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# Structural Differences among Alkali-Soluble Arabinoxylans from Maize (*Zea mays*), Rice (*Oryza sativa*), and Wheat (*Triticum aestivum*) Brans Influence Human Fecal Fermentation Profiles

Devin J. Rose,[†,§] John A. Patterson,[‡] and Bruce R. Hamaker*,[†]

[†]Whistler Center for Carbohydrate Research and Department of Food Science, Purdue University, 745 Agriculture Mall Drive, West Lafayette, Indiana 47906 and [‡]Department of Animal Science, Purdue University, 915 West State Street, West Lafayette, Indiana 47906. [§]Present address: USDA-ARS-NCAUR, 1815 North University Street, Peoria, Illinois 61615.

Human fecal fermentation profiles of maize, rice, and wheat bran and their dietary fiber fractions released by alkaline-hydrogen peroxide treatment (principally arabinoxylan) were obtained with the aim of identifying and characterizing fractions associated with high production of short chain fatty acids and a linear fermentation profile for possible application as a slowly fermentable dietary fiber. The alkali-soluble fraction from maize bran resulted in the highest short chain fatty acid production among all samples tested, and was linear over the 24 h fermentation period. Size-exclusion chromatography and $^1$H NMR suggested that higher molecular weight and uniquely substituted arabinose side chains may contribute to these properties. Monosaccharide disappearance data suggest that maize and rice bran arabinoxylans are fermented by a debranching mechanism, while wheat bran arabinoxylans likely contain large unsubstituted xylose regions that are fermented preferentially, followed by poor fermentation of the remaining, highly branched oligosaccharides.

**KEYWORDS: Corn; gut; human; microbiota; prebiotic; short chain fatty acids**

## INTRODUCTION

It is clear that individuals on a normal Western diet do not consume enough dietary fiber. In the United States, adult men and women, on average, consume an inadequate 17.8 and 14.1 g/d, respectively (*1*). This is in comparison with recommendations of 38 and 25 g/d, respectively (*2*, *3*).

There are numerous adverse effects of inadequate dietary fiber intake. In addition to constipation, recent literature ties insufficient dietary fiber consumption to increased risk of colon cancer (*4*, *5*), type 2 diabetes (*6*, *7*), heart disease (*8*), obesity (*9*), and inflammatory bowel disease (*10*). Three of these diseases (i.e., cancer, diabetes, and heart disease) are among the top 10 leading causes of death in the United States (*11*). It thus falls incumbent on health care professionals to educate the public on the importance of adequate dietary fiber intake and food scientists to create foods that will help consumers meet their dietary fiber needs.

Not all dietary fibers are created equal. In general, insoluble dietary fibers such as cellulose and water-unextractable arabinoxylan pass through the colon largely intact and contribute mainly to stool bulking, while soluble dietary fibers, such as $\beta$-glucan and water-extractable arabinoxylans, ferment rapidly in the cecum and proximal colon, leaving little carbohydrate substrate for bacteria in distal regions of the colon (*12*). The rapid utilization of the majority of fermentable dietary fibers creates a gradient of SCFAs in the colon, with high concentrations in the proximal and low concentrations in the distal colon (*13*). The latter leads to putrefactive fermentation or bacterial fermentation of protein, which produces undesirable metabolites such as hydrogen sulfide, phenols, and ammonia (*14*). Thus, a dietary fiber with certain composition and structure such that it is capable of sustaining the production of SCFAs by the microbiota with prolonged fermentation, and accompanying changes in microbiota composition, would be desirable.

Cereal brans present an interesting source of dietary fiber because they can be obtained cheaply, contain high concentrations of dietary fiber, and their consumption has been associated with lower risk of diseases such as coronary heart disease, certain types of cancer, inflammatory bowel disease, and disordered laxation (*15*). Unfortunately, cereal brans are generally poorly fermented (*16*) and thus not desirable dietary fibers for stimulating a healthy colonic production of SCFAs. Certain treatments, however, such as alkali or alkaline-hydrogen peroxide, can break down the complex cell wall structure of cereal brans and improve fermentability (*17*, *18*).

Maize, rice, and wheat bran contain high concentrations of arabinoxylans. The basic structure of arabinoxylans consists of a (1→4)-linked $\beta$-D-xylopyranosyl (Xyl*p*) backbone, with $\alpha$-L-arabinofuranosyl (Ara*f*) side units attached to O-2 and/or O-3 of backbone Xyl*p* units (*19*). Additionally, ferulic acid can be esterified to the arabinose side-chains and create cross-links with

*To whom correspondence should be addressed. Phone (765) 494-5668. Fax: (765) 494-7953. E-mail: hamakerb@purdue.edu.


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other cell wall components to yield insoluble complexes (*20*). Variations in the complex structure of arabinoxylans are likely to result in polysaccharides with different fermentation properties (*21*). The purpose of this research was to determine the fermentation profiles of alkali-soluble dietary fiber fractions isolated from cereal brans, with the aim of identifying specific chemical structures that may be associated with a slowly, completely fermented fiber.

