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In Vitro Fermentation Profiles, Gas Production Rates, and Microbiota Modulation as Affected by Certain Fructans, Galactooligosaccharides, and Polydextrose

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It is of interest to benefit from the positive intestinal health outcomes of prebiotic consumption but with minimal gas production. This study examined gas production potential, fermentation profile, and microbial modulation properties of several types of oligosaccharides. Substrates studied included short-chain, medium-chain, and long-chain fructooligosaccharides, oligofructose-enriched inulin, galactooligosaccharide, and polydextrose. Each substrate was fermented in vitro using human fecal inoculum, and fermentation characteristics were quantified at 0, 4, 8, and 12 h. Gas and short-chain fatty acid (SCFA) production data showed that short-chain oligosaccharides were more rapidly fermented and produced more SCFA and gas than substrates with greater degrees of polymerization. Lactobacilli increased similarly among substrates. Short-chain oligosaccharides fermentation resulted in the greatest increase in bifidobacteria concentrations. Mixing short- and long-chain oligosaccharides attenuated short-chain oligosaccharide fermentation rate and extent. This study provides new information on the fermentation characteristics of some oligosaccharides used in human nutrition.

KEYWORDS: In vitro fermentation; gas production; fructans; GOS; Polydextrose; degree of polymerization; prebiotic

INTRODUCTION

The human gut microbiota plays a major role in host health, and there is much interest in its manipulation toward a potentially more remedial community (*1*). Dietary intervention with prebiotics has been shown to selectively stimulate the growth and/or activity of one or a limited number of intestinal bacteria associated with health and well-being, such as bifidobacteria and lactobacilli (*1, 2*). Among the nondigestible food ingredients studied for their prebiotic potential, fructooligosaccharides (FOS; oligofructose and inulin) and galactooligosaccharides (GOS) are widely accepted as prebiotics, a fact supported in many human trials (*3*−*6*).

In addition to their selective stimulation of bacterial growth in the large intestine, prebiotics influence many aspects of bowel function through fermentation. Short-chain fatty acids are a major product of prebiotic breakdown, but gases are also inevitable products of fermentation that represent the major discouragement to consumption of prebiotics. Several human studies reported meteorism (production of copious amounts of gas), bloating, and loose stool occurring with ingestion of large doses of prebiotics (*7*). Some in vitro studies showed that inulin resulted in the highest amount of gas production compared with FOS, polydextrose, GOS, and isomaltooligosaccharides (*8, 9*). Such unwanted symptoms raised questions about the feasibility of daily use of certain prebiotics and, therefore, more recent research has focused on assessing these side effects (*10*).

Responses to fermentation of prebiotics are affected by individual variation as well as by the chemical structure of the specific carbohydrates (*7*). Both gas production and prebiotic properties of the carbohydrates are likely to be influenced by the monosaccharide composition (prebiotics are primarily composed of glucose, fructose, galactose, and xylose), the glycosidic linkages between the monosaccharide residues, and the degree of polymerization (DP) of the prebiotic (*7, 11*). Several studies suggested that substances with longer chain lengths (DP > 10) were fermented more slowly, with less gas production, and with less bifidogenic capacities than short-chain carbohydrates (DP < 10) (*11*−*13*).

Humans are interested in experiencing the intestinal health benefits of prebiotics, but with minimal gas production. This study examined the in vitro fermentation profile, prebiotic potential, and gas production capacity of certain fructans varying in chain length, GOS, and polydextrose. The objective of this

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Table 1. Composition of Microbiological Medium Used in in Vitro Experiments

component	concentration in medium, mL/L
solution A[a]	330.0
solution B[b]	330.0
trace mineral solution[c]	10.0
water-soluble vitamin solution[d]	20.0
folate:biotin solution[e]	5.0
riboflavin solution[f]	5.0
hemin solution[g]	2.5
short-chain fatty acid mix[h]	0.4
resazurin[i]	1.0
distilled H_2O	296.1
Na_2CO_3	4.0
cysteine HCl−H_2O	0.5
trypticase	0.5
yeast extract	0.5

[a] Composition (g/L): NaCl, 5.4; KH_2PO_4, 2.7; $CaCl_2-H_2O$, 0.16; $MgCl_2-6H_2O$, 0.12; $MnCl_2-4H_2O$, 0.06; $CoCl_2-6H_2O$, 0.06; $(NH_4)_2SO_4$, 5.4. [b] Composition (g/L): K_2HPO_4, 2.7. [c] Composition (mg/L): ethylenediaminetetraacetic acid (disodium salt), 500; $FeSO_4-7H_2O$, 200; $ZnSO_4-7H_2O$, 10; $MnCl_2-4H_2O$, 3; H_3PO_4, 30; $CoCl_2-6H_2O$, 20; $CuCl_2-2H_2O$, 1; $NiCl_2-6H_2O$, 2; $Na_2MoO_4-2H_2O$, 3. [d] Composition (mg/L): thiamin-HCl, 100; D-pantothenic acid, 100; niacin, 100; pyridoxine, 100; *p*-aminobenzoic acid, 5; vitamin B_{12}, 0.25. [e] Composition (mg/L): folic acid, 10; D-biotin, 2; NH_4HCO_3, 100. [f] Composition: riboflavin, 10 mg/mL in 5 mmol/L of Hepes. [g] Composition: hemin, 500 mg/mL in 10 mmol/L of NaOH. [h] Composition: 250 mL/L each of *n*-valerate, isovalerate, isobutyrate, and DL-α-methylbutyrate. [i] Composition: resazurin, 1 g/L in distilled H_2O.

experiment was to determine those oligosaccharides that produce the least gas but with preservation of SCFA production and microbiota enhancement properties.

