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In Vitro Fermentation by Human Fecal Microflora of Wheat Arabinoxylans

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The fermentation of three arabinoxylan (AX) fractions from wheat by the human fecal microflora was investigated in vitro. Three AX fractions, with average molecular masses of 354, 278, and 66 kDa, were incorporated into miniature-scale batch cultures (with inulin as a positive prebiotic control) with feces from three healthy donors, aged 23–29. Microflora changes were monitored by the culture-independent technique, fluorescent in situ hybridization, and short chain fatty acid (SCFA) and lactic acid production were measured by high-performance liquid chromatography. Total cell numbers increased significantly in all treated cultures, and the fermentation of AX was associated with a proliferation of the bifidobacteria, lactobacilli, and eubacteria groups. Smaller but statistically significant increases in bacteroides and clostridia groups were also observed. All AX fractions had comparable bifidogenic impacts on the microflora at 5 and 12 h, but the 66 kDa AX was particularly selective for lactobacilli. Eubacteria increased significantly on all AX fractions, particularly on 66 kDa AX. As previously reported, inulin gave a selective increase in bifidobacteria. All supplemented cultures showed significant rises in total SCFA production, with a particularly high proportion of butyric acid being produced from AX fermentation. The prebiotic effect, that is, the selectivity of AX for bifidobacteria and lactobacilli groups, increased as the molecular mass of the AX decreased. This suggests that molecular mass may influence the fermentation of AX in the colon.

KEYWORDS: Prebiotics; in vitro fermentation; arabinoxylan; polysaccharide molecular mass

INTRODUCTION

Cereal grains provide an important source of dietary fiber. Nonstarch polysaccharides (*1*) from cereal cell walls can resist digestion and absorption in the upper human gut and enter the colon, where they undergo fermentation by the colonic microbiota. The consequences of fermentation by the gut microbiota include increased fecal bulk through bacterial proliferation and the formation of microbial metabolites, for example, short chain fatty acids (SCFA) and gas (*2*). Dietary fiber has been shown to improve health and prevent diseases, with the more soluble, viscous, and fermentable fiber sources being proposed to reduce the glycemic index, insulin sensitivity (*3*), and cholesterol absorption (*4*). Fiber also dilutes the energy density of the diet and prolongs the intestinal digestive processes (*5*), which aids the control of satiety. Stool weight increases with fiber content (*6*), which increases the water and bacterial contents of stools (*7*, *8*).

Arabinoxylan (AX) is the primary NSP in the endosperm of wheat kernels, accounting for about 60–70% of the cell wall polysaccharides, which in turn account for about 9.5% of the wholemeal and 3% of white flour (*9*).

Previous work (*10*) showed that AX supplementation to the diet of rats resulted in reduced epithelial proliferation indices, the impact being greater than with whole wheat bran, guar gum, or a control with no fiber added. In addition, AX supplementation stimulated the greatest increases in fecal bulk, as reported by Eastwood et al. (*11*) and McIntosh et al. (*12*). The benefits of AX even stretch to increased natural killer cell activity, increased IL-2 and INF-γ production, and reduction in the symptoms of atopic dermatitis in mice (*13*). Work by Adam et al. (*14*) showed that a highly viscous and AX-rich wheat flour could increase the steroid content (bile acids and sterols) in excreted feces by 78% above that excreted from rats fed a control diet of purified wheat starch.

There is growing interest in the use of prebiotic oligosaccharides as functional food ingredients. A prebiotic is a "nondigestible food ingredient that affects the host by selectively targeting the growth and/or activity of one or a limited number of bacteria in the colon, and thus has the potential to improve

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host health" (*15*). This is currently accepted as including increases in the populations of *Bifidobacterium* spp. and *Lactobacillus* spp. (*16*), as both genera are viewed as positive for host health and have a long history of use as probiotics.

Current prebiotics, such as fructo-oligosaccharides (*17*), galacto-oligosaccharides, and inulin, are limited in their persistence to the distal colon and are predominantly fermented in the proximal colon, where the microbiota is known to have a saccharolytic metabolism (*18*). However, most chronic colonic diseases (for example, ulcerative colitis and colorectal cancer) originate in the distal colon (*19*, *20*); consequently, there is much greater interest in finding prebiotics that can persist to more distal regions of the colon.

