due to one being linked to Glc and the other to a second Man in the oligosaccharide backbone, or to the difference between branched and unbranched Man residues. The presence of β-1,4-Glc in the backbone was confirmed by anomeric NMR signals at 4.45 and 102.5 ppm, which COSY showed to be coupled to β-1,4-Glc H-2 at 3.30 ppm. The anomeric values are in good agreement with those reported for β-1,4-linked mannose residues[28,29] and with previous assignments for galactoglucomannan.[23]

Proton NMR signals for the α-H-1 and β-H-1 of the reducing mannose residues were observed at 5.12 and 4.85 ppm, respectively. These were coupled to overlapping ^{13}C NMR signals at 94 ppm, as reported previously.[30] Two small α-anomeric ($J \sim 4$ Hz) peaks were observed at 4.95 and 4.97 ppm, coupled to overlapping H-2 protons at 3.79 ppm. Coupled carbon-13 signals for these protons were observed by HSQC to overlap at 98.5 ppm. These signals were assigned as due to α-1,6-galactosyl side chains, in agreement with previous assignments.[31] Several smaller anomeric signals were observed, even for the gel fitration-purified samples. These are likely due to compositional heterogeneity arising from the nonselective nature of the mild acid hydrolysis. The HSQC experiment was used to assign the C-6 (60.5 ppm) and H-6a/H-6b (3.65 and 3.80 ppm) signals due to the 6-methylene group as the only carbon atom coupled to two protons. The C-6 ^{13}C resonance is in good agreement with β-D-mannopyranosyl residues unbranched at the 6-OH, as branching at this position tends to shift the signal upfield to 67.4 ppm.[28] Other HSQC signals were assigned for H-3/C-3 (3.74, 71.5 ppm), H-4/C-4 (3.75, 77.0 ppm), and H-5/C-5 (3.50, 75.0 ppm) on the basis of published assignments.[23,28,29]

In summary, most prebiotic oligosaccharides are currently produced by transglycosylation or enzymatic cleavage of polysaccharides and include fructo-oligosaccharides, gentio-oligosaccharides, xylo-oligosaccharides, and galacto-oligosaccharides. Moreover, there is a growing need in the food and feed industries for novel carbohydrates and new prebiotic oligosaccharides. Galactoglucomannan oligosaccharides (GGMO) are attractive alternative carbohydrates having biological activities worthy of study. The structural analysis reported indicates that GGMO can be recovered from Temulose molasses by a straightforward ethanol precipitation after mild acid hydrolysis of xyloarabinan components. The DP of the GGMO (mean DP 5−6) is similar to that of proven prebiotic oligosaccharides, and GGMO is able to maintain the selective growth of bifidobacterium.[32,33] Because Temulose molasses is produced in large amounts (about 0.9 tonne per day), it is a raw material that provides an abundant source of potential bioactive novel oligosaccharides.

■ ASSOCIATED CONTENT

Supporting Information. Additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: neil.price@ars.usda.gov. Phone: (309) 681-6246. Fax: (309) 681-6040.

Notes

We declare no competing financial interests.

■ ACKNOWLEDGMENT

We thank Anne C. Hopkins of Temple-Inland, Diboll, TX, for the gift of Temulose molasses and Dr. J. O. Rich (ARS, USDA, Peoria, IL) for help with editing the manuscript.

■ REFERENCES

(1) Timell, T. E. Wood hemicelluloses. II. *Adv. Carbohydr. Chem.* **1965**, *20*, 448–458.

(2) Spiridon, I.; Popa, V. I. Hemicelluloses: structure and properties. In *Polysaccharides: Structural Diversity and Functional Versatility*, 2nd ed.; Dumitriu, S., Ed.; Dekker; New York, 2004; pp 475−491.

(3) Puls, J.; Schorn, B.; Schuseil, J. Acetylmannanesterase: a new component in the arsenal of wood mannan degrading enzymes. In *Biotechnology in the Pulp and Paper Industry*; Kuwahara, M., Shimada, M., Eds.; Uni Publishers; Tokyo, Japan, 1992; pp 357−363.