MATERIALS AND METHODS

Substrates. Substrates used in this study were as follows: (A) fructans: short-chain FOS (NutraFlora, GTC Nutrition, Golden, CO), medium-chain FOS (Beneo P95 (Orafti, Oreye, Belgium), and Frutalose L90 (Sensus, Monmouth Junction, NJ)), long-chain FOS (inulin: Beneo ST and Beneo HP (Orafti), Oliggo-Fiber (Cargill, Minneapolis, MN), and agave inulin (Nekutli, Guadalajara, Mexico)); (B) oligofructose-enriched inulin (Beneo Synergy 1 (Orafti) and Frutafit HD (Sensus)); (C) GOS (Vivinal GOS, Friesland Foods Domo, Zwolle, The Netherlands); (D) polydextrose (STA-LITE, Tate & Lyle, Decatur, IL). Pectin (pectin HM rapid, Tic Gums, Belcamp, MD) was used as a standard in this in vitro fermentation experiment. Dry matter (DM) and organic matter (OM) concentrations of all substrates were analyzed using Association of Official Analytical Chemists (AOAC) methods (*14*). Substrates were analyzed in duplicate and an error between duplicate samples ≤5% was considered acceptable.

Donors and Collection Method. Three human fecal samples, from male volunteers, were pooled to serve as the source of inoculum for the in vitro fermentation experiment. All donors consumed their normal diet, were greater than 18 yr old, were free of gastrointestinal disease, and had not received antibiotics at least three months prior to or during the study. The experimental protocol was approved by the University of Illinois at Urbana−Champaign Institutional Review Board, and all subjects signed an informed consent prior to initiation of the experiment.

On the morning of the experiment, each donor provided a fresh fecal sample, collected using a Commode Specimen Collection System (Sage Products, Crystal Lake, IL). Samples were brought to the laboratory within 15 min of defecation to ensure viability of microbial populations.

In Vitro Fermentation Model: Gas Production. For each pull time (4, 8, and 12 h fermentation), 115 mg of substrate were weighed in triplicate into 16 mL Balch tubes that were used in a model that simulated large bowel fermentation (*15*). The composition of the in vitro medium is presented in **Table 1**. All components except vitamin and SCFA mixes were added before autoclave sterilization of the medium. Filter-sterilized vitamin solutions were added just before dispensing the medium that was maintained under anaerobic conditions at all times after preparation. An aliquot (10 mL) of medium was aseptically transferred into the appropriate Balch tubes, capped with butyl rubber stoppers, and sealed with aluminum caps. All tubes were stored at 4 °C for approximately 12 h to enable hydration of the substrates before initiating fermentations. Tubes were placed in a 37 °C water bath approximately 30 min before inoculation.

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 (wt/vol) in anaerobic dilution solution (*16*) 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 blank tubes were aseptically inoculated with 1.5 mL of diluted feces. Tubes were incubated at 37 °C with periodic mixing for 4, 8, and 12 h. At the appropriate time, tubes were removed from the 37 °C incubator and processed immediately for analyses. First, gas production was determined by fluid displacement (water with 5% HCl and resazurin) at equal pressure using a manometer (*17*). Corrections were made for temperature, pressure, and headspace contained in the Balch tube prior to the initiation of fermentation. Gas production (mL) was calculated as gas produced from the substrate minus gas produced from the blank divided by original sample weight expressed on a DM basis. A 1 mL aliquot was taken with a gastight syringe for gas composition (CH_4 and H_2) on a Quintron gas analyzer. The pH of tube contents was measured with a standard pH meter (Denver Instrument Co., Arvada, CO). Then, a 2 mL subsample was taken from each tube for SCFA and lactate analyses. Finally, a 2 mL subsample was taken and frozen at −80 °C for bacterial analyses.

Short-Chain Fatty Acid and Lactate Analyses. The 2 mL aliquot of fluid removed from the sample tubes for SCFA and lactate analyses was immediately added to 0.5 mL of 25% metaphosphoric acid, precipitated for 30 min, and centrifuged at 20000*g* for 20 min. The supernatant was decanted and frozen at −20 °C in microfuge tubes. After freezing, the supernatant was thawed and centrifuged in microfuge tubes at 10000*g* for 10 min. Concentrations of acetate, propionate, and butyrate were determined in the supernatant using a Hewlett-Packard 5890A series II gas chromatograph (Palo Alto, CA) and a glass column (180 cm × 4 mm id) packed with 10% SP-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Oven temperature, detector temperature, and injector temperature were 125, 175, and 180 °C. Short-chain fatty acid concentration values also were corrected for blank tube production of SCFA. The supernatants were analyzed for lactate concentration by the spectrophotometric method described by Barker and Summerson (*18*). All samples were run in duplicate and an error ≤5% was considered acceptable.