One approach to increase the persistence of prebiotics is regulation of molecular mass (*21*, *22*). We have therefore used commercial AX fractions differing in molecular mass as substrates in small-scale fecal batch cultures, as similar small-scale cultures (*23*) have been shown previously to be a valid system for the initial screening of novel candidate prebiotics, where the supply of substrate is limited (*23*−*27*), and for obtaining structure:function information (*25*−*27*).

MATERIALS AND METHODS

Materials. The high, medium, and low AX viscosity fractions were purchased from Megazyme (Co. Wicklow, Ireland), and inulin (Raftiline-HP) was provided by Orafti (Tienen, Belgium).

Methods. *Gas Chromatographic (GC) Analysis of Monosaccharide.* The AX fractions were hydrolyzed following the method of ref *28*. Samples (3 mg) were weighted in Pyrex screw-cap vials with a PTFE-faced septum and dissolved in 1 mL of 2 N trifluoroacetic acid. Hydrolysis was carried out at 120 °C for 2 h. After the samples were cooled, 1 mL of phenyl-β-D-glucoside (1 mg/mL) was added as an internal standard and the samples were dried under nitrogen. The derivatization process was carried out following the method of Troyano et al. (*29*). Carbohydrates were dissolved in 100 μL of pyridine and silylated using 100 μL of trimethylsilylimidazol and 100 μL of trimethylchlorosilane and kept for 30 min at room temperature. One hundred microliters of hexane and 200 μL of water were added to the samples, and 1 μL of the upper layer was injected on the GC.

GC analysis was carried out using a 25 m × 0.25 mm i.d. × 0.25 μm film thickness fused silica column, coated with SPB-1 (crosslinked methyl silicone from Supelco, Bellefonte, PA). Analyses were carried out in a Carlo Erba chromatograph with a flame ionization detector (MFC800 HRGC Megaseries 2, Milan, Italy). Injector and detector temperatures were 300 °C; the oven temperature was held at 180 °C for 20 min, then increased to 290 °C at a heating rate of 15 °C min^{-1}, and held for 10 min. Chromatographic peaks were measured using a Chrom-Card 1.20 acquisition system (CE Instruments).

GC-MS analyses were carried out using the same capillary column installed in a HP-5890 chromatograph with a MD 5971 quadrupole mass detector (both from Hewlett-Packard) working in EI mode at 70 eV. Helium was used as the carrier gas, and injections were made in the split mode, with a split flow of 40 mL min^{-1}. Acquisition was done using an HPChem Station software (Hewlett-Packard, Palo Alto, CA).

Peaks were identified by comparison of their retention times with those of standard compounds and confirmed by mass spectrometry. Quantitative values were calculated from flame ionization detection peak areas. Standard solutions containing different proportions of each carbohydrate were prepared to calculate the response factor (RF) relative to phenyl-β-D-glucoside (internal standard) over the expected range.

Analysis of Phenolic Content. The internal standard used was 1.53 mg of 3,5-dichlor-4-hydroxybenzoic acid, dissolved in 1 mL of 80:20 ethanol and water. Twenty-five milligrams of sample was weighed into a 2 mL Eppendorf tube in preparation for three separate extraction techniques for each class of phenolics: free (I), soluble conjugated (II), and bound phenolics (III).

For the extraction of free phenolics and soluble conjugated phenolics, the internal standard was added (5 μL for I and 10 μL for II) and the flour was extracted twice with 1 mL of an 80:20 ethanol−water solution. Solutions were then vortexed, and the sample tube was sonicated for 10 min. Extracts were then centrifuged (20000*g*) for 15 min before the suspension was vacuum centrifuged to dryness. The residue was dissolved in 500 (I) or 400 μL (II) of a 2% acetic acid.

For the extraction of bound phenolics, the sample was extracted twice with 1 mL of an 80:20 ethanol−water solution. The supernatants were removed, and 10 μL of internal standard solution was added to the residue. The sample was hydrolyzed with 1 mL of 2 M NaOH for 4 h at room temperature and then centrifuged (20000*g*) for 15 min.

All extracts (free, bound, and soluble conjugated) were then acidified to pH 2 by the addition of 2 μL of 1 M HCL. The phenolic acids in each sample were extracted three times with ethyl acetate (500 μL for I and II and 1 mL for III), and the combined supernatants were evaporated to dryness. For high-performance liquid chromatography (HPLC) analysis, 100 μL of 2% acetic acid solution was added prior to injection.

