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In Vitro Digestion and Fermentation Characteristics of Temulose Molasses, a Coproduct of Fiberboard Production, and Select Temulose Fractions Using Canine Fecal Inoculum

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ABSTRACT: It is of interest to discover new fermentable carbohydrate sources that function as prebiotics. This study evaluated the hydrolytic digestibility, fermentative capacity, and microbiota modulating properties of Temulose molasses, four hydrolyzed fractions of Temulose molasses, short-chain fructooligosaccharides (scFOS), and a yeast cell wall preparation (Safmannan). These substrates resisted in vitro hydrolytic digestion. Each substrate was fermented in vitro using dog fecal inoculum, and fermentation characteristics were quantified at 0 and 12 h. All Temulose molasses substrates decreased pH by at least 0.64 unit and resulted in greater ($P < 0.05$) butyrate and total short-chain fatty acid (SCFA) production compared to scFOS and Safmannan. Temulose molasses substrates resulted in higher ($P < 0.01$) or equal *Bifidobacterium* spp. concentrations compared to scFOS. Temulose molasses substrate and its fractions demonstrated prebiotic characteristics as indicated by low hydrolytic digestibility, high fermentability, and enhanced growth of microbiota considered to be beneficial to health.

KEYWORDS: In vitro fermentation, Temulose molasses, degree of polymerization, prebiotic, short-chain fatty acids

■ INTRODUCTION

Fermentable carbohydrates have garnered increased attention as a result of their ability to improve bowel health of both humans and animals. These carbohydrates are able to resist hydrolytic digestion and are fermented in the large bowel, yielding short-chain fatty acids (SCFA), particularly butyrate, an energy source for colonocytes.[1] In addition, fermentable carbohydrates may potentially increase populations of beneficial bacteria, such as bifidobacteria and lactobacilli, and decrease concentrations of pathogenic bacteria, such as *Escherichia coli* and *Clostridium perfringens*.

Temulose molasses, derived from the fiberboard manufacturing process, may be a beneficial fermentable carbohydrate source. Production of this novel carbohydrate involves steaming wood chips obtained from southern yellow pine, which includes *Pinus taeda*, *Pinus echinata*, *Pinus palustris*, and *Pinus elliotii*, using high temperature and pressure. When the pressure is released quickly, soluble wood sugars and oligosaccharides are released and washed into the surrounding water. The resulting sugar solution is condensed through evaporation, resulting in a viscous ingredient referred to as Temulose molasses.

Temulose molasses is composed of numerous types of oligosaccharides, including mannanoligosaccharides (MOS), xylooligosaccharides (XOS), and glucooligosaccharides (GOS).[2] Several in vitro studies have reported that these oligosaccharides are moderately to well fermented as indicated by increased production of SCFAs, lowered pH, and altered microbial populations.[3−8] In addition, studies have indicated that fermentative end-products may vary depending on the degree of polymerization (DP) of the oligosaccharide.[9,10]

Temulose molasses and select fractions of Temulose molasses, varying in DP, were evaluated for fermentative and microbiota modulating properties in vitro using canine fecal inoculum.

■ MATERIALS AND METHODS

Temulose Molasses Substrate. Production of Temulose molasses involves wood chips, water, and pressure but does not use strong acids or bases, unlike other wood pulping production processes.[11] This results in an ingredient safe for consumption by animals.[11] The effects of time and steam pressure on the "wood chip digester" result in depolymerization of cellulose, hemicelluloses, and lignin that releases soluble sugars and polyphenolic compounds that are recovered by water washing. The resulting solution contains high concentrations of sugars (3−4%), which is further condensed into a molasses with a 60−65% solids content. Sugars are mostly in the form of oligosaccharides as compared to free sugars. Temulose molasses has a DP ranging from 2 to 50 units.