Polymerase Chain Reaction. Microbial populations were measured by DNA extraction from fermented samples, followed by quantitative PCR (qPCR). Deoxyribonucleic acid was extracted from frozen samples using the repeated bead beating plus column (RBB+C) method described by Yu and Morrison (*19*). Briefly, cells were lysed by employing bead beating in the presence of high concentrations of sodium dodecyl sulfate (SDS), salt, and EDTA. Then, DNA was purified using QIAamp columns (QIAamp DNA stool mini kit, Qiagen, Valencia, CA). Extracted DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Nano-Drop Technologies, Wilmington, DE).

Quantitative PCR was performed for bifidobacteria, lactobacilli, and *Escherichia coli* genera, as well as *Clostridium perfringens* (*20*). Specific primers were used for bifidobacteria (*21*), lactobacilli (*22*), *E. coli* (*23*), and *C. perfringens* (*24*). Amplification was performed according to DePlanke et al. (*25*). Briefly, a 10 μL final volume contained 5 μL 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 logarithmic 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 fecal DNA to create triplicate standard curves using an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City,

CA). Colony forming units in each dilution were determined by plating in triplicate on specific agars; lactobacilli MRS (Difco, Detroit, MI) for lactobacilli, reinforced clostridial medium (bifidobacteria, *C. perfringens*), and Luria−Bertani 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/tube.

Statistical Analysis. Data were analyzed as a randomized complete block design using the Proc Mixed procedure of SAS (SAS Inst., Inc., Cary, NC). Treatments included substrate and length of fermentation. Therefore, substrate, time, and substrate × time were used in the statistical model. The general linear model (GLM) procedures of SAS were used to analyze data from this experiment. Least squares means were reported along with the pooled SEM for all response criteria. When significant ($P < 0.05$) differences were detected, individual means were compared using the least significant difference method of SAS (*26*).

The data for gas and SCFA production were fitted to a logistic model equation to determine the rate of production and the time to attain maximal rate of production for each substrate. This function is frequently used to model biological and, especially, microbial growth (*27*). It is a sigmoidal curve that can describe accelerating and, after passing through an inflection point, decelerating phases of growth (*28*). The time at which maximal rate of gas or SCFA production occurred was calculated according to the following equation:

$$Y = A/(1 + e^{-(t-C)/B}) \quad (1)$$

where Y = gas or SCFA production, A = asymptote, or maximal gas or SCFA production, t = incubation time in hours, C = time in hours at which the rate of gas or SCFA production is maximum (the inflection point), and B = a measure of the duration of gas or SCFA production expressed in milliliters and milligrams per gram of DM, respectively.

Variables (A, B, and C) were estimated for each substrate using nonlinear regression (NLREG; (*29*)). The model explained 93% or more of the variation in gas and SCFA production in all cases.

Additionally, maximal rates of gas and SCFA production were estimated using the derivative of the logistic function according to the following equation:

$$(A \times e^{(C+t)/B})/(B \times [e^{(C/B)} + e^{(t/B)}]^2) = dY/dt \quad (2)$$

where A = asymptote, or maximal gas or SCFA production, t = time in hours, C = time in hours at which maximal rate of gas or SCFA production occurs, and B = measure of the duration of gas or SCFA production expressed as milliliters and milligrams per gram of DM, respectively.

Maximal rate and time to attain maximal rate of production values were obtained from logistic model equations fit to values obtained from a single mixed inoculum; therefore, only one estimate per substrate was obtained and these parameters could not be compared in a classical statistical manner.

RESULTS AND DISCUSSION

Chemical Composition. Dry matter (DM) concentrations were higher in all Beneo substrates (97.1−97.8%). The lowest DM concentration was found in the Frutalose L90 (76.1%), followed by the Vivinal GOS (78.0%) and the pectin standard (92.2%). All substrates had an OM concentration very close to 100% on a DM basis. The pectin standard had the lowest OM concentration (98.4% DM basis) (data not shown).

Gas Production. Total gas production data, rate of gas production, and time to attain maximal rate of gas production are presented in **Table 2**. Except for STA-LITE polydextrose, all substrates generated some gas after 4 h of fermentation. Despite the greatest amount of gas generated at 4 h, Frutalose L90 fermentation did not follow a logistical model for gas production; therefore, rate of production of gas and time to attain maximal rate of gas production could not be calculated. At all time points, total gas and H_2 production (**Table 3**) was lowest ($P < 0.05$) for STA-LITE polydextrose, followed by two long-chain FOS, Oligo-Fiber, and Beneo HP. Previous studies have shown that the nature of the glycosidic bonds in polydextrose renders it only partially fermented by intestinal microflora and