HPLC Analysis of Phenolic Acid Extracts. HPLC analysis was carried out on 20 μL injections of each of the different phenolic extracts described above, using an Agilent 1100 HPLC and a Discovery Reverse-Phase-Amide C16 column (250 mm × 4.6 mm, 5 μm). The column was fitted with a precolumn (Discovery Reverse-Phase-Amide C16, 20 mm × 4.0 mm, 5 μm). The mobile phases used were acetonitrile (solvent A) and 2% aqueous acetic acid (solvent B) under the following stepped gradient: 0−30 min 100% B; 30−50 min 15% A, 85% B; 50−55 min 50% A, 50% B; and 55−65 min 70% A, 30% B. The flow rate was 1 mL/min using a column temperature of 30 °C and detection at 280/320 nm.

Size Exclusion (SE)-HPLC. AX fractions were dissolved at 1% (w/v) in HPLC grade water and subjected to SE-HPLC using TSK G6000$_{XL}$ and two TSK G4000 PW columns (Polymer Laboratories, Shropshire, United Kingdom) attached with decreasing pore size. The eluent was 0.02% (w/v) sodium azide, with a 0.6 mL min^{-1} flow rate, at ambient temperature. Dextran standards, with molecular masses between 11 and 260 kDa were used to calibrate the column.

Fecal Samples and in Vitro Fermentation. Each fermentation was conducted in triplicate on each of three fecal donors. The three donors were healthy, one female and two males, aged 23−29, who had not received antibiotic treatment for at least 3 months prior to experimentation and had no history of bowel disorders. Carbohydrate (90 mg) was mixed in autoclaved medium to give a final concentration of 1% (w/v) with a control sample prepared without any carbohydrate addition. This medium contained per liter: 2 g of peptone water (Oxoid Ltd., Basingstoke, United Kingdom), 2 g of yeast extract (Oxoid), 0.1 g of NaCl, 0.04 g of K_2HPO_4, 0.01 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $CaCl_2 \cdot 6H_2O$, 2 g of $NaHCO_3$, 0.005 g of hemein (Sigma), 0.5 g of l-cysteine HCl (Sigma), 0.5 g of bile salts (Oxoid), 2 mL of Tween 80, 10 μL of vitamin K (Sigma), and 4 mL of 0.025% (w/v) resazurin solution. Samples were inoculated with 1 mL of fecal slurry, which was prepared by homogenizing fresh human feces (10%, w/v) in phosphate-buffered saline (PBS; 8 g/L NaCl, 0.2 g/L KCl, 1.15 g/L Na_2HPO_4, and 0.2 g/L KH_2HPO_4), pH 7.3 (Oxoid), with a manual homogenizer (Fisher, Loughborough, United Kingdom). All additions, inoculations, and incubations were carried out inside an anaerobic cabinet (10% H_2, 10% CO_2, and 80% N_2) at 37 °C, in Hungate tubes, that were shaken at 300 rpm to ensure a homogeneous distribution of carbohydrate. Sampling time points (0, 5, and 12 h) were determined by analysis of total carbohydrate using the phenol−sulfuric acid assay (*30*) in a preliminary miniature batch culture (data not shown).

Bacterial Counting by Fluorescent in Situ Hybridization (FISH). The FISH technique was used to quantify bacteria at three time points in the incubations. Samples were centrifuged at 1500*g* for 2 min to remove particulate matter, then 400 μL of sample was fixed overnight at 4 °C with 4% (w/v) filtered paraformaldehyde (pH 7.2) in a ratio of 1:3 (v/v). Samples were washed twice with filtered PBS and resuspended in 200 μL of a mixture of PBS/ethanol (1:1, v/v) and then stored at −20 °C for up to 3 months. The hybridization was carried out as previously described (*31*, *32*) using genus- and group-specific 16S rRNA-targeted oligonucleotide probes labeled with Cy3 (MWG Biotech,

Ebersberg, Germany) or the nucleic acid stain 4′,6-diamidino-2-phenylindole (DAPI) for total cell counts. The bacterial groups were chosen based on their high abundance within, and contribution to, the colonic microflora. The probes (previously validated for the bacterial groups) were as follows: Bif164, specific for *Bifidobacterium* (*33*); Bac303, specific for *Bacteroides* (*34*); His150, for the *Clostridium histolyticum* subgroup (*35*); Erec482 for the *Clostridium coccoides*−*Eubacterium rectale* group (*35*); Lab158, for *Lactobacillus*/*Enterococcus* (*36*); and Ato291 for the *Atopobium* cluster, including most *Coriobacteriaceae* species (*37*).