## MATERIALS AND METHODS

**Sample Preparation.** Finely ground (<200 μm) maize bran was a gift from Bunge Milling (St. Louis, MO), and heat-stabilized rice bran and wheat bran were obtained from a local market. Rice bran and wheat bran were finely ground in a cyclone mill to pass through a 0.5 mm screen. Brans were partially defatted with two volumes of hexane (bran:hexane, 1:7 [w/v]) for 30 min each.

Alkaline-hydrogen peroxide extraction was then carried out as previously described (*22*, *23*), with some modifications. Bran was suspended in water (1:9 w/w) and the pH adjusted to 7.0. Under constant stirring, the mixture was boiled, and then 4 mL of heat stable α-amylase (150 U, A-3403, Sigma-Aldrich Corp., St. Louis, MO) were added. Starch was hydrolyzed at 90−95 °C for 30 min, and then the mixture was cooled in an ice bath to 50 °C. The pH was adjusted to 6.0, and 5 mL of neutral protease (4 U, P-1236, Sigma-Aldrich) were added. Protein was hydrolyzed at 50 °C for 4 h, and then the mixture was brought to a boil to inactivate enzymes, cooled in an ice bath, and the pH adjusted to 7.0. The slurry was centrifuged at 10000*g* for 10 min. The residue was washed three times with water, centrifuged, dried in a forced draft oven (40 °C, 48 h), and then ground in a cyclone mill.

The enzyme-treated bran from above (50 g) were suspended in 1 L of 1 M sodium hydroxide. Under constant mixing at 60 °C, 42 mL of 30% hydrogen peroxide were slowly added to the mixture, which was stirred for a total of 4 h and then centrifuged (10000*g* for 10 min), followed by the addition of 3 volumes of ethanol (95% v/v) to the supernatant. The mixture was held overnight at 4 °C, and then the aqueous ethanol portion, containing the liberated ferulic acid, was siphoned off, discarded, and the precipitated material was washed with 80% ethanol, anhydrous ethanol, and acetone. The resulting powder was air-dried until no solvent could be detected by odor and then further dried in an oven at 40 °C for 24 h. To further purify the alkali-soluble fractions, enzymatic treatments with heat-stable α-amylase and neutral protease, ethanol precipitations, and washings were repeated as described above.

**In Vitro Digestion.** Throughout this paper, in vitro digestion refers to simulated upper gastrointestinal digestion, i.e., mouth, stomach, and small intestine, which was carried out according to Lebet et al. (*24*), except pancreatin was suspended in phosphate buffer (20 mM pH 6.9, containing 10 mM calcium chloride) instead of water, and the concentrations of pepsin and pancreatin were increased by factors of 10 to enhance breakdown of digestible components. Following digestion, the suspensions were dialyzed (molecular weight cut off 10000, Spectrum Laboratories, Rancho Dominguez, CA) against distilled water for 24 h with three changes of distilled water and then freeze-dried (Virtis, Gardiner, NY).

**In Vitro Fermentation.** Throughout this paper, in vitro fermentation refers to simulated lower gastrointestinal fermentation, i.e., large intestine, which was carried out according to Lebet et al. (*25*) with some modifications. Fifty mg of equivalent carbohydrate (neutral sugars + uronic acids) were weighed into each of 5 serum tubes for each replicate (i.e., one tube per replicate for each sampling period: 0, 4, 8, 12, and 24 h). Fermentations were carried out in duplicate, and duplicate blank tubes containing no substrate were included for each time point. Anaerobic carbonate-phosphate buffer (*26*) was prepared and then sterilized by autoclaving for 20 min at 121 °C. Immediately following autoclaving, 0.25 mg/L of cysteine hydrochloride was added as a reducing agent, and carbon dioxide was bubbled through the buffer. During use, a constant stream of carbon dioxide was bubbled through the buffer to maintain anaerobiosis. Four mL of this buffer were added, along with 100 μL of Oxyrase for Broth (Oxyrase, Inc., Mansfield, OH) to each tube. The Oxyrase was added to scavenge any residual oxygen, and the tubes were sealed anaerobically (by flushing headspace with carbon dioxide) with a rubber stopper and metal crimp cap and placed at 4 °C overnight to hydrate.

The next morning, feces were collected from three healthy volunteers consuming unspecified and varied diets and who had not taken antibiotics in the last 3 months. Once feces were collected, they were kept on ice in tightly sealed plastic bags with air expelled and used within 2 h. This storage time was unlikely to affect bacterial fermentation (*27*). The feces were pooled and homogenized with 3 parts sterile anaerobic carbonate-phosphate buffer (prepared and maintained as described above) and then filtered through four layers of cheesecloth. Tubes were opened and 1 mL of filtrate was used to inoculate each tube under constant carbon dioxide flushing. The tubes were then resealed and incubated at 37 °C with gentle shaking. At predetermined time intervals (0, 4, 8, 12, or 24 h), the tubes were opened and microbial activity was halted by the addition of 0.4 mL of 2.75 mg/mL copper sulfate (containing 12.5 mg/mL of *myo*-inositol, as an internal standard for residual carbohydrate analysis). The pH was recorded, and aliquots of slurry were removed for SCFA, BCFA, and ammonia quantification and frozen (−40 °C) until analysis. The remainder of the reaction mixture was also frozen and then freeze-dried for monosaccharide analysis.