Another substrate, ethanol-precipitated Temulose brown sugar (TBS), was produced according to the procedure described by Price et al.[2] Briefly, 5 mL of Temulose molasses was partially hydrolyzed with 7 mL of 0.2 M aqueous trifluoroacetic acid (TFA), precipitated in ethanol, and freeze-dried. The hydrolysis procedure removed arabinose and xylose from Temulose molasses, producing a galactoglucomannan oligosaccharide (GGMO) product. This fraction had a DP of 4−13, with the major component having a DP of 5−8. An aliquot of TBS then was

fractionated using size exclusion chromatography (SEC) into select DP ranges according to the procedure of Price et al.[2] One substrate, SEC-purified small GGMO oligosaccharides (GS), had a DP of 2−5, whereas SEC-purified medium GGMO oligosaccharides (GM) had a DP of 6−8 and SEC-purified large GGMO oligosaccharides (GL) had a DP of 9−13.

The yeast cell wall preparation, Safmannan (Lesaffre Yeast Corp., Milwaukee, WI), and short-chain fructooligosaccharide (scFOS; Nutra-Flora, GTC Nutrition Co., Johnstown, CO) were obtained commercially and evaluated for comparison to the Temulose substrates.

Temulose molasses and fractions were analyzed for dry matter (DM), organic matter (OM), and ash using AOAC[12] methods. Free monosaccharide concentrations were determined according to the method of Smiricky et al.[13] Hydrolyzed monosaccharides (i.e., sugars obtained after acid hydrolysis) were determined according to the methods of Hoebler et al.[14] and Bourquin et al.[15] Briefly, an internal standard of 1 mg of inositol/mL in 72% (w/w) sulfuric acid was prepared. One milliliter of the internal standard was added to a screw-cap tube, containing 50 mg of finely ground sample, and vortexed gently. After 30 min, the samples were diluted to 2 N sulfuric acid by adding 11 mL of distilled, deionized water. Samples were hydrolyzed for 2 h in a boiling water bath. The hydrolyzed samples were filtered through Whatman GF/D glass fiber filters (Whatman Inc., Florham Park, NJ) and then neutralized by passage through a preparation column containing 15 g of AG 4-X4 anion exchange resin (Bio-Rad, Hercules, CA). Effluents were collected in 200 mL volumetric flasks and brought up to volume with distilled, deionized water. Hydrolyzed monosaccharides were quantified using a Dionex DX500 high-performance liquid chromatography (HPLC) system (Dionex Corp., Sunnyvale, CA). Standards for quantification included arabinose, fucose, galactose, glucose, inositol, mannose, rhamnose, and xylose.

In Vitro Digestion. The method described by Boisen[16] was used to simulate gastric and small intestinal (hydrolytic) digestion. Briefly, at each fermentation sampling time (0 and 12 h), 0.2 g of substrate was weighed in triplicate and incubated with pepsin/hydrochloric acid for 6 h and with pancreatin for 18 h. Tubes containing reagents, but no substrate, were run as blanks. The tubes were analyzed for free released monosaccharides using HPLC[13] following simulated hydrolytic digestion. The remaining residues were lyophilized and used for the in vitro fermentation stage.

The released monosaccharide values correspond to the amount of monosaccharides resulting from hydrolytic digestion and would be expected to be absorbed in vivo. These sugars cannot be extracted from the in vitro tubes after the second stage of simulated digestion. Therefore, the released monosaccharide values were used to prepare a set of pure monosaccharide blank tubes for each substrate. These free sugar blank tubes went through the in vitro fermentation experiment along with the residues from the hydrolytic digestion stage. SCFAs measured after 12 h of in vitro fermentation then were corrected with the appropriate blank (control) tube values.

Donors. Purpose-bred, healthy, female, adult dogs (*n* = 3; Butler Farms USA, Clyde, NY) with hound bloodlines, an average initial body weight of 23.1 kg (18.2−26.6 kg), and an average age of 4.4 years (1−6 years) served as sources of feces from which inoculum was prepared. Dogs consumed the same commercial diet (Iams Weight Control; The Iams Co., Lewisburg, OH) composed of corn meal, chicken, whole grain sorghum, chicken byproduct meal, ground whole grain barley, and fish meal. The dogs had not been exposed to antibiotics for 6 months prior to the experiment. Dogs were housed individually in kennels in a temperature-controlled room (21 °C) at the animal care facility in the Edward R. Madigan Laboratory, University of Illinois at Urbana−Champaign. Animal care procedures were approved by the University of Illinois Animal Care and Use Committee prior to initiation of the experiment. On the designated collection day, fresh feces from three dogs were collected in plastic bags, which were sealed after expressing excess air, and maintained at 37 °C until inoculum was prepared. Anaerobic inoculum was prepared from fresh fecal samples within 15 min of defecation.