Prebiotic Index (PI). The PI was used as a comparative measure of the selectivity of fermentation of carbohydrates (*38*). The PI is calculated from changes in the proportions of organisms generally considered to be beneficial (e.g., bifidobacteria and lactobacilli) and those generally considered detrimental (e.g., clostridia and bacteroides) in relation to the initial population. The equation used was $PI = \alpha + \beta - \delta - \epsilon$, where α = (Bif12/Bif0)/total, β = (Lac12/Lac0)/total, δ = (Bac12/Bac0)/total, ϵ = (His12/His0)/total, total = total cell count (12 h)/total count (0 h), Bif12 = bifidobacterial count at 12 h, Bif0 = bifidobacterial count at 0 h, etc.

SCFA and Lactic Acid Analysis. Samples were centrifuged at 13000*g* for 20 min to remove all particulate matter, and the SCFA and lactic acid contents of the supernatant were quantified using a BioRad HPX-87H HPLC column (Watford, United Kingdom), at 50 °C, with a 0.005 mM H_2SO_4 eluant at a flow rate of 0.6 mL min^{-1} (*23*).

Statistical Analysis. Statistical analysis was performed using SPSS for windows, version 12.0.1. Univariate analysis of variance (ANOVA) and the posthoc Tukey test were used to determine the significance of the effect of difference AX fractions on bacterial group populations and SCFA and lactic acid production. Differences were deemed significant when $P < 0.05$.

RESULTS

Molecular Mass Distribution of the AX Fractions. Three AX fractions described as high, medium, and low viscosity were obtained from Megazyme. The mean molecular masses reported by the manufacturer were 354, 278, and 66 kDa, and SE-HPLC showed that each comprised a mixture of components with the molecular mass distribution of the fractions overlapping (**Figure 1**). The physicochemical properties of the three AX fractions are described in **Table 1**.

Monosaccharide Composition of the AX Fractions. Table 1 shows the monosaccharide compositions of the three AX fractions obtained by GC analysis. As expected, arabinose and xylose were the major sugars with lower amounts of glucose and fructose. The 66 kDa AX fraction had a higher ratio of arabinose:xylose than the 354 and 278 kDa fractions, which had similar ratios.

Phenolics Content of AX Fractions. The 354 and 66 kDa fractions had comparable concentrations of the three forms of free and soluble ferulic acid (**Table 1**), but the 66 kDa fraction contained nearly 40% more bound ferulic acid than the 354 kDa fraction. The 278 kDa contained no detectable levels of ferulics. The 354 kDa fraction also contained significantly more diferulic acid than the 66 kDa fraction. The 278 kDa contained no diferulic acid.

Effect of AX Fractions on Bacterial Populations. Table 2 shows the bacterial populations after 0, 5, and 12 h of incubation in anaerobic batch culture following supplementation with inulin or the AX fractions (1% w/v). All substrates gave significant increases in total cell numbers with the highest values being recorded at 5 h. The total cell numbers were lower at 12 h in all cultures except that with the 66 kDa AX fractions. The microflora in these fermenters was selective in its ability to ferment AX, with considerable proliferation of the bifidobacteria, lactobacilli, and eubacteria groups; lesser increases in bacteroides and clostridia groups occurred, but these were still statistically significant.