Table 2. Gas Production, Rate of Gas Production, and Time to Attain Maximal Rate of Gas Production for Select Fructans, Galactooligosaccharides, Polydextrose, and Pectin Fermented with Human Fecal Microflora

	gas production (mL/g DM)			rate of gas production	time to attain maximal rate of
substrate[a]	4 h	8 h	12 h	(mL/g DM·h^{-1})	gas production (h)
			Fructans		
Nutraflora	120.2	134.2	147.6	73.2	3.2
Beneo P95	120.7	130.2	140.5	81.2	3.1
Frutalose L90[b]	148.1	137.4	142.8		
Oliggo-Fiber	49.3	96.5	115.0	17.2	4.6
Beneo HP	46.8	88.6	110.3	14.9	4.8
Beneo ST	82.6	126.8	127.4	62.8	3.7
agave inulin	52.2	143.3	148.0	38.7	4.6
Beneo Synergy 1	104.6	127.2	145.6	53.3	3.2
Frutafit HD	103.5	131.2	147.3	52.4	3.3
			Others		
Vivinal GOS	110.0	114.2	126.2	79.3	3.1
Sta-Lite polydextrose	0.6	63.3	90.2	33.3	7.4
			Standard		
pectin HM rapid	89.1	111.0	117.9	51.9	3.3
SEM[c]		6.54			

[a] The interaction of substrate and time of fermentation for gas production resulted in a P-value <0.0001. [b] Frutalose L90 fermentation did not follow a logistic model; therefore, rate of gas production and time to attain maximal rate of gas production could not be determined. [c] Standard error of the mean.

Table 3. Average Production of Hydrogen (H_2) and Methane (CH_4) after 4, 8, and 12 h of in Vitro Fermentation of Select Fructans, Galactooligosaccharides, Polydextrose, and Pectin Using Human Fecal Microflora

	H_2			CH_4		
substrate[a]	4 h mg/g of DM	8 h mg/g of DM	12 h mg/g of DM	4 h mg/g of DM	8 h mg/g of DM	12 h mg/g of DM
			Fructans			
Nutraflora	3.9	3.4	7.8	0.0	1.6	2.2
Beneo P95	4.9	6.8	8.0	2.1	1.6	2.8
Frutalose L90	5.3	5.3	6.0	2.3	2.2	3.2
Oliggo-Fiber	0.8	1.8	2.8	0.7	2.2	3.2
Beneo HP	0.5	1.0	2.2	0.5	2.2	3.0
Beneo ST	3.2	6.1	7.6	1.6	1.6	3.2
agave inulin	0.9	6.3	7.2	1.1	2.2	3.0
Beneo Synergy 1	2.7	5.4	7.0	1.1	2.1	2.7
Frutafit HD	2.8	5.9	7.8	1.5	2.1	2.6
			Others			
Vivinal GOS	3.9	4.1	5.0	0.0	2.4	2.5
Sta-Lite polydextrose	0.03	0.5	1.1	0.1	1.5	2.9
			Standard			
pectin HM rapid	0.1	1.0	1.9	0.9	2.3	3.2
SEM[b]		0.4			0.3	

[a] The interaction of substrate and time of fermentation for hydrogen and methane production resulted in a P-value <0.0001 and $P = 0.0007$, respectively. [b] Standard error of the mean.

resistant to enzymatic attack (*30*, *31*). Compared with highly fermentable fructans, this explains the low gas production after fermentation of STA-LITE polydextrose. Moreover, polydextrose resulted in the lowest increase in breath hydrogen excretion compared with lactitol and isomalt when given in equal doses to healthy subjects (*12*). When comparing all long-chain fructans, Oligo-Fiber and Beneo HP had lower total gas and H_2 productions than Beneo ST and agave inulin at all time points. Also, they had the lowest rates of gas production and the longest times to attain maximal rate of gas production among fructans. Beneo ST contains 90% inulin with a DP ranging between 10 and 60, and Beneo HP contains 100% inulin with a DP > 23. In view of the influence of chain length on fermentation rate, we hypothesize that Oligo-Fiber and Beneo HP would contain more fructans of longer chain-length than Beneo ST and agave inulin.