**Sample Analyses.** Moisture content of samples was determined by loss in weight upon drying at 105 °C for 16 h. Protein was determined using a nitrogen analyzer (Perkin-Elmer Series II nitrogen analyzer, model 2410, Norwalk, CT), with a conversion factor from % nitrogen to % protein of 6.25. Starch was determined using an assay kit (Total Starch (AA/AMG method), Megazyme, Wicklow, Ireland). Total dietary fiber was determined as the sum of neutral sugars, uronic acids, and lignin, according to AACC International Official Method 32−25 (*28*), except the procedure was modified to accommodate a smaller sample size (50 mg) and, because the starch content was known, the digestion procedure was skipped, and the glucose contributed by starch was subtracted from the total glucose content to obtain nonstarch glucose. In preliminary experimentation, these modifications were found to give values for nonstarch glucose and total dietary fiber that were statistically indistinguishable from those using the method as published (data not shown). Each of these analyses was performed before and after in vitro upper-gastrointestinal digestion.

Additional characterization of the alkali-soluble fractions after in vitro upper-gastrointestinal digestion was performed using size-exclusion chromatography (SEC) and $^1$H NMR spectroscopy. For the chromatography, samples (2.0 mg/mL) were dissolved in 0.02% (w/v) sodium azide at 50 °C for 1 h and filtered (5 μm cutoff). The filtered sample was injected onto a SEC system consisting of a pump (Varian, Walnut Creek, CA), an injection valve (Rheodyne, Rohnert Park, CA) with a 500 μL sample loop, a column (50 cm × 2 cm) packed with Sephacryl S-500 (Amersham Biosciences, Piscataway, NJ) at room temperature, and a refractive index detector (Varian, Walnut Creek, CA). Mobile phase was 0.02% sodium azide at 1.3 mL/min. Data were collected using Galaxie software (Varian, Walnut Creek, CA). Molecular weight was estimated using the retention times of known pullulan standards. Normalized peak areas were calculated by dividing the area of the peak of interest by the total area of all peaks.

Samples for $^1$H NMR spectroscopy were prepared by dissolving samples in deuterium oxide (10 mg/mL) for 8 h at room temperature, followed by freeze-drying. The dissolving and freeze-drying steps were repeated twice more, and then spectra were recorded on a 300 MHz spectrometer at 85 °C. Sixty-four pulses were collected, with an acquisition time of 1.7 s, a relaxation delay of 2 s, and a pulse angle of 45°. Partial structural assignments of the peaks were made by comparison with previously published data (*29*−*32*).

**Fermentation Analyses.** For SCFA and BCFA quantification, 0.4 mL of fermentation slurry was combined with 0.1 mL of 5% phosphoric acid (containing 50 mM 4-methyl valeric acid, as an internal standard), mixed with a vortex mixer, and allowed to rest for 30 min. Samples were then centrifuged at 13000 rpm for 10 min, and a 4 μL aliquot was injected onto a HP 5890 GC equipped with a Nukol capillary column (30 m × 0.25 mm ID, 0.25 μm bonded phase, Supelco, Bellefonte, PA) under conditions defined by the manufacturer.

Ammonia was determined using an enzymatic method (glutamate dehydrogenase/ NADH/2-oxoglutarate method; Boehringer, Mannheim, Germany), and residual carbohydrates (neutral sugars and uronic acids) were measured in freeze-dried fermentation residues using AACC International Official Method 32−25 (*28*) with the modifications described above (see Sample Analyses).

**Table 1.** Composition of Starting Material (SM) and Alkali-Soluble (AS) Fractions Prior to in Vitro Digestion and Dermentation[a]

| | maize bran | | rice bran | | wheat bran | |
|---|---|---|---|---|---|---|
| constituent | SM | AS | SM | AS | SM | AS |
| fraction of total | 100 | 38.3 ± 0.1[b] | 100 | 13.1 ± 0.2 | 100 | 27.3 ± 0.1 |
| protein[c] | 4.75 ± 0.07 | 1.28 ± 0.11 | 19.5 ± 0.5 | 6.94 ± 0.25 | 19.3 ± 0.0 | 6.05 ± 0.13 |
| starch | 9.66 ± 0.03 | ND[d] | 29.8 ± 1.18 | 0.97 ± 0.07 | 14.4 ± 0.0 | 1.14 ± 0.28 |
| total dietary fiber | 69.7 ± 1.8 | 61.5 ± 2.6 | 31.4 ± 0.9 | 38.1 ± 0.1 | 52.4 ± 0.2 | 62.7 ± 1.76 |
| arabinose[e] | 14.2 ± 0.6 | 17.0 ± 1.1 | 4.89 ± 0.08 | 14.2 ± 0.2 | 11.0 ± 0.1 | 26.0 ± 0.5 |
| xylose | 27.3 ± 0.9 | 33.2 ± 1.5 | 4.38 ± 0.12 | 14.6 ± 0.1 | 16.2 ± 0.3 | 30.4 ± 0.7 |
| mannose | 0.39 ± 0.03 | 0.16 ± 0.03 | 0.97 ± 0.00 | 0.17 ± 0.02 | 0.63 ± 0.02 | 0.10 ± 0.00 |
| galactose | 4.33 ± 0.07 | 5.27 ± 0.11 | 1.21 ± 0.02 | 3.00 ± 0.01 | 1.38 ± 0.03 | 1.09 ± 0.04 |
| glucose[f] | 18.3 ± 0.5 | 0.93 ± 0.04 | 12.6 ± 0.7 | 1.68 ± 0.03 | 16.4 ± 0.0 | 1.76 ± 0.17 |
| uronic acids | 2.59 ± 0.02 | 4.64 ± 0.00 | 1.16 ± 0.02 | 3.35 ± 0.04 | 1.27 ± 0.02 | 2.23 ± 0.03 |
| lignin | 2.63 ± 0.24 | 0.30 ± 0.14 | 6.10 ± 0.40 | 1.15 ± 0.16 | 5.59 ± 1.09 | 1.06 ± 0.33 |