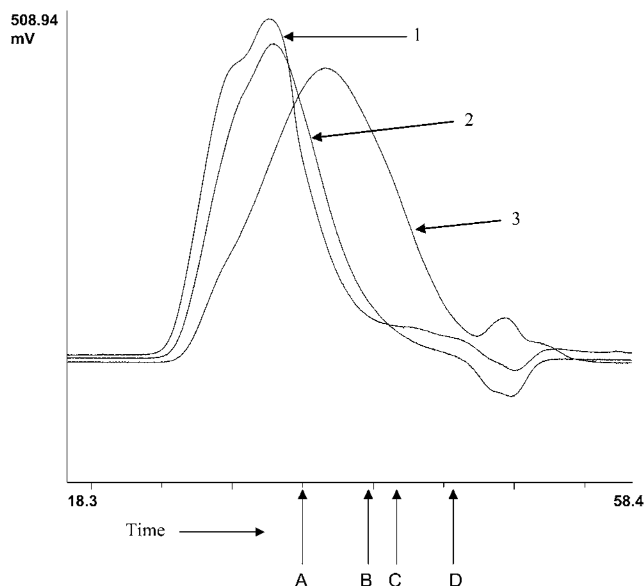

Figure 1. SE-HPLC of AX fractions on a Progel TSK G6000 PWXL column. AX fraction peaks: 1, 354 kDa AX fraction; 2, 278 kDa AX fraction; and 3, 66 kDa AX fraction. Dextran standard elution peaks: A, 260 kDa; B, 77 kDa; C, 42 kDa; and D, 11 kDa.

There were marked differences in the effects of the AX fractions on the different bacterial groups. The 66 kDa AX was more selective for lactobacilli and eubacteria groups at 12 h. The populations of bacteroides were highest with the lower molecular mass AX fractions at 12 h, but bacteroides peaked at 5 h and decreased by 12 h in the other AX treatments. Clostridia increased most with 354 kDa AX fraction.

As expected from previous studies (*39*−*41*), inulin resulted in a selective increase in beneficial bacteria. This included significant increases in bifidobacteria (at 12 h), eubacteria (5 and 12 h), and lactobacilli (12 h). Although there was a significant increase in the clostridia group at 5 and 12 h from inulin, it was significantly less than the other treated cultures (except 66 kDa at 12 h). There was also a significant contribution of the atopobia group to the increase in total cell counts.

A significant increase in bifidobacteria occurred early in the carbohydrate-negative control cultures. However, this was not sustained beyond 5 h. Other significant changes in the populations of bacterial groups in these cultures included an increase in bacteroides and clostridia and a decrease in lactobacilli.

Prebiotic Index. The PI provides a comparative tool for evaluating the prebiotic efficacy of carbohydrates and is calculated for the different cultures in **Figure 2** (*38*).

In the absence of added carbohydrate, the PI became negative after 12 h as a result of more sustained increases in bacteroides and clostridia.

Inulin gave a relatively consistent PI for all donors, but there were substantial differences in the responses of the microflora from the three donors to the AX fractions (data not shown for individual donors). In general, however, the PI increased with decreasing AX molecular mass: 354 kDa AX gave a lower PI than inulin, and 66 kDa AX gave the highest PI, 9.7 at 12 h as compared to 5.9 with inulin.

There were differences in the selectivity of the three AX fractions for different bacteria with the major contribution of the 354 kDa and 278 kDa AX fractions to the high PI being increased populations of bifidobacteria. The 66 kDa AX resulted

Table 1. Characteristics of the Three AX Fractions[a]

			monosaccharide composition (%)[c]				ferulic content (μg/g)			diferulic content (μg/g)		
fraction	mass (kDa)[b]	viscosity (cSt)[b]	A	X	F	G	free ferulic acid	soluble ferulic acid	bound ferulic acid	free diferulic acid	soluble diferulic acid	bound diferulic acid
1	66	2	27.03	67.54	2.53	2.90	6.79	11.47	67.13	4.51	8.79	10.17
2	278	27	37.46	60.93	0.54	1.07	ND[d]	ND	ND	ND	ND	ND
3	354	48	35.45	58.57	2.96	3.02	6.35	12.05	40.73	5.96	14.47	65.34

[a] Monosaccharide composition (% of total carbohydrates) of AX fractions by GC analysis was carried out using a 25 m × 0.25 mm i.d. × 0.25 μm film thickness fused silica column, coated with SPB-1 (cross-linked methyl silicone). Analyses were carried out in a Carlo Erba chromatograph with a flame ionization detector. Chromatographic peaks were measured using a Chrom-Card 1.20 acquisition system (CE Instruments). Ferulic acid content: HPLC analysis was carried out using an Agilent 1100 HPLC and a Discovery Reverse-Phase-Amide C16 column (250 mm × 4.6 mm, 5 μm), fitted with a precolumn (Discovery Reverse-Phase-Amide C16, 20 mm × 4.0 mm, 5 μm). [b] Data provided by Megazyme. [c] A, arabinose; X, xylose; F, fructose; and G, glucose. [d] ND, not detectable.