At 8 and 12 h of fermentation, the highest ($P < 0.05$) gas volume occurred for agave inulin followed by the short- and medium-chain FOS (Nutraflora, Beneo P95, Frutalose L90) and the oligofructose-enriched inulin substrates (Frutafit HD, Beneo S1). Particularly at 12 h, these substrates produced similar ($P > 0.05$) volumes of gas (>140 mL/g DM). Agave inulin is a branched-type fructan with a DP ranging between 10 and 60. Because of its specific molecular geometry, its structure is more accessible to enzymes and is easily fermented compared with the other long-chain fructan compounds tested in this study. This explains why agave inulin had the highest gas production observed among substrates and had similar gas production values as the short- and medium-chain fructans. Smiricky-Tjardes et al. (*32*) showed that, using swine fecal inoculum, short-, medium-, and long-chain fructans produced similar amounts of gas at similar rates when compared to each other. At all time points, fructans were followed closely by two different trans-galactooligosaccharides that had similar rates of gas production and times to attain maximal rate of gas production. In our study, we found a similar pattern, with the Vivinal GOS producing moderate amounts of gas (less than most of the fructans) and having a similar rate of gas production as short- and medium-chain fructans (Nutraflora scFOS and Beneo P95) (average; 78 $mL \cdot g^{-1} \cdot h^{-1}$). Several studies suggested that compounds with longer chain lengths (DP > 10) are fermented more slowly, with less gas production than short-chain carbohydrates (DP < 10) (*7*, *12*). In the present study, the highest rates of gas production were observed with Nutraflora scFOS, Beneo P95, and Vivinal GOS. Nutraflora contains 95% FOS with a DP ranging between 2 and 5 (GTC Nutrition, Golden, CO), and Beneo P95 contains 95% oligosaccharides with a DP < 8 and 5% monomers (glucose/fructose/sucrose) (Orafti, Oreye, Belgium). Amaretti et al. (*33*) reported that Vivinal GOS contains 18.8% glucose, 3.5% galactose, 19.4% lactose, and 58.3% GOS, with a DP ranging between 3 and 9. Thus, our results confirmed that carbohydrates with a DP < 10 are fermented more quickly than longer chain compounds (*34*). Moreover, the highest H_2 productions were found with Nutraflora scFOS and Beneo P95 compared to long-chain FOS, supporting previous in vitro and in vivo reports that suggested less net hydrogen excretion after long-chain carbohydrate fermentation (*7*). After 8 and 12 h of fermentation, the greatest H_2 production ($P < 0.05$) occurred for Beneo P95. In general, the greatest amounts of H_2 produced at 8 and 12 h occurred with the short- and medium-chain FOS, the two long-chain FOS, Beneo ST and agave inulin, and the two oligofructose-enriched inulin products (Beneo S1 and Frutafit HD) (≥7 mg/g DM at 12 h). Both at 8 and 12 h, all substrates produced a similar ($P > 0.05$) amount of CH_4.

Table 4. Initial pH and pH Change after 4, 8, and 12 h of in Vitro Fermentation of Select Fructans, Galactooligosaccharides, Polydextrose, and Pectin with Human Fecal Microflora

		pH change		
substrate[a]	initial pH	4 h	8 h	12 h
	Fructans			
Nutraflora	6.52	−1.20	−1.41	−1.32
Beneo P95	6.47	−1.29	−1.35	−1.23
Frutalose L90	6.50	−1.02	−1.03	−0.96
Oliggo-Fiber	6.53	−0.47	−0.91	−0.95
Beneo HP	6.51	−0.45	−0.85	−0.90
Beneo ST	6.47	−0.92	−1.23	−1.24
agave inulin	6.48	−0.90	−1.26	−1.19
Beneo Synergy 1	6.48	−0.88	−1.25	−1.21
Frutafit HD	6.52	−0.90	−1.26	−1.21
	Others			
Vivinal GOS	6.51	−0.84	−0.96	−0.96
Sta-Lite polydextrose	6.53	−0.33	−0.72	−0.91
	Standard			
pectin HM rapid	6.32	−0.76	−1.04	−0.99
SEM[b]			0.02	

[a] The interaction of substrate and time of fermentation for pH change resulted in a *P*-value <0.0001. [b] Standard error of the mean.

The two oligofructose-enriched inulin substrates produced approximately the same amounts of gas as the short- and medium-chain FOS but more gas than most of the long-chain FOS after 12 h of fermentation. Moreover, Beneo S1 and Frutafit HD had intermediate rates of gas production as compared to both short- and long-chain fructans. These results suggest that gas production and rate of gas production resulting from short-chain fructan fermentation could be attenuated by mixing with longer chain carbohydrates.

Changes in pH. Table 4 presents the pH change after 4, 8, and 12 h of in vitro fermentation. Generally, the pH decrease was greater at 8 h for most of the substrates. Except for Frutalose L90, Oliggo-Fiber, and Beneo HP, fructans fermentation resulted in the greatest pH decrease compared to Vivinal GOS and STA-LITE polydextrose. Flickinger et al. (*35*) reported lower pH values and a more rapid decline in pH with FOS compared to GOS after in vitro fermentation with canine fecal inoculum. In the present study, Nutraflora resulted in the greatest decrease in pH ($P < 0.02$) both at 8 and 12 h, followed by the medium-chain FOS, Beneo P95, suggesting that these substrates were the most rapidly fermented among those tested. These results are in accordance with the highest rates of gas production found for these two substrates. After 8 and 12 h of in vitro fermentation, the smallest decreases in pH were observed with the medium-chain FOS, Frutalose L90, the two long-chain FOS, Oliggo-Fiber and Beneo HP, the Vivinal GOS, and the STA-LITE polydextrose, which were not significantly different from each other.