In Vitro Fermentation Model. The lyophilized residue remaining after simulated stomach and small intestinal digestion (in vitro hydrolytic digestion) was used in a model that simulated large bowel fermentation.[17] The composition and the preparation of the in vitro medium have been described in detail elsewhere.[18] An aliquot (26 mL) of the medium was aseptically transferred to the tubes containing the lyophilized residue remaining after simulated hydrolytic digestion and to the control tubes containing the appropriate amount of free monosaccharides. All tubes were stored at 4 °C for approximately 12 h to enable hydration of the substrates before initiation of fermentations. Tubes were placed in a 37 °C water bath approximately 30 min before inoculation.

Fresh fecal samples were maintained at 37 °C until inoculum was prepared (within 10 min). Equal amounts of each fecal sample were mixed together and diluted 1:10 (w/v) in anaerobic dilution solution[19] by blending for 15 s in a Waring blender under a stream of CO_2. Blended, diluted feces were filtered through four layers of cheesecloth and sealed in 125 mL serum bottles under CO_2.

Appropriate samples and control tubes were aseptically inoculated with 4 mL of diluted feces. Tubes were incubated at 37 °C with periodic mixing for 12 h. After 12 h, tubes were removed from the 37 °C incubator and processed immediately for analyses. First, the pH of the tube contents was measured with a standard pH-meter (Denver Instrument Co., Arvada, CO). Then, a 2 mL aliquot was taken from each tube for SCFA analyses. A second 2 mL aliquot was taken and frozen at −80 °C for bacterial analyses.

Chemical Analysis. SCFA and branched-chain fatty acid (BCFA) concentrations were determined by gas chromatography according to the method of Erwin et al.[20] using a Hewlett-Packard 5890A series II gas chromatograph (Palo Alto, CA) and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Nitrogen was the carrier with a flow rate of 75 mL/min. Oven, detector, and injector temperatures were 125, 175, and 180 °C, respectively.

Microbial Analysis. Microbial populations were measured by DNA extraction from fermented samples, followed by quantitative PCR (qPCR). DNA was extracted from frozen samples using the RBB+C method described by Yu and Morrison.[21] Briefly, cells were lysed by employing bead beating in the presence of high concentrations of sodium dodecyl sulfate (SDS), salt, and EDTA. The DNA was purified using QIAamp columns (QIAamp DNA stool mini kit, Qiagen, Valencia, CA) according to the manufacturer's instructions. Extracted DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Nano-Drop Technologies, Wilmington, DE).

Quantitative PCR was performed for bifidobacteria, lactobacilli, and *E. coli* genera, as well as *C. perfringens*. Specific primers were used for bifidobacteria,[22] lactobacilli,[23] *E. coli*,[24] and *C. perfringens*.[25] Amplification was performed according to the method of DePlanke et al.[26] Briefly, a 10 μL final volume contained 5 μL of 2X SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), 15 pmol of the forward and reverse primers for the bacterium of interest, and 10 ng of extracted DNA from the sample. Standard curves were obtained by harvesting pure cultures of the bacterium of interest in the log growth phase in triplicate followed by serial dilution. Bacterial DNA was extracted from each dilution using a QIAamp DNA stool mini-kit and amplified with the bacterial DNA to create triplicate standard curves using an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). Colony forming units (cfu) in each dilution were determined by plating on specific agars: lactobacilli MRS (Difco, Detroit, MI) for lactobacilli, reinforced clostridial medium (bifidobacteria, *C. perfringens*), and Luria−Bertani