Table 2. Bacterial Populations (log10 Cells/g Feces) in Small-Scale Batch Cultures at 0 (Inoculum), 5, and 12 h Using Inulin and Three Fractions of AX as Substrates, as Compared to No Treatment[a]

treatment	time	total cells	*Bifidobacterium*	*Bacteroides*	*Clostridium*	*Eubacterium*	*Lactobacillus*	*Atopobium*
no treatment	0	8.94 (0.03) a	7.11 (0.03) a	8.17 (0.04) a	7.76 (0.04) ab	8.23 (0.04) a	7.33 (0.04) bc	7.70 (0.04) a
	5	8.92 (0.03) a	7.29 (0.04) bc	8.21 (0.05) ab	7.58 (0.05) a	8.24 (0.03) a	7.28 (0.04) b	7.86 (0.04) ab
	12	9.03 (0.04) a	7.07 (0.03) a	8.40 (0.04) b	7.84 (0.04) b	8.16 (0.04) a	7.05 (0.03) a	7.81 (0.04) ab
inulin	5	9.43 (0.03) bcdd	7.20 (0.03) ab	8.28 (0.04) de	7.88 (0.04) bc	8.50 (0.04) b	7.49 (0.05) c	8.31 (0.03) e
	12	9.37 (0.03) b	7.87 (0.03) e	8.30 (0.03) cd	7.81 (0.04) b	8.50 (0.04) b	7.71 (0.03) ef	8.39 (0.04) e
354 kDa AX	5	9.54 (0.02) d	7.32 (0.04) bc	8.58 (0.03) de	8.19 (0.05) d	8.83 (0.03) c	7.88 (0.04) fg	7.93 (0.04) bc
	12	9.39 (0.03) bc	7.92 (0.04) e	8.51 (0.04) cd	8.27 (0.05) d	8.95 (0.04) d	7.61 (0.05) de	8.05 (0.03) cd
278 kDa AX	5	9.54 (0.03) cd	7.40 (0.04) d	8.63 (0.04) de	8.09 (0.04) cd	8.87 (0.04) c	8.15 (0.05) hi	7.89 (0.03) bc
	12	9.47 (0.04) bcd	7.95 (0.04) e	8.60 (0.04) de	7.84 (0.05) b	8.73 (0.04) c	7.62 (0.05) de	7.94 (0.03) bcd
66 kDa AX	5	9.49 (0.04) bcd	7.40 (0.04) d	8.64 (0.04) de	8.06 (0.03) c	8.96 (0.04) d	8.02 (0.05) gh	7.97 (0.04) bcd
	12	9.70 (0.04) e	7.92 (0.03) e	8.74 (0.04) e	8.18 (0.04) d	9.19 (0.03) e	8.30 (0.05) i	8.10 (0.03) d

[a] Univariate ANOVA and Tukey tests were used to determine significant differences for each bacterial population ($n = 135$). Different letters indicate significant differences ($P < 0.05$) for each bacterial genus (i.e., within column) between the different carbohydrate sources and the time points. The standard error is given in parentheses.

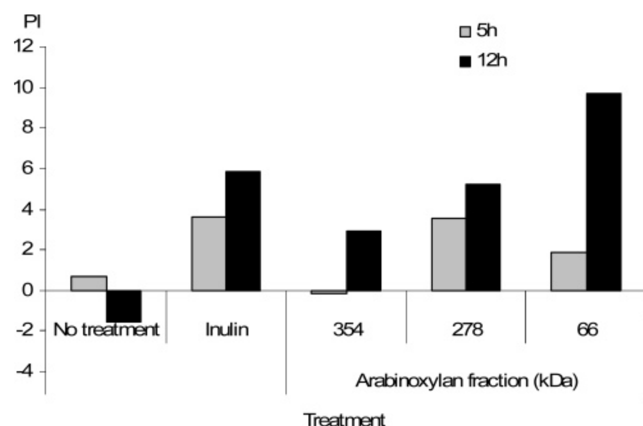

Figure 2. PI scores from batch cultures after 5 and 12 h of fermentation of a no treatment control, inulin, and three molecular mass fractions of AX.

in similar increases in bifidobacteria but a greater increase in lactobacilli, resulting in a higher PI.