[a] Expressed as g/100 g of dm; $n = 2$. [b] Mean ± standard deviation. [c] $N \times 6.25$. [d] ND, none detected. [e] Expressed in polysaccharide form (0.88 and 0.9, for pentoses and hexoses, respectively, for conversion of monosaccharide into polysaccharide form). [f] Non-starch glucose.

**Table 2.** Short and Branched Chain Fatty Acid and Ammonia Production after 24 h of in Vitro Fecal Fermentation[a]

| | maize bran | | rice bran | | wheat bran | |
|---|---|---|---|---|---|---|
| metabolite | SM | AS | SM | AS | SM | AS |
| acetate[b] | 1.70 (0.11)$^{E}$ | 12.0 (1.0)$^{A}$ | 6.59 (0.07)$^{C}$ | 9.49 (0.33)$^{B}$ | 4.72 (0.49)$^{D}$ | 6.21 (0.20)$^{CD}$ |
| propionate | 0.185 (0.134)$^{E}$ | 5.55 (0.56)$^{A}$ | 1.41 (0.17)$^{CD}$ | 2.77 (0.04)$^{B}$ | 0.730 (0.183)$^{ED}$ | 2.17 (0.42)$^{BC}$ |
| butyrate | 0.490 (0.059)$^{C}$ | 2.02 (0.15)$^{A}$ | 1.98 (0.17)$^{A}$ | 1.99 (0.01)$^{A}$ | 1.89 (0.16)$^{AB}$ | 1.56 (0.00)$^{B}$ |
| *i*-butyrate | −0.001 (0.005)$^{D}$ | 0.161 (0.018)$^{A}$ | 0.113 (0.000)$^{B}$ | 0.037 (0.001)$^{C}$ | 0.086 (0.005)$^{B}$ | 0.037 (0.004)$^{C}$ |
| *i*-valerate | −0.025 (0.000)$^{D}$ | 0.132 (0.012)$^{A}$ | 0.025 (0.006)$^{C}$ | 0.040 (0.002)$^{C}$ | 0.074 (0.010)$^{B}$ | 0.098 (0.000)$^{B}$ |
| ammonia[c] | 49.8 (0.0)$^{C}$ | 9.93 (1.67)$^{D}$ | 61.4 (1.1)$^{B}$ | 12.3 (0.9)$^{D}$ | 75.9 (4.7)$^{A}$ | 8.02 (0.23)$^{D}$ |

[a] Short and branched chain fatty acids expressed as μmol/mg carbohydrate; ammonia expressed as μmol/sample tube; blank has been subtracted from these data. [b] SM, starting material; AS, alkali-soluble. [c] Mean (standard error); within row, different capital letter superscripts are significantly different ($p < 0.05$); $n = 2$.

**Data Analysis.** SAS software (version 9.1, SAS Institute, Cary, NC) was used to calculate statistical differences, which were defined as $P < 0.05$. Differences among metabolites (SCFAs, BCFAs, and ammonia) produced after 24 h of in vitro fermentation and residual carbohydrate remaining at each time point during fermentation were analyzed using ANOVA followed by Fisher's least significant difference test used to determine differences among least-squares means. To determine the point at which fermentation samples reached a plateau in SCFA production, repeated measures ANOVA was used and contrast statements were used to determine significant differences among each time point and the average of subsequent time points. Significant linear and quadratic trends for the change in arabinose:xylose ratio over time during in vitro fermentation were calculated using least-squares regression.

## RESULTS AND DISCUSSION

**Sample Composition and in Vitro Digestion.** The compositions of the bran starting materials and their alkali-soluble fractions are shown in **Table 1**. The starting materials contained small to moderate concentrations of starch and protein, while these components were substantially lower in the alkali-soluble fractions. This was due to the amylase and protease treatments used during the extraction and isolation processes. A higher percentage of the alkali-soluble fraction was found in maize bran compared to rice and wheat brans. The dietary fiber composition of maize, rice, and wheat bran alkali-soluble fractions revealed that each contained mostly arabinoxylan, as these two monosaccharides (arabinose and xylose) represented the majority of neutral monosaccharides in the fractions.

Prior to performing in vitro fermentation with human fecal microbiota, each sample was treated using an in vitro procedure mimicking the digestion occurring in the upper gastrointestinal tract. This was performed to remove components that may be digested there if the product were consumed, namely starch and protein.