Table 1. Dry Matter, Organic Matter, Free Sugar Concentrations, and Hydrolyzed Monosaccharide Concentrations of Control Substrates, Temulose Molasses, Temulose Brown Sugar (TBS), and Size Exclusion Chromatography-Purified Galactogluco-mannan Oligosaccharides

	fraction						
item	ScFOS[a]	Safmannan	Temulose molasses	TBS[b]	GL[c]	GM[d]	GS[e]
dry matter, %	94.6	96.4	59.2	92.7	94.3	98.1	98.6
			DM basis				
organic matter, %	100.0	95.6	94.0	86.8	100.0	99.9	98.3
free sugars, mg/g							
fucose	0.0	0.0	1.7	0.6			
arabinose	0.8	0.2	41.6	11.1			
rhamnose	0.0	0.0	2.3	0.9			
galactose	0.0	0.1	11.5	14.8			
glucose	1.6	0.3	10.5	13.1			
xylose	0.0	0.0	18.1	22.3			
mannose	0.4	1.2	5.9	37.2			
fructose	8.5	0.3	9.2	2.0			
total	11.3	2.1	100.8	102.7			
hydrolyzed monosaccharides[f]							
fucose			8.1	0.0	0.0	0.0	0.0
arabinose			12.7	12.4	10.0	12.1	14.8
rhamnose			3.7	0.0	0.0	0.0	0.0
galactose			73.2	82.8	118.8	114.2	108.8
glucose			115.0	148.8	284.5	273.8	261.0
xylose			132.8	24.2	15.9	34.0	53.3
mannose			364.3	347.6	622.3	580.4	522.1
total			697.9	615.8	1051.4	1014.5	960.1

[a] ScFOS, short-chain fructooligosaccharides. [b] TBS, Temulose molasses hydrolyzed with 0.2 M trifluoroacetic acid and precipitated with ethanol. [c] GL, SEC-purified galactoglucomannan oligosaccharide with a high degree of polymerization. [d] GM, SEC-purified galactoglucomannan oligosaccharide with a medium degree of polymerization. [e] GS, SEC-purified galactoglucomannan oligosaccharide with a low degree of polymerization. [f] Hydrolyzed monosaccharide concentrations were corrected for free sugar concentrations.

medium (*E. coli*). The calculated log cfu/mL of each serial dilution was plotted against the cycle threshold (Ct) to create a linear equation to calculate cfu/mL.

Statistical Analysis. Data were analyzed as a completely randomized design using the Mixed Models procedure of SAS (SAS Institute, Inc., Cary, NC). All treatment least-squares means were compared with each other, and a Tukey adjustment was used to control for experiment-wise error. Least-squares means were reported along with the pooled SEM for all response criteria. A probability of $P < 0.05$ was accepted as statistically significant for released monosaccharides, SCFA, and pH and a probability of $P < 0.01$ was accepted as statistically significant for microbiota. Means were separated using the least significant difference method of SAS.

■ RESULTS

Substrates. All substrates were similar in DM content except Temulose molasses, which had a considerably lower DM content (59.2%) (Table 1). Organic matter was high for all substrates except TBS. SEC-purified GGMO oligosaccharides (GS, GM, and GL) contained no free sugars. Besides SEC-purified GGMO oligosaccharides, Safmannan contained the lowest total concentration of free sugars (2.1 mg/g), whereas Temulose molasses and TBS had the highest free sugar concentrations (100.8 and 102.7 mg/g, respectively). The Temulose molasses contained a high concentration of free arabinose (41.6 mg/g), whereas TBS

contained high concentrations of mannose (37.2 mg/g) and xylose (22.3 mg/g).

All Temulose substrates contained high concentrations of total hydrolyzed monosaccharides ranging between 615.8 and 1051.4 mg/g. Fucose and rhamnose were detected only in the Temulose molasses. Arabinose and galactose concentrations were low but similar among all Temulose substrates. The Temulose molasses contained more than twice the amount of xylose compared to TBS and SEC-purified GGMO oligosaccharides. The glucose concentration was much lower in Temulose molasses compared to TBS and the SEC-purified GGMO oligosaccharides. Mannose concentrations were high for all Temulose substrates, with GL and GM being greatest (622.3 and 580.4 mg/g DMB, respectively). Safmannan and scFOS were not analyzed for hydrolyzed monosaccharide concentrations.