SCFA and Lactic Acid Production. All of the supplemented cultures contained significantly higher concentrations of total as compared with the control cultures at all time points, with acetic acid being the dominant component (**Table 3**). The increase in total SCFA and lactic acid production with AX supplementation resulted from increases in all SCFAs, and particularly in butyric acid, which increased three-fold from fermentation of the 66 kDa AX fraction. Butyric acid production can be correlated to the significant increase in the populations of eubacteria and clostridia, which are known producers of butyric acid (*42*−*44*).

The absolute amounts of propionic acid were similar in the cultures with AX and inulin. However, the proportion of total SCFA accounted for by propionic acid was greater in inulin-fermented cultures. It has been shown (*45*) that bacteroides produce propionic acid and that the amount produced can be related to the population increase. However, it is difficult to attribute a product to a specific bacterial group in a mixed culture system.

DISCUSSION

The greatest difference between the AX fractions was in molecular mass, with the viscosity of the solutions increasing as the molecular mass increased. The undetectable level of ferulic acid in the 278 kDa fraction suggested that it may be derived from the same starting material as 354 kDa but had the ferulic acid removed, thus reducing the overall molecular mass and viscosity of 278 kDa because no inter- and intramolecular di- and triferulic acid cross-linking could be established (*46*). AXs with longer chain length are accepted as having greater cross-linking capacity and a resulting capability to entrap and hold water, which in turn increases viscosity (*47*). The 66 kDa AX had a vastly different xylose:arabinose ratio of 2.5 (as compared to roughly 1.6 for the other fractions) giving reason for the comparative difficulty in solubilizing the 66 kDa fraction and its resulting low viscosity (*48*).

The 66 kDa fraction was more selective for purportedly "health-promoting" bacterial groups, as shown by its high PI across all donors and the elevated production of SCFA and lactic acid. This may relate to the fact that the shorter polysaccharides in the 66 kDa fraction have more nonreducing ends per unit mass, which are susceptible to attack by the exo-enzymes produced by colonic bacteria than higher molecular mass AX fractions. All AX fractions gave increases in eubacteria with the most noticeable differences being the effects of the 278 and

Table 3. SCFA Concentration (mM) in Small-Scale Batch Cultures at 0 (Inoculum), 5, 12, and 24 h Using Inulin and Three Different Molecular Mass Fractions of AX as Substrates, as Compared to No Treatment[a]

			SCFA			
treatment	time	lactic	acetic	propionic	butyric	total SCFA
no treatment	0	1.8 (0.2) a	4.4 (0.5) a	1.1 (0.6) a	0.5 (0.2) (0.2)	7.8 (1.3) a
	5	0.7 (0.1) a	13.1 (0.4) ab	3.8 (0.6) ab	2.6 (0.3) ab	20.2 (0.9) a
	12	0.3 (0.1) a	12.8 (0.9) ab	3.8 (0.2) ab	2.3 (0.1) ab	19.2 (1.0) a
	24	0.3 (0.1) a	13.9 (1.1) ab	3.8 (0.3) ab	2.4 (0.1) ab	20.5 (1.2) a
inulin	5	6.0 (1.2) ab	25.0 (1.9) bc	7.6 (1.4) bc	6.6 (0.9) abc	45.1 (3.0) b
	12	8.6 (3.3) b	29.7 (4.4) cd	9.2 (0.9) bc	9.2 (1.2) bcde	56.6 (6.9) b
	24	6.5 (2.4) ab	30.4 (4.4) cd	7.7 (1.1) bc	11.0 (2.4) cde	55.6 (5.2) b
354 kDa AX	5	3.1 (0.9) ab	34.4 (3.0) cd	8.0 (1.1) c	8.2 (1.0) bcd	53.6 (5.9) b
	12	2.3 (0.5) ab	40.8 (5.1) d	7.9 (1.0) c	12.9 (1.6) cde	63.9 (6.9) b
	24	4.7 (2.4) ab	36.1 (2.4) cd	7.3 (0.7) bc	13.3 (2.4) e	61.3 (3.6) b
278 kDa AX	5	3.1 (1.0) ab	32.3 (2.4) cd	8.0 (1.1) c	7.9 (0.7) bcd	51.3 (5.0) b
	12	3.5 (1.0) ab	34.8 (1.9) cd	6.5 (0.4) bc	10.7 (1.3) cde	55.3 (2.2) b
	24	4.2 (1.3) ab	39.1 (2.9) d	6.2 (0.3) bc	12.5 (2.0) cde	62.0 (3.6) b
66 kDa AX	5	1.0 (0.1) a	39.9 (1.5) d	8.5 (0.7) c	9.6 (1.0) bcde	59.0 (2.9) b
	12	0.5 (0.2) a	34.5 (2.8) cd	7.5 (0.4) bc	13.2 (1.5) cde	55.7 (3.7) b
	24	0.5 (0.2) a	28.7 (3.7) cd	6.5 (0.8) bc	14.7 (2.7) de	50.4 (6.6) b