In vitro digestion decreased the starch content of the bran starting materials to <2%, while the alkali-soluble fractions already showed low starch contents due to the amylase used during extraction (data not shown). The protein content of all samples remained roughly constant before and after in vitro digestion, indicating that the in vitro digestion procedure was ineffective at removing protein, possibly due to the intimate association of protein with dietary fiber (*33*). Lebet et al. (*24*) also experienced difficulty in removing protein from cereal products during in vitro digestion; however, contaminating protein did not interfere with the fermentation profiles.

**SCFA Production during in Vitro Fermentation.** The alkali-soluble fraction from maize bran produced significantly more acetate and propionate than any of the other samples tested, whereas butyrate production was not significantly different from the other alkali-soluble fractions and the rice bran starting material (**Table 2**). This indicates that the maize sample was relatively highly fermentable, with efficient conversion of carbohydrate to SCFAs by the fecal bacteria. This may be beneficial due to the numerous trophic effects SCFAs have on the colonic environment (*34*). In particular, dietary fibers that have high production of propionate may help reduce cholesterol (*35*).

The alkali-soluble fraction from maize bran did not display a plateau in SCFA production during 24 h of in vitro fermentation as was experienced with the other samples tested (**Figure 1**). As mentioned, the ability to sustain high SCFA production after prolonged fermentation may be beneficial by maintaining high concentrations of SCFAs in distal regions of the colon (*36*).

**Branched Chain Fatty Acid (BCFA) and Ammonia Production during in Vitro Fermentation.** The alkali-soluble fraction from maize bran produced more BCFAs than any other sample (**Table 2**). Production of BCFAs is often used as a marker for protein putrification. Although no detrimental effects of BCFAs themselves have been demonstrated, their evolution often accompanies the production of undesirable, toxic metabolites (*14*). The alkali-soluble fraction from maize bran did not result in higher

production of ammonia during fermentation compared to the other alkali-soluble samples. Thus, the higher BCFA production by the microbiota exposed to the alkali-soluble fraction from maize bran was likely due to the induction of higher bacterial metabolism, an observation also suggested by the SCFA production data.

**Carbohydrate Loss and Changes in A/X Ratio during in Vitro Fermentation.** There were some discrepancies when comparing the SCFA production and carbohydrate disappearance data. The SCFA data revealed that the alkali-soluble fraction from maize bran fermented in a linear fashion with significant production of SCFAs even after 12 h of fermentation, while the alkali-soluble fractions from rice and wheat bran showed asymptotic productions of SCFAs, reaching plateaus in after 12 h (**Figure 1**). This suggests that there would be more residual carbohydrate in the alkali-soluble fraction from maize bran at $t = 12$ h, which could then be fermented during the latter half of the fermentation period. Instead, the alkali-soluble fraction from wheat bran showed the highest concentration of residual carbohydrate at both 8 and 12 h of fermentation when compared to the other alkali-soluble fractions (**Table 3**). This may be due to differences in the fine structures of the alkali-soluble fractions from the various sources. For instance, the alkali-soluble fraction from maize bran may contain structural components that are conducive to slow, complete fermentation, with efficient bacterial conversion to SCFAs, while the alkali-soluble fraction from wheat bran may contain some structural components that are rapidly degraded, accompanied by others that are more difficult to metabolize and are not converted well to SCFAs as fermentation progresses.

Because arabinoxylans consist, basically, of a xylose backbone with arabinose side chains (*19*), the arabinose to xylose ratio is a rough estimate of the degree of branching. Trends for the change in arabinose:xylose ratios during fermentation among the alkali-soluble fractions showed substantial differences (**Figure 2**). Wheat bran arabinoxylans, for instance, displayed a parabolic-shaped relationship, suggesting that these polysaccharides fermented by a two-stage mechanism. A possible explanation for this is that the arabinoxylans found in wheat bran contain unsubstituted backbone xylosyl regions that are easily hydrolyzed by bacterial xylanases and then rapidly ferment. Indeed, enzymatic degradation studies of alkali-soluble fractions from wheat bran have suggested regions of up to six repeating unsubstituted xylosyl units (*37*). The more efficient fermentation of these regions would result in an increase in the arabinose:xylose ratio initially. Once the

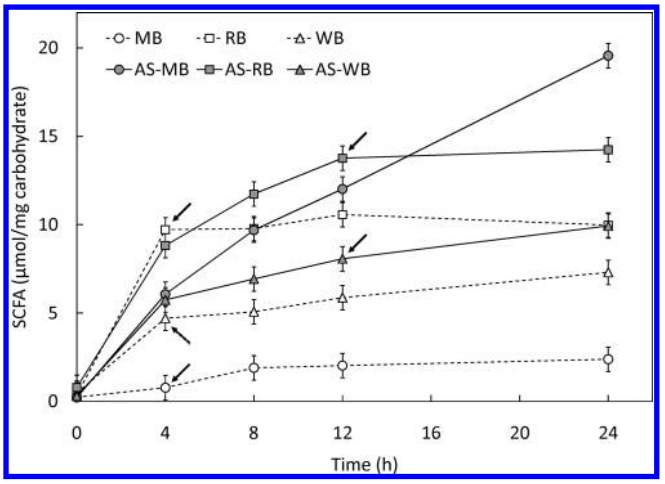


**Figure 1.** Short chain fatty acid (SCFA) production during in vitro fecal fermentation. Data points marked with arrows indicate the first time point that is not significantly different from average of subsequent time points in each series (i.e., when the SCFA production reaches a plateau); SM, starting material; AS, alkali-soluble; MB, maize bran; RB, rice bran; WB, wheat bran; blank has been subtracted from these data; error bars show standard error; $n = 2$.