After in vitro hydrolytic digestion, all substrates released similar concentrations of fucose, rhamnose, and galactose (Table 2). The Temulose molasses released a greater ($P < 0.05$) concentration of arabinose (13.9 mg/g DMB) than the other test substrates. Glucose release was lowest ($P < 0.05$) for Temulose molasses (4.9 mg/g DMB) and greatest ($P < 0.05$) for Safmannan (27.8 mg/g DMB). The Temulose molasses released a greater ($P < 0.05$) concentration of xylose (1.1 mg/g) compared to scFOS, TBS, and GM. Temulose brown sugar resulted in the greatest ($P < 0.05$) release of mannose among substrates. Short-chain FOS was the only substrate to release

Table 2. Released Monosaccharide Concentrations of Control Substrates, Temulose Molasses, Temulose Brown Sugar (TBS), and Size Exclusion Chromatography-Purified Galactoglucomannan Oligosaccharides after Simulated Hydrolytic Digestion[a,b]

item	ScFOS[c]	Safmannan	Temulose molasses	TBS[d]	GL[e]	GM[f]	GS[g]	SEM
			mg/g, DMB					
fucose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00
arabinose	0.0 a	0.0 a	13.9 b	0.6 a	0.0 a	0.1 a	0.3 a	0.19
rhamnose	0.0 a	0.0 a	0.1 b	0.0 a	0.0 a	0.0 a	0.0 a	0.02
galactose	0.0 a	0.0 a	0.6 ab	1.2 b	0.2 a	0.1 a	0.4 a	0.15
glucose	21.5 e	27.8 f	4.9 a	13.6 b	19.3 de	17.3 cd	16.8 c	0.51
xylose	0.3 a	0.7 ab	1.1 b	0.0 a	0.6 ab	0.3 a	0.6 ab	0.13
mannose	0.0 a	0.0 a	0.4 b	4.9 c	0.0 a	0.1 ab	0.0 a	0.07
fructose	203.5 b	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	1.00
total	225.3 c	28.5 b	21.0 ab	20.3 ab	20.1 a	17.9 a	18.1 a	1.57

[a] Sugars released from substrate after 6 h of HCl–pepsin digestion and 18 h of digestion with pancreatin. Concentrations were corrected for free sugars. [b] Within a row, means without a common letter differ ($P < 0.05$). [c] ScFOS, short-chain fructooligosaccharides. [d] TBS, Temulose molasses hydrolyzed with 0.2 M trifluoracetic acid and precipitated with ethanol. [e] GL, SEC-purified galactoglucomannan oligosaccharide with a high degree of polymerization. [f] GM, SEC-purified galactoglucomannan oligosaccharide with a medium degree of polymerization. [g] GS, SEC-purified galactoglucomannan oligosaccharide with a low degree of polymerization.

Table 3. pH Change and Short-Chain Fatty Acid (SCFA) and Branched-Chain Fatty Acid (BCFA) Production following 12 h of in Vitro Fermentation of Control Substrates, Temulose Molasses, Temulose Brown Sugar (TBS), and Size Exclusion Chromatography-Purified Galactoglucomannan Oligosaccharides[a]