[a] Univariate ANOVA and Tukey tests were used to determine significant differences for each SCFA concentration ($n = 9$). Different letters indicate significant differences ($P < 0.05$) for each bacterial genus (i.e., within column) between the different carbohydrate sources and time points. The standard error is given in parentheses.

354 kDa fractions on bifidobacteria and of the 66 kDa fraction on both bifidobacteria and lactobacilli.

The bifidogenic effect agrees with previous work by Arrigoni et al. (*1*) who used in situ hybridization to show that digestion in vitro of a wheat germ preparation exerted a bifidogenic effect. AX (derived from rye) has also been shown to increase the growth of three strains of *Bifidobacterium longum* by 80−120% as compared with glucose (*49*). In addition, *B. longum* was able to utilize free arabinose monomers far more efficiently than free xylose or xylooligosaccharides. *Bifidobacterium adolescentis* has also been shown (*50*) to produce arabinofuranohydrolase, which is able to remove side chains from the doubly substituted xylose subunits. However, studies with pure culture studies are limited as they consider the response of only one or a few isolated strains and, therefore, cannot be used to make predictions concerning the entire microbiota.

None of the lactobacilli strains tested by Crittenden et al. (*49*) grew on AX, while Jaskari et al. (*51*) showed that *Lactobacillus rhammosus GG* and *Lactobacillus acidophilus* did not grow on arabinoxylooligosaccharides. However, it is clear that *Lactobacillus* species must ferment AX or products of its breakdown by other species, to account for the abundance of lactobacilli at 12 h with the 66 kDa fraction.

Bacteroides are a metabolically versatile group with respect to their ability to utilize many types of plant polysaccharides as substrates (*52*−*54*). This correlates with the significant increases in bacteroides observed in response to all AX fractions. However, the increases were modest as compared to those in bifidobacteria and lactobacilli. Considering that bacteroides were a dominant group in the donors' inoculum, the lower populations of the bifidobacteria and lactobacilli groups were clearly able to compete successfully for AX and proliferate, to a greater extent, than bacteroides.

The bifidogenic response of the colonic and fecal microflora to inulin supplementation in vitro and in vivo has been well-documented (*39*, *55*, *56*), which is consistent with the reproducible bifidogenic effect observed in batch cultures inoculated with fecal samples from all three donors. The atopobia group also responded positively to inulin supplementation.

The SCFA and lactic acid profile resulting from the fermentation of AX in different experimental models is variable and inconsistent in the literature (*1*, *10*, *54*). However, specific increases in butyric acid were reported by Salvador et al. (*57*) from xylose-rich substrates and by Adam et al. (*14*) using a viscous AX-rich wheat flour as the substrate. Eubacteria are involved in the production of butyric acid (*58*), which possibly relates the proliferation of eubacteria to the increased butyric acid production from AX fermentation.

Roland and Nugon-Baudon et al. (*59*) suggested that a positive correlation existed between solubility and fermentability. A study on dietary fiber (*60*) led to the premise that the digestion of soluble fiber was influenced by its chemical composition (i.e., monosaccharide composition, linkage type, and MW) whereas the digestion of insoluble fiber was influenced by its degree of susceptibility to microflora digestion (i.e., access to enzyme target sites). These concepts could explain the differences between effects of the AX fractions on the microflora reported here.

Future work should investigate further the relationship between the physicochemical characteristics of NSPs and their activity in vivo in order to establish an understanding of structure−function relationships. The use of three-stage anaerobic batch−culture fermenters would help to establish the effect of structure on the persistence of cereal polysaccharides to more distal regions of the colon.

In conclusion, AX had a donor-dependent ability to selectively promote the growth of beneficial groups of bacteria, as shown by the variation in the PI for the three donors (data not shown). Nevertheless, the structure−activity relationship was demonstrated in that the PI increased with decreasing molecular mass.

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