SCFA Production. When data were pooled across substrates, the interaction of substrate and time was significant ($P < 0.0001$) for acetate, propionate, butyrate, total SCFA, and lactate production (**Table 5**). Beneo HP fermentation produced the lowest amount of total SCFA ($P < 0.0001$), followed by STA-LITE polydextrose and Oliggo-Fiber. These three substrates also were among the lowest acetate and propionate producers and had lower rates of SCFA production and attained a maximal rate of SCFA production later in time when compared to other

Table 5. Acetate, Propionate, Butyrate, Total Short-Chain Fatty Acid (SCFA), and Lactate Production Following 4, 8, and 12 h of in Vitro Fermentation of Select Fructans, Galactooligosaccharides, Polydextrose, and Pectin Using Human Fecal Microflora

	acetate			propionate			butyrate			total SCFA			lactate		
substrate[a]	4 h, mg/g of DM in sample	8 h, mg/g of DM in sample	12 h, mg/g of DM in sample	4 h, mg/g of DM in sample	8 h, mg/g of DM in sample	12 h, mg/g of DM in sample	4 h, mg/g of DM in sample	8 h, mg/g of DM in sample	12 h, mg/g of DM in sample	4 h, mg/g of DM in sample	8 h, mg/g of DM in sample	12 h, mg/g of DM in sample	4 h, mg/g of DM in sample	8 h, mg/g of DM in sample	12 h, mg/g of DM in sample
							Fructans								
Nutraflora	201	242	265	62	74	77	66	139	152	329	455	495	18	3	0
Beneo P95	232	258	263	55	65	67	73	144	149	360	468	479	22	0	0
Frutalose L90	246	254	278	61	68	71	77	128	141	384	450	490	15	0	0
Oliggo-Fiber	104	157	169	26	48	51	50	131	163	181	335	384	2	0	0
Beneo HP	107	155	141	28	45	51	48	122	144	183	322	337	1	0	0
Beneo ST	174	183	192	43	55	57	68	179	197	285	417	446	9	1	0
agave inulin	173	226	229	43	52	52	72	163	172	287	441	452	15	0	0
Beneo Synergy 1	162	196	187	47	60	63	60	176	185	269	432	435	9	0	0
Frutafit HD	170	190	187	43	56	57	59	178	194	272	424	438	11	0	0
							Others								
Vivinal GOS	234	282	278	62	72	74	72	121	137	368	475	489	12	0	0
Sta-Lite polydextrose	121	210	249	28	56	71	28	41	50	176	307	369	0	0	0
							Standard								
pectin HM rapid	196	324	318	36	48	51	34	71	85	265	443	455	0	0	0
SEM[b]		4.9			1.2			2.3			6.4			0.7	

[a] The interaction of substrate and time of fermentation for acetate, propionate, butyrate, total SCFA, and lactate production resulted in a *P*-value <0.0001 for each variable. [b] Standard error of the mean.

Table 6. Rate of Production and Time to Attain Maximal Rate of Production of Acetate, Propionate, and Butyrate as a Result of in Vitro Fermentation of Select Fructans, Galactooligosaccharides, Polydextrose, and Pectin with Human Fecal Microflora

	rate of production (mg/g DM·h^{-1})			time to attain maximal rate of production (h)		
substrate	acetate	propionate	butyrate	acetate	propionate	butyrate
			Fructans			
Nutraflora	112.4	39.4	29.4	3.2	3.3	4.3
Beneo P95	178.3	38.3	42.0	3.2	3.3	4.0
Frutalose L90	178.0	42.2	33.0	3.1	3.2	3.7
Oliggo-Fiber	50.8	11.9	23.8	3.5	3.9	5.6
Beneo HP	334.0	10.9	22.7	3.9	3.7	5.2
Beneo ST	133.8	27.8	37.5	3.1	3.4	4.9
agave inulin	123.0	81.5	39.7	3.5	3.8	4.4
Beneo Synergy 1	423.7	33.0	43.9	3.8	3.4	4.8
Frutafit HD	410.9	29.7	39.7	3.7	3.5	5.0
			Others			
Vivinal GOS	697.9	44.6	28.5	3.8	3.3	3.8
Sta-Lite polydextrose	40.4	9.4	9.8	4.1	5.1	3.6
			Standard			
pectin HM rapid	741.5	19.5	12.3	4.0	3.4	4.9