item	ScFOS[b]	Safmannan	Temulose molasses	TBS[c]	GL[d]	GM[e]	GS[f]	SEM
pH change	−1.22 e	−0.29 a	−0.86 d	−1.22 e	−0.77 c	−0.64 b	−0.67 b	0.17
SCFA			mg/g DM					
acetate	86.1 b	23.1 a	192.5 d	102.1 c	201.6 d	196.3 d	198.9 d	5.64
propionate	76.1 b	25.3 a	119.2 d	106.4 c	170.7 f	142.8 e	106.7 c	4.01
butyrate	0.0 a	15.8 b	16.2 b	17.8 b	17.1 b	18.7 c	22.5 d	0.72
total	162.2 b	64.1 a	327.9 d	226.3 c	389.4 f	357.8 e	328.1 d	5.01
BCFA			mg/g DM					
isobutyrate	0.0 a	1.1 b	0.0 a	0.3 a	0.0 a	0.0 a	0.0 a	0.18
isovalerate	0.0 a	4.1 b	0.0 a	0.0 a	3.7 b	4.3 b	4.3 b	0.71
valerate	0.0 a	2.4 b	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.23
total	0.0 a	7.6 c	0.0 a	0.3 a	3.7 b	4.4 b	4.3 b	1.06

[a] Within a row, means without a common letter differ ($P < 0.05$). [b] ScFOS, short-chain fructooligosaccharides. [c] TBS, Temulose molasses hydrolyzed with 0.2 M trifluoracetic acid and precipitated with ethanol. [d] GL, SEC-purified galactoglucomannan oligosaccharide with a high degree of polymerization. [e] GM, SEC-purified galactoglucomannan oligosaccharide with a medium degree of polymerization. [f] GS, SEC-purified galactoglucomannan oligosaccharide with a low degree of polymerization.

fructose (203.5 mg/g). Only glucose was released upon hydrolytic digestion of Safmannan. Short-chain FOS resulted in the greatest ($P < 0.05$) release of total monosaccharides, whereas GS, GM, and GL resulted in the lowest ($P < 0.05$) release of total monosaccharides. The hydrolytic digestion value for the Temulose substrates was only approximately 2%.

Fermentation Metabolites. Safmannan fermentation resulted in the smallest ($P < 0.05$) pH change among test substrates (−0.29) (Table 3). Short-chain FOS and TBS fermentation resulted in the greatest ($P < 0.05$) decrease in pH (−1.22) among test substrates. SEC-purified GGMO oligosaccharides resulted in intermediate changes in pH.

Safmannan fermentation resulted in the lowest ($P < 0.05$) concentrations of acetate, propionate, and total SCFA among substrates. Short-chain FOS fermentation resulted in lower ($P < 0.05$) concentrations of total SCFA, acetate, propionate, and butyrate compared to the Temulose substrates. The Temulose molasses and SEC-purified GGMO oligosaccharides resulted in a similar concentration of acetate, with these values being greater ($P < 0.05$) than that for TBS. Propionate production was similar between GS and TBS, but values were lower ($P < 0.05$) than that for the Temulose molasses. Large DP GGMO fermentation produced the greatest ($P < 0.05$) concentration of propionate (170.7 mg/g), whereas GM fermentation resulted in the next highest concentration (142.8 mg/g). Among SEC-purified GGMO oligosaccharides, as DP increased, propionate production increased ($P < 0.05$). Butyrate production was similar for Safmannan, Temulose molasses, TBS, and GL. Small DP GGMO

Table 4. Microbiota Concentration in Batch Culture Fermentation with Control Substrates, Temulose Molasses, Temulose Brown Sugar (TBS), and Size Exclusion Chromatography-Purified Galactoglucomannan Oligosaccharides after 12 h of Fermentation with Dog Fecal Inoculum[a]

	fraction							
item	ScFOS[b]	Safmannan	Temulose molasses	TBS[c]	GL[d]	GM[e]	GS[f]	SEM
	CFU, log10/mL of in vitro fluid							
Bifidobacterium spp.	5.8 bc	4.8 a	6.4 e	6.1 d	5.7 b	5.7 b	5.9 cd	0.06
Lactobacillus spp.	8.2 ab	8.1 a	8.3 b	8.2 ab	8.1 a	8.1 ab	8.2 ab	0.04
Escherichia coli	10.0 c	10.0 c	9.8 bc	9.8 bc	9.6 a	9.6 a	9.7 ab	0.05
Clostridium perfringens	7.3 bc	7.0 a	7.2 ab	7.4 c	7.1 ab	7.1 ab	7.3 bc	0.06