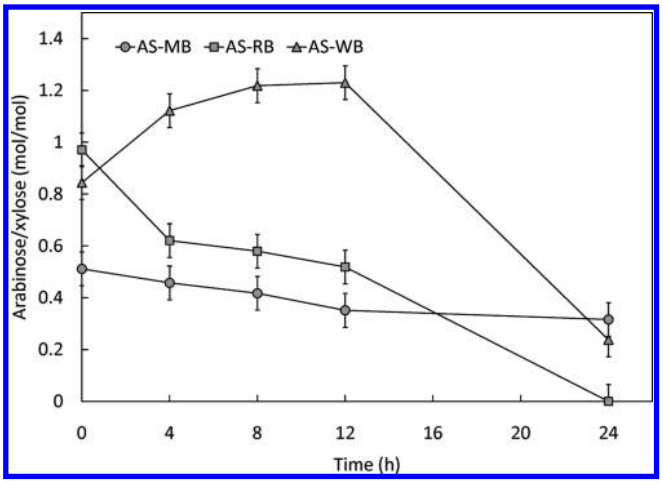


**Figure 2.** Changes in arabinose/xylose ratio during in vitro fecal fermentation. For the AS-RB sample at $t = 24$ h, the arabinose/xylose ratio was 0, meaning that no arabinose, but some xylose, was detected in the fermentation medium; SM, starting material; AS, alkali-soluble; MB, maize bran; RB, rice bran; WB, wheat bran; blank has been subtracted from these data; error bars show standard error; $n = 2$; $p$-values for linear trends over time are 0.0084, 0.0119, and 0.0019 and for quadratic trends are 0.8890, 0.7904, and 0.0003 for AS-MB, AS-RB, and AS-WB, respectively.

**Table 3.** Total Carbohydrate (Arabinose, Xylose) Remaining in Fecal Surries during in Vitro Fecal Fermentation[a]

| | maize bran | | rice bran | | wheat bran | |
|---|---|---|---|---|---|---|
| time (h) | SM[b] | AS | SM | AS | SM | AS |
| 0 | 100 | 100 | 100 | 100 | 100 | 100 |
| | (21.8, 40.2) | (29.5, 57.7) | (16.6, 16.7) | (39.8, 41.0) | (25.1, 39.1) | (42.2, 50.0) |
| 4[c] | 79.5$^{A}$ | 61.4$^{B}$ | 31.0$^{C}$ | 28.5$^{C}$ | 61.6$^{B}$ | 54.8$^{B}$ |
| | (18.0, 34.5) | (15.5, 33.9) | (8.73, 11.0) | (6.51, 10.5) | (15.5, 23.5) | (24.9, 22.2) |
| 8 | 77.6$^{A}$ | 26.7$^{D}$ | 36.8$^{C}$ | 14.9$^{E}$ | 55.9$^{B}$ | 41.2$^{C}$ |
| | (17.0, 32.9) | (4.33, 10.4) | (7.96, 9.51) | (2.73, 4.70) | (16.8, 21.1) | (19.2, 15.8) |
| 12 | 70.1$^{A}$ | 15.0$^{D}$ | 26.7$^{C}$ | 11.6$^{D}$ | 50.2$^{B}$ | 25.8$^{C}$ |
| | (14.4, 28.2) | (1.24, 3.56) | (4.80, 6.11) | (2.07, 3.99) | (14.0, 16.6) | (11.2, 9.08) |
| 24 | 59.0$^{A}$ | 5.95$^{D}$ | 23.6$^{C}$ | 5.33$^{D}$ | 44.8$^{B}$ | 5.05$^{D}$ |
| | (12.2, 23.9) | (0.47, 1.47) | (3.80, 5.38) | (0.00, 1.84) | (13.9, 13.4) | (0.37, 1.51) |

[a] Expressed as a percentage of the initial total carbohydrate content ($t = 0$ h). [b] SM, starting material; AS, alkali-soluble. [c] Means within row with different capital letter superscripts are significantly different ($p < 0.05$); $n = 2$.