(4) Willför, S.; Sundberg, A.; Hemming, J.; Holmbom, B. Polysaccharides in some industrially important softwood species. *Wood Sci. Technol.* **2005**, *39*, 245–258.

(5) Aspinall, G. O.; Wood, T. M. The structures of two water-soluble polysaccharides from Scots pine (*Pinus sylvestris*). *J. Chem. Soc.* **1963**, *1963*, 1686–1696.

(6) Aspinall, G. O.; Begbie, R.; McKay, J. E. The hemicelluloses of European larch (*Larix decidua*). Part II. The glucomannan component *J. Chem. Soc.* **1962**, *1962*, 214–219.

(7) Brasch, D. J. The chemistry of *Pinus radiata*. VI. The water-soluble galactoglucomannan. *Aust. J. Chem.* **1983**, *36*, 947–954.

(8) Willfor, S.; Sjoholm, R.; Laine, C.; Roslund, M.; Hemming, J.; Holmbom, B. Characterisation of water-soluble galactoglucomannans from Norway spruce wood and thermomechanical pulp. *Carbohydr. Polym.* **2003**, *52*, 175–187.

(9) Lundqvist, J.; Teleman, A.; Junel, L.; Zacchi, G.; Dahlman, O.; Tjerneld, F.; Stålbrand, H. Isolation and characterization of galactoglucomannan from spruce (*Picea abies*). *Carbohydr. Polym.* **2002**, *48*, 29–39.

(10) Lundqvist, J.; Jacobs, A.; Palm, M.; Zacchi, G.; Dahlman, O.; Stålbrand, H. Characterization of galactoglucomannan extracted from spruce (*Picea abies*) by heat-fractionation at different conditions. *Carbohydr. Polym.* **2003**, *51*, 203–211.

(11) Xu, C.; Leppänen, A.-S.; Eklund, P.; Holmlund, P.; Sjöholm, R.; Sundberg, K.; Willför, S. Acetylation and characterization of spruce (*Picea abies*) galactoglucomannans. *Carbohydr. Res.* **2010**, *345*, 810–816.

(12) Willfor, S.; Holmbom, R. B. Isolation and characterization of water-soluble polysaccharides from Norway spruce and Scots pine. *Wood Sci. Technol.* **2004**, *38*, 173–179.

(13) Capek, P.; Alfoldi, J.; Liskova, D. An acetylated galactoglucomannan from *Picea abies* L. Karst. *Carbohydr. Res.* **2002**, *337*, 1033–1037.

(14) Capek, P.; Kubacková, M.; Alfoldi, J.; Bilisics, L.; Liskova, D.; Kakoniova, D. Galactoglucomannan from the secondary cell wall of *Picea abies* L. Karst. *Carbohydr. Res.* **2000**, *329*, 635–645.

(15) Hamilton, J. K.; Partlow, E. V. The hemicelluloses of western red cedar: the constitution of a glucomannan. *J. Am. Chem. Soc.* **1958**, *80*, 4880–4885.

(16) Hamilton, J. K.; Kircher, H. W. The constitution of a gluco-mannan associated with wood cellulose from western hemlock. *J. Am. Chem. Soc.* **1958**, *80*, 4703–4709.

(17) Hamilton, J. K.; Thompson, N. S. Graded acid hydrolysis studies of a xylan polyuronide associated with wood cellulose from western hemlock. *J. Am. Chem. Soc.* **1957**, *79*, 6464–6469.

(18) Jones, J. K. N.; Painter, T. J. The hemicelluloses of loblolly pine (*Pinus taeda*) wood. Part I. The isolation of five oligosaccharide fragments. *J. Chem. Soc.* **1957**, *1957*, 669–673.