[a] Within a row, means without a common letter differ ($P < 0.01$). [b] ScFOS, short-chain fructooligosaccharides. [c] TBS, Temulose molasses hydrolyzed with 0.2 M trifluoracetic acid and precipitated with ethanol. [d] GL, SEC-purified galactoglucomannan oligosaccharide with a high degree of polymerization. [e] GM, SEC-purified galactoglucomannan oligosaccharide with a medium degree of polymerization. [f] GS, SEC-purified galactogluco-mannan oligosaccharide with a low degree of polymerization.

fermentation resulted in the greatest ($P < 0.05$) butyrate concentration (22.5 mg/g) among substrates, whereas GM fermentation resulted in the next greatest concentration (18.7 mg/g). Among SEC-purified GGMO oligosaccharides, as DP increased, butyrate production decreased ($P < 0.05$). Short-chain FOS fermentation resulted in no butyrate production after 12 h. Total SCFA production was greatest ($P < 0.05$) for the SEC-purified GGMO oligosaccharides compared with TBS. Total SCFA production increased ($P < 0.05$) as the DP of the GGMO fraction increased. Short-chain FOS and Temulose molasses fermentation did not result in any BCFA production. Also, very low concentrations of BCFA were produced by any of the test substrates.

Microbiota. After 12 h of fermentation, Temulose molasses resulted in the greatest ($P < 0.01$) *Bifidobacterium* count among substrates, whereas Safmannan resulted in the lowest ($P < 0.01$) count (Table 4). *Lactobacillus* counts for Temulose molasses were greater ($P < 0.01$) than those for Safmannan and GL, whereas the remaining substrates were intermediate. *E. coli* counts for scFOS and Safmannan were greater ($P < 0.01$) than for GL and GM, whereas other counts were intermediate. Temulose brown sugar resulted in the greatest ($P < 0.01$) *C. perfringens* count, whereas Safmannan resulted in the lowest ($P < 0.01$) count.

■ DISCUSSION

The objective of this in vitro study was to evaluate the hydrolytic digestibility, fermentability, and prebiotic potential of Temulose molassses and purified GGMO fractions isolated from the molasses. After 12 h of fermentation, test substrates resulted in a significant drop in pH and produced greater concentrations of SCFA than did the control substrates, scFOS, or Safmannan. The Temulose substrates also resulted in beneficial shifts in microbial populations compared to the control substrates. To be classified as a prebiotic, the substrate must "be a selectively fermented ingredient that allows specific changes, both in composition and/or activity, in the gastrointestinal microflora that confers benefits upon host well-being and health".[27] On the basis of this definition, the Temulose molasses and SEC-purified GGMO oligosaccharides demonstrated potential to be prebiotic ingredients.

Saffmannan and scFOS were used as control substrates as considerable information is known about each. Safmannan is a mannanoligosaccharide (MOS) derived from the cell wall of yeast.

Short-chain FOS is a fermentable oligosaccharide proven to be a prebiotic.

Substrates tested were similar in OM content, except for TBS, which contained a much lower OM content. This substrate may contain an unknown inorganic substance, perhaps a residual salt from the fractionation step. The TBS fraction contained a lower OM concentration than did the Temulose molasses from which TBS was derived. SEC removed the inorganic impurity as indicated by fractions of select DP having an OM concentration near 100%. For discussion of the oligosaccharide composition, refer to the accompanying paper by Price et al.[11]

All substrates, except scFOS, had low hydrolytic digestibility values, resulting in a large amount of substrate for fermentation. The scFOS substrate used in this study was of low purity, which allowed a portion of the fructose to be cleaved during in vitro hydrolytic digestion.

It is evident from pH change data and total SCFA production data that Temulose substrates were well fermented compared to control substrates. The Temulose substrates contained various bound monosaccharides with high concentrations of xylose, mannose, glucose, and galactose. Fermentative end-product concentrations were similar to those reported from other in vitro studies evaluating pure forms of these oligosaccharides, with all studies noting that these oligosaccharides were well fermented.[2−7] The GL fraction contained the highest concentration of mannose and total oligosaccharides, which resulted in greater fermentation as indicated by greater acetate, propionate, and total SCFA production compared to the other substrates evaluated.