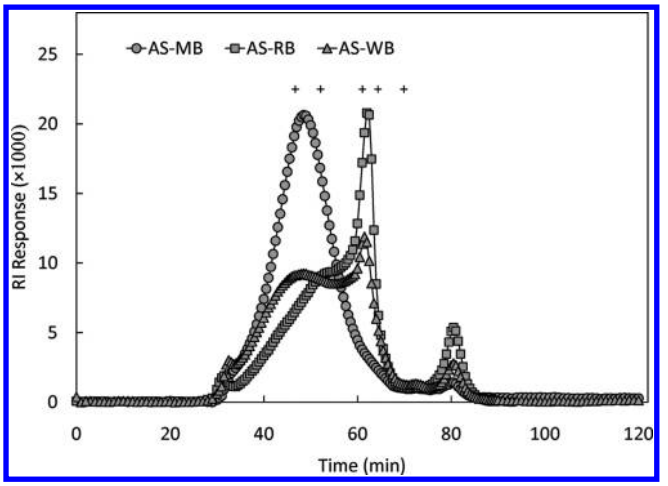


**Figure 3.** Size-exclusion chromatography elution profiles of alkali-soluble bran fractions. Peak retention times of pullulan standards of molecular weight $78.8 \times 10^4$, $21.2 \times 10^4$, $4.73 \times 10^4$, $1.18 \times 10^4$, and $0.74 \times 10^4$ are indicated by + (left to right); AS, alkali-soluble; MB, maize bran; RB, rice bran; WB, wheat bran; RI, refractive index.

unbranched regions of wheat arabinoxylans were metabolized, however, the remaining densely branched oligosaccharides would provide resistance to bacterial hydrolysis due to steric hindrance, explaining the eventual decrease in the arabinose:xylose ratio.

The maize bran and rice bran fractions showed decreasing, linear trends for the change in arabinose:xylose ratios during fermentation (**Figure 2**). Because arabinosyl moieties generally constitute the side chains and xylosyl moieties constitute the backbone (*19*), these decreasing trends suggest that fermention proceeds by a debranching mechanism. Clearly, however, from the slopes of the lines, these two fractions were not metabolized in exactly the same fashion: there was a much more rapid decrease in the arabinose:xylose ratio in the alkali-soluble fraction from rice bran compared the maize bran fraction. Thus, bacteria were effectively required to remove the arabinosyl moieties from the rice bran arabinoxylans before the xylosyl moieties could be utilized, whereas for maize bran arabinoxylans, the bacteria utilized both sugars relatively simultaneously. This could be the result of the more highly branched nature of the rice bran arabinoxylans compared to the maize bran arabinoxylans, as demonstrated by the substantially higher arabinose:xylose ratio for rice bran compared to corn bran initially. Alternatively, maize bran arabinoxylans may also have a higher proportion of branches that contain both arabinose and xylose than rice bran arabinoxylans. This is suggested from $^1$H NMR spectra (discussed below) and previous works that have shown that alkali-soluble arabinoxylans from rice bran contain only 2.2−2.9% terminal xylose units (*38*), while alkali-soluble maize bran arabinoxylans contain 18.4−19.0% terminal xylose units (*33*). Thus, in the process of debranching maize bran arabinoxylans, bacteria would concomitantly metabolize arabinosyl and xylosyl moieties, while in the latter case, mostly arabinosyl moieties would be metabolized initially.

**Size-Exclusion Chromatography of the Alkali-Soluble Fractions.** The alkali-soluble fraction from maize bran contained a less complex SEC profile than the fractions from rice and wheat brans with the former showing a single peak (normalized peak area, 98.5%) that crested at 49 min (~500 kDa) (**Figure 3**). Both the rice bran and wheat bran fractions showed a small peak (normalized peak areas, 1 and 3%, respectively) at 31 min. This represented the void volume of the column and was far too large to represent a single polymer, thus likely representing polymer aggregation.

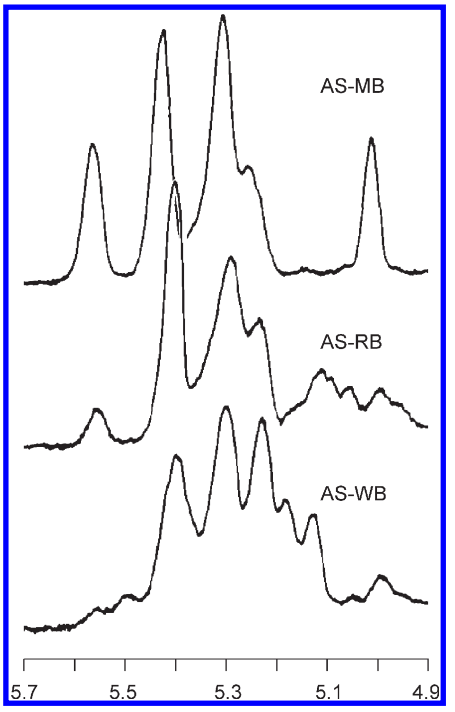


**Figure 4.** Anomeric region of $^1$H NMR spectra of alkali-soluble fractions. AS, alkali-soluble; MB, maize bran; RB, rice bran; WB, wheat bran.

The chromatograms for the rice and wheat fractions were immediately followed by two large unresolved peaks. The first peak crested at 53 (~200 kDa) and 49 min (~500 kDa) for the rice and wheat fractions, respectively, and the second peak showed a maximum at 62 min (~30 kDa) in both fractions. In the rice bran fraction, the peak area was nearly equally divided between the high and low molecular weight peaks, with normalized peak areas of 41 and 48%, respectively. The wheat bran fraction, however, contained a predominance of the higher molecular weight polymers (normalized peak area, 60%) compared to low molecular weight (normalized peak area, 33%). Both the rice and wheat bran fractions also showed a very low molecular weight peak at 81 min (~600 Da), with normalized peak areas of 9 and 3%, respectively.