(19) Dutton, G. G. S.; Hunt, K. The constitution of the hemicellulose of Sitka Spruce (*Picea sitchensis*). II. Structure of the mannan portion. *J. Am. Chem. Soc.* **1958**, *80*, 5697–5701.

(20) Hoffmann, G. C.; Timell, T. E. Isolation and characterization of a galactoglucomannan from red pine (*Pinus resinosa*) wood. *Tappi* **1970**, *53*, 1896–1899.

(21) Hamilton, J. K.; Partlow, E. V.; Thompson, N. S. The nature of a galactoglucomannan associated with wood cellulose from southern pine. *J. Am. Chem. Soc.* **1960**, *82*, 451–457.

(22) Tyminsk, A.; Timell, T. E. The constitution of a glucomannan from white spruce (*Picea glauca*). *J. Am. Chem. Soc.* **1960**, *82*, 2823–2827.

(23) Kubačková, M.; Karácsonyi, Š.; Bilisics, L. Structure of galactoglucomannan from *Populus monilifera* H. *Carbohydr. Polym.* **1992**, *19*, 125–129.

(24) Price, N. P. J. Acylic sugar derivatives for GC/MS analysis of ^{13}C-enrichment during carbohydrate metabolism. *Anal. Chem.* **2004**, *76*, 6566–6574.

(25) Jones, T. M.; Albersheim, P. A gas chromatographic method for the determination of aldose and uronic acid constituents of plant cell wall polysaccharides. *Plant Physiol.* **1972**, *49*, 926–936.

(26) Ciucano, I.; Kerek, F. A simple and rapid method for the permethylation of carbohydrates. *Carbohydr. Res.* **1984**, *131*, 209–217.

(27) Xu, C.; Pranovich, A.; Vähäsalo, L.; Hemming, J.; Holmbom, B.; Schols, H. A.; Willför, S. Kinetics of acid hydrolysis of water-soluble spruce *O*-acetyl galactoglucomannans. *J. Agric. Food Chem.* **2008**, *56*, 2429–2435.

(28) Hannuksela, T.; Hervé du Penhoat, C. NMR structural determination of dissolved *O*-acetylated galactoglucomannan isolated from spruce thermomechanical pulp. *Carbohydr. Res.* **2004**, *339*, 301–312.

(29) Ramesh, H. P.; Yamaki, K.; Ono, H.; Tsushida, T. Two-dimensional NMR spectroscopic studies of fenugreek (*Trigonella foenum-graecum* L.) galactomannan without chemical fragmentation. *Carbohydr. Polym.* **2001**, *45*, 69–77.

(30) Sato, K.; Akai, S.; Youda, H.; Kojima, M.; Sakuma, M.; Inaba, S.; Kurosawa, K. Practical synthesis of D-[1-^{13}C]mannose, L-[1-^{13}C] and L-[6-^{13}C]fucose. *Tetrahedron Lett.* **2005**, *46*, 237–243.

(31) Vieira, I. G. P.; Mendes, F. N. P.; Gallao, M. I.; de Brito, E. S. NMR studies of galactomannans from the seeds of mesquite tree (*Prosopis juliflora* (Sw) DC. *Food Chem.* **2007**, *101*, 70–73.

(32) Sela, D. A.; Chapman, J.; Adeuya, A.; Kimble., J. H.; Chen, F.; Whitehead, T. R.; Lapidus, A.; Rokhsar, D. S.; Lebrilla, C. B.; German, J. B.; Price, N. P. J.; Richardson, P. M.; Mills, D. A. The genome sequence of *Bifidobacterium longum* subsp. *infantis* reveals adaptations for milk utilization within the infant microbiome. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 18964–18969.

(33) Faber, T. A.; Bauer, L. L.; Price, N. P.; Hopkins, A. C.; Fahey, G. C., Jr. In vitro digestion and fermentation characteristics of temulose molasses, a coproduct of fiberboard production, and select temulose fractions using canine fecal inoculum. *J. Agric. Food Chem.* **2011**, 10.1021/jf103737y.