substrates (**Table 6**). This confirmed the slower fermentation rate of long-chain compared to short-chain fructans (*34*). Moreover, fermentation of STA-LITE polydextrose and pectin resulted in the lowest production of butyrate ($P < 0.0001$) at all time periods. Studies in rats and humans showed that polydextrose is partially fermented in the large intestine (*30, 31*), and Wang and Gibson (*36*) reported a lower SCFA production after polydextrose fermentation compared with inulin and oligofructose. The authors reported a molar ratio of acetate: propionate:butyrate of 61:25:14 for polydextrose; our results showed a similar ratio for STA-LITE polydextrose (67:19:14). Short-chain fatty acid data obtained for Oliggo-Fiber and Beneo HP were in accordance with gas production and pH change data and suggested that these two long-chain FOS compounds are slowly fermented and did not reach their potential for maximal gas or SCFA production at 12 h of fermentation. Rycroft et al. (*9*) and Ghoddusi et al. (*8*) showed that an inulin similar to Beneo HP produced more gas than scFOS after 24 and 32 h of in vitro fermentation, respectively. This supports the proposition that Oliggo-Fiber and Beneo HP were the longest chain molecules tested in the present study and, in contrast to short-chain fructans, were not completely fermented at 12 h but can produce more fermentation products than scFOS if given adequate time (i.e., 32 h of fermentation). Fermentation of the short- and medium-chain FOS (Nutraflora, Beneo P95, and Frutalose L90) and the Vivinal GOS resulted in the highest ($P < 0.05$) production of total SCFA at all time points (>479 mg/g DM at 12 h), and were among the greatest acetate and propionate producers. Only these four substrates produced more total SCFA than pectin after 8 and 12 h of in vitro fermentation. Moreover, Vivinal GOS in particular had the highest rate of acetate production. At 8 and 12 h, butyrate production was highest ($P < 0.02$) for Beneo ST and Frutafit HD, followed by Beneo S1 and agave inulin. Fermentation of these four substrates resulted in similar ($P > 0.05$) intermediate concentrations of total SCFA (average; 443 mg/g DM). When considering all fructans, the SCFA concentration pattern correlated well with the gas production values. From these results, short- and medium-chain FOS are more fermentable than long-chain FOS. Wang and Gibson (*36*) reported a molar ratio of acetate:propionate:butyrate of 72:19:8 for inulin and 78:14:8 for oligofructose after 24 h of in vitro fermentation. We found different molar ratios at 12 h, notably a higher proportion of butyrate (55:15:30 and 45:13:42, respectively, for the short-/medium-chain and long-chain fructans). These variations may be due to disparities in the molecular structure of substrates used and to the difference in fermentation times. These data suggest that fructans switch their acetate to butyrate production ratio between 12 and 32 h of in vitro fermentation. On average, the two oligofructose-enriched inulins (Beneo S1 and Frutafit HD) decreased production of acetate, propionate, and total SCFA by 30%, 17%, and 10%, respectively, compared with short- and medium-chain FOS at 12 h. Mixing short- and long-chain FOS together decreased rate of production of propionate by 22% and increased rate of production of acetate and butyrate by 167% and 20%, respectively, compared with short- and medium-chain FOS at 12 h. This can explain the higher ($P < 0.05$) butyrate concentrations observed at 8 and 12 h with Beneo S1 and Frutafit HD compared with the short- and medium-chain FOS. Along with the reduction in rate of gas production observed with these two products, our results suggest that the fermentation of short-chain fructans can be attenuated by mixing with longer chain molecules.

Finally, in agreement with Wang and Gibson (*36*), no lactate production was noted for STA-LITE polydextrose and pectin at any time point. Generally speaking, we did not detect any lactate at 8 h of fermentation for most of the substrates, and 12 h for all substrates. Any lactate produced can be rapidly processed by bacteria and not be present in tubes in sufficient amounts to be detected. Nevertheless, this is not in accordance with Wang and Gibson (*36*), who reported some lactate production with inulin and oligofructose after 24 h of fermentation. However, in the present study, lactate concentrations observed at 4 h were higher ($P < 0.0005$) for short/medium chain fructans compared with the long-chain molecules, as reported by Wang and Gibson (*36*), after 24 h.

Changes in Bacterial Populations. At each time point, bacterial populations were measured using qRT-PCR (**Table 7**). This is a culture-independent procedure that allows bacterial enumeration in environmental samples. This technique has been reported to be reliable and as specific as the FISH procedure, a common technique to quantify bacteria in biological samples

Table 7. Bacterial Population (log_{10} cfu/tube) in Batch Culture Fermentation with Select Fructans, Galactooligosaccharides, Polydextrose, and Pectin Immediately after Inoculation (0 h)[a], and after 4, 8, and 12 h Fermentation with Human Fecal Inoculum

	bifidobacteria			lactobacilli			*Escherichia coli*		
substrate[b]	4 h	8 h	12 h	4 h	8 h	12 h	4 h	8 h	12 h
Fructans									
Nutraflora	8.7	8.6	8.5	9.5	9.5	9.2	8.6	8.6	8.4
Beneo P95	8.8	8.4	8.5	9.6	9.4	8.6	8.7	8.4	8.3
Frutalose L90	8.6	8.1	8.4	9.5	9.4	9.2	8.7	8.5	8.3
Oliggo-Fiber	7.6	7.8	7.4	9.5	9.6	9.3	8.6	8.5	8.3
Beneo HP	7.8	7.6	7.3	9.4	9.6	9.4	8.6	8.5	8.4
Beneo ST	8.3	8.1	8.0	9.5	9.5	9.3	8.6	8.4	8.4
agave inulin	8.0	8.0	7.6	9.5	9.7	9.3	8.7	8.6	8.3
Beneo Synergy 1	8.6	8.5	8.3	9.6	9.7	9.3	8.6	8.5	8.4
Frutafit HD	8.3	8.2	8.1	9.5	9.6	9.2	8.7	8.6	8.3
Others									
Vivinal GOS	8.6	8.5	8.5	9.4	9.3	9.1	8.7	8.7	8.4
Sta-Lite polydextrose	8.2	8.2	7.9	9.2	9.3	8.9	8.7	8.5	8.3
Standard									
pectin HM rapid	8.3	8.2	7.9	9.3	9.5	9.1	8.6	8.6	8.3
SEM[c]		0.08			0.11			0.04	

[a] Mean values ± SD for the 0 h tubes are 7.4 ± 0.3 for bifidobacteria, 8.6 ± 0.2 for lactobacilli, and 8.4 ± 0.2 log_{10} cfu /tube for *Escherichia coli.* [b] The interaction of substrate and time of fermentation for bifidobacteria, lactobacilli, and *E. coli* resulted in a *P*-value = 0.0208, 0.2171, and 0.0024, respectively. [c] Standard error of the mean.