The finding that alkali-soluble maize and wheat arabinoxylan molecular weight distributions each showed two fractions may explain their high initial productions of SCFAs, followed by a plateau (**Figure 1**); one of the fractions may have fermented more rapidly than the other. Alternatively, types of linkages among monosaccharide residues (e.g., α vs $\beta$) or the degree of substitution in a given polysaccharide may have influenced the fermentation profile (*39*, *40*).

**$^1$H NMR of the Alkali-Soluble Fractions.** The portion of the $^1$H NMR spectrum for each of the alkali-soluble arabinoxylans where the anomeric protons of the α-linked arabinose units in an arabinoxylan resonate is shown in **Figure 4**. The resonances at 5.40, 5.30, and 5.23 ppm present in all of the alkali-soluble fractions are characteristic of the anomeric protons of terminal arabinose units linked to main chain xylose residues. The resonance at 5.40 ppm represents the anomeric protons of Ara*f* linked to O-3 of Xyl*p* on the main chain (*30*, *31*). The two peaks at 5.30 and 5.23 ppm represent the anomeric protons of Ara*f* linked to O-3 and O-2 of the same Xyl*p* residue on the backbone (*30*, *31*). The peak at 5.30 ppm is larger than the peak at 5.23 ppm because the peak at 5.30 also represents the anomeric protons of Ara*f* linked to O-2 of monosubstituted Xyl*p* residues on the main chain (*32*).

Each spectrum contained additional resonances that may be attributed the anomeric protons of arabinose residues, including

peaks between 5.00 and 5.20 and at 5.53 ppm. The resonance at 5.53, which is present in the alkali-soluble fractions from maize bran and rice bran, may be attributed to a disaccharide side chain with the structure: β-D-Xyl*p*-(1→2)-α-L-Ara*f* linked to O-3 of Xylp on the main chain (*29*). For this side chain to be present, the $^1$H NMR spectrum must also show a resonance corresponding to the anomeric proton of the Xyl*p* residue, which occurs at 4.56 ppm (*41*). The alkali-soluble fraction from maize bran contained a clear resonance at this position, and although the fraction from rice bran did not, it may have been buried under other peaks present in this region (data not shown). The alkali-soluble fraction from maize bran contained the highest proportion of this disaccharide side chain. The presence of this side chain, particularly if it were evenly distributed along the xylan backbone, may be a contributing factor to the linear fermentation profile of this sample through the difficulty in hydrolyzing the unusual (1→2) linkage.

Structural assignments for the resonances between 5.00 and 5.20 ppm were more difficult to define. Literature data suggest that these resonances represent substituted arabinose units (multiunit branches or branched branches) (*42*−*44*). The peak at 5.00 ppm in particular has been shown in a number of previous reports (*45*−*47*) but has not been specifically identified. The peaks at 5.19 and 5.12 ppm, present in the alkali-soluble fraction from wheat bran, have also been previously found in rye bran after barium and potassium hydroxide extraction (*46*) and in barley after barium hydroxide extraction (*48*), but were not defined.

Because the $^1$H NMR spectra suggest that not all arabinose units are involved in single unit branches, the equations of Roels et al. (*49*) for calculating the distribution of un-, mono-, and disubstituted xylose residues on arabinoxylan, are not valid; however, from the quantitative integrals of the resonances at 5.40, 5.30, and 5.23, the ratio of monosubstituted to disubstituted xylose residues containing single-unit arabinose branches could be calculated. For the alkali-soluble fractions from maize, rice, and wheat brans, this ratio was 1.70, 1.44, and 0.85. This indicates that the alkali-soluble fraction from wheat bran contained a higher proportion of disubstituted xylose residues (and more unsubstituted regions), which was expected from the parabolic relationship observed in arabinose to xylose ratio during fermentation (**Figure 2**). Additionally, this may explain why the alkali-soluble fraction from wheat bran was comparably poorly fermented during latter stages of fermentation (12−24 h), i.e. the disubstituted xylose residues were more difficult to digest.

In conclusion, these data reveal two important findings regarding the fermentation of alkali-soluble fractions from cereal brans. First, the fermentation profile of the alkali-soluble fraction from maize bran is linear with a high production of SCFAs during 24 h of in vitro fermentation. These unique characteristics are not present for the same fractions from rice and wheat bran. Second, arabinoxylans may be degraded by different mechanisms depending on structure. Changes in the arabinose:xylose ratio during fermentation suggest that maize and rice bran arabinoxylans are degraded by a debranching mechanism, while wheat bran arabinoxylans are degraded by a two-phase mechanism wherein bacteria utilize unsubstituted xylose regions initially and then arabinose side chains are subsequently metabolized. Additional research is necessary to substantiate these claims and elucidate the true benefits of these dietary fiber fractions in humans.

## ABBREVIATIONS USED

Ara*f*, α-L-arabinofuranose; AS, alkali-soluble; BCFA, branched chain fatty acids; MB, maize bran; RB, rice bran, SCFA, short chain fatty acid; SEC, size-exclusion chromatography; SIEM, standard ileal efflux medium; SM, starting material; WB, wheat bran; Xyl*p*, (1→4)-linked β-D-xylopyranose.

## SAFETY

The materials used in this study require no special safety considerations beyond basic laboratory safety.

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