The TBS substrate produced much less acetate (approximately 100 mg/g) compared to the other Temulose substrates. Bound monosaccharide compositional differences may have been a factor. The TBS substrate contained much less mannose and glucose than did the SEC-purified GGMO oligosaccharides. However, the SEC-purified GGMO oligosaccharides and the Temulose molasses produced similar concentrations of acetate, despite Temulose molasses and TBS being similar in monosaccharide composition except for bound xylose concentration. In this study, we analyzed the six commonly produced SCFA. It is possible that other SCFAs such as formate, succinate, or malate were produced from TBS instead of acetate, but these were not analyzed. The TBS fraction resulted in the greatest pH change among all substrates tested, potentially indicative of higher fermentability and higher SCFA production.

Even though the SEC-purified GGMO oligosaccharides were derived from TBS, they produced greater concentrations of

isovalerate compared to TBS that produced no BCFA. Safmannan, a yeast cell wall-derived MOS, produced a high concentration of BCFA, likely due to the protein commonly found in this ingredient.

The DP of a carbohydrate affects the rate and site of fermentation in the large bowel and also potentially alters the microbial species that utilize the substrate.[8,9,28,29] Both Hernot et al.[28] and Roberfroid et al.[29] noted that fructans with a DP of <10 increased bifidobacteria populations and were fermented at a faster rate than fructans with a DP of >10. Van Laere et al.[9] demonstrated that bifidobacteria preferentially fermented low DP substrates first and that bacteroides were able to ferment substrates with a high DP.

Substrates fermented in this study were either SEC-purified GGMO oligosaccharides (GL, GM, and GS) or a mixture of DP fractions (Temulose molasses and TBS). The large DP fraction resulted in greater propionate and total SCFA production. The small and medium DP fractions, which both had a DP of <10, resulted in greater butyrate production than did GL. The GS substrate, but not GM, resulted in a greater bifidobacteria population. This result is similar to findings by Van Laere et al.,[9] who noted that lower DP oligosaccharides promote bifidobacteria populations. The mixed DP fractions, Temulose molasses and TBS, resulted in the lowest butyrate and total SCFA concentrations, but resulted in the highest bifidobacteria populations. Substrates with an array of DPs promoted bifidobacteria growth to a greater extent than select fractions of similar DP.

Roberfroid et al.[29] indicated "the observation of 1 log-fold increase in bifidobacteria is a clear indication of a modification of the intestinal flora". In the current study, Safmannan had a final bifidobacteria population of 4.8 cfu, $\log_{10}$/mL. All other substrates were noted to be at least 0.9 log unit greater than Safmannan, indicating that Temulose substrates were able to clearly modify the bifidobacteria population. The Temulose substrates resulted in either an equal or significantly greater bifidobacteria population compared to the proven prebiotic, scFOS. The Temulose molasses resulted in the greatest increase in the bifidobacteria population after 12 h of fermentation. Moreover, Temulose substrates inhibited the growth of *E. coli* to a greater extent than did Safmannan or scFOS. Inhibition of growth of *E. coli* and *C. perfringens* is viewed as beneficial to large bowel health. Only slight differences in *C. perfringens* and *Lactobacillus* spp. populations were detected among substrates.

In conclusion, Temulose substrates resisted hydrolytic digestion and were well fermented as indicated by a decrease in pH, increased SCFA production, and beneficial microbial changes in comparison to control substrates. On the basis of these results, all Temulose substrates exhibited prebiotic-like effects. Among substrates, TBS appears to be least fermentable based on total SCFA production. The Temulose molasses has the greatest prebiotic potential based on SCFA production and promotion of bifidobacteria and lactobacilli growth and inhibition of *E. coli* growth. Overall, Temulose substrates were highly fermentable and may positively affect large bowel health.

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dx.doi.org/10.1021/jf103737y |*J. Agric. Food Chem.* 2011, 59, 1847–1853