(*3*). In the present study, no *C. perfringens* was detected in the tubes at any time period for any of the substrates. The probe used has been successfully utilized in our laboratory to detect *C. perfringens* in fecal samples in previous experiments but, even in fecal samples, bacterial counts can be too low for adequate quantification. Perhaps there were low *C. perfringens* concentrations in the original fecal samples used to prepare the inoculum (interindividual variation).

All substrates had similar ($P > 0.05$) counts for each bacterial population measured at 0 h (7.4 ± 0.3, 8.6 ± 0.2, and 8.4 ± 0.2 log_{10} cfu/tube, respectively, for bifidobacteria, lactobacilli, and *E. coli*). In general, substrates tested in the present study increased ($P < 0.02$) bifidobacteria, lactobacilli, and *E. coli* populations after 4 h of fermentation. This increase did not continue over time and, for all bacteria populations measured, the 12 h values were equal to or lower than the 4 h values. When considering the 12 h values, fermentation of all substrates, except Oliggo-Fiber, Beneo HP, and agave inulin, resulted in an increase ($P < 0.0001$) in bifidobacteria populations compared to the 0 h values. Even low fermented substrates, such as polydextrose, increased bifidobacteria as reported before (*37*). Except for Beneo P95, STA-LITE polydextrose, and pectin, fermentation of all substrates resulted in a similar ($P > 0.05$) increase in the number of lactobacilli ($P < 0.05$) after 12 h. Finally, after 12 h of fermentation, no increase in *E. coli* was observed compared to the 0 h value. Our results are in accordance with the prebiotic effect of fructans and GOS reported in several studies (*3*−*6*). It has been shown that prebiotic properties are likely to be influenced by the monosaccharide composition, the glycosidic linkage between the monosaccharide residues, and the DP of the prebiotic (*11*). Roberfroid et al. (*13*) reported that the ability to stimulate growth of bifidobacteria by short-chain carbohydrates (DP between 2 and 10) appears to be almost an order of magnitude higher than that of higher DP substrates (DP > 10). In the present study, the greatest increase in bifidobacteria ($P < 0.05$) was observed with Vivinal GOS, Beneo P95, and Nutraflora scFOS (all oligosaccharides with a DP < 10). The long-chain fructans increased bifidobacteria to a lesser extent than short-chain fructans at 4 h, and no differences were observed in bifidobacteria populations between 0 and 12 h. The oligofructose-enriched inulin products (Beneo S1 and Frutafit HD) had intermediate bifidobacteria numbers as compared to both short- and long-chain fructans. Nearly all substrates increased lactobacilli populations after 12 h of fermentation but to a lesser extent than the increase in bifidobacteria, as reported by Ghoddusi et al. (*8*). These authors also showed that FOS and FOS + inulin gave rise to the greatest increase in bifidobacteria populations among substrates. Along with our results, this reveals that mixing short- and long-chain FOS still produced more bifidobacteria than the long-chain inulin alone. After 12 h of fermentation, no increase in *E. coli* was observed compared to the 0 h values and, particularly, most of the substrates significantly decreased *E. coli* populations. This could be associated with the prebiotic effect of fructans but, to the best of our knowledge, *E. coli* populations have not been measured in in vitro experiments aiming to determine the prebiotic potential of substrates in humans. Several canine studies reported *E. coli* in feces after dogs were fed oligosaccharides (i.e., FOS and mannanoligosaccharides), but no changes in *E. coli* populations were found (*38*, *39*).

The type of oligosaccharide fermented influences the nature of the fermentative end-products and their ability to modulate the gut microbiota. Higher amounts of gas and SCFA production indicate faster rates of production and earlier times to attain maximal rates of production. In our study, this was the case for the short-chain and medium-chain oligosaccharides (Nutraflora, Beneo P95, Frutalose L90, and Vivinal GOS). Moreover, these compounds had the highest bifidogenic capacities among substrates. Lower amounts of gas and SCFA production were indicative of slower rates of production and longer times to attain maximal rates of production. This concerned mostly the long-chain fructans and STA-LITE polydextrose. Nevertheless, among long-chain fructans, Oliggo-Fiber and Beneo HP behave differently and were much more slowly fermented. We hypothesize that these substrates may contain more fructans of longer chain-length than Beneo ST and agave inulin. Moreover, our results revealed that mixing short- and long-chain oligosaccharides attenuated the fermentation rate and fermentability of short-chain substrates. This could be of interest, particularly because of the gas production capacity of short-chain oligosaccharides. For the same reasons, Beneo ST and Vivinal GOS appeared to be of particular interest as they produce large quantities SCFA, particularly butyrate, and only moderate amounts of gas. In vitro fermentation profiles for oligosaccharides appear to be useful for predicting fermentation patterns in vivo. Therefore, the results presented in the present study and our conclusions do not take into account the adaptation of human microflora after a long-term consumption of prebiotics.

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