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# In Vitro Fermentation of Arabinoxylan Oligosaccharides and Low Molecular Mass Arabinoxylans with Different Structural Properties from Wheat (*Triticum aestivum* L.) Bran and Psyllium (*Plantago ovata* Forsk) Seed Husk

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**ABSTRACT:** Ball milling was used for producing complex arabinoxylan oligosaccharides (AXOS) and low molecular mass arabinoxylans (AX) from wheat bran, pericarp-enriched wheat bran, and psyllium seed husk. The arabinose to xylose ratio of the samples produced varied between 0.14 and 0.92, and their average degree of polymerization (avDP) ranged between 42 and 300. Their fermentation for 48 h in an in vitro system using human colon suspensions was compared to enzymatically produced wheat bran AXOS with an arabinose to xylose ratio of 0.22 and 0.34 and an avDP of 4 and 40, respectively. Degrees of AXOS fermentation ranged from 28% to 50% and were lower for the higher arabinose to xylose ratio and/or higher avDP materials. Arabinose to xylose ratios of the unfermented fractions exceeded those of their fermented counterparts, indicating that molecules less substituted with arabinose were preferably fermented. Xylanase, arabinofuranosidase, and xylosidase activities increased with incubation time. Enzyme activities in the samples containing psyllium seed husk AX or psyllium seed husk AXOS were generally higher than those in the wheat bran AXOS preparations. Fermentation gave rise to unbranched short-chain fatty acids. Concentrations of acetic, propionic, and butyric acids increased to 1.9−2.6, 1.9−2.8, and 1.3−2.0 times their initial values, respectively, after 24 h incubation. Results show that the human intestinal microbiota can at least partially use complex AXOS and low molecular mass AX. The tested materials are thus interesting physiologically active carbohydrates.

**KEYWORDS:** *wheat bran, psyllium seed husk, ball milling, arabinoxylan oligosaccharides, in vitro fermentation, short-chain fatty acids, enzyme activity*

## INTRODUCTION

Arabinoxylan oligosaccharides (AXOS), hydrolysis products of plant cell wall arabinoxylans (AX), selectively stimulate the growth and activity of beneficial colon bacteria and meet the criteria to be considered as prebiotics.[1] Effects of prebiotics in the gut include the growth of health promoting bacteria such as lactobacilli and bifidobacteria, the increase in production of short-chain fatty acids such as butyric and propionic acid, which are believed to be positive for colonic health, and the decrease of toxic bacterial metabolites such as polyamines and ammonia. AXOS are fermented very well in vitro and exert bifidogenic effects.[1,2] Administration of AXOS to mice, rats, chickens, and humans selectively increases colon bifidobacteria levels.

Millers' wheat bran is an AX-rich, low-cost byproduct of conventional wheat milling and, therefore, a logical starting material for producing AXOS. Wheat bran AXOS have a backbone of $\beta$-1,4-linked xylopyranose residues, with some arabinofuranose residues on the C(*O*)-2 and/or C(*O*)-3 positions of xylose. Some uronic acids, mostly glucuronic acid, can also occur on the C(*O*)-2 position of xylose.[1] During enzymatic release of AXOS, mostly wheat bran aleurone AX with a low degree of xylose substitution by arabinose, or, stated otherwise, with a low arabinose to xylose ratio are hydrolyzed. Indeed, the highly substituted wheat bran pericarp AX are only poorly enzymatically degradable.[3] This implies that the structural diversity of the components examined so far is limited, with most preparations containing AXOS with an arabinose to xylose ratio of ca. 0.2−0.3 and an average degree of polymerization (avDP) below 60.[1,4,5] An alternative method for producing AXOS from wheat bran is ball mill treatment.[6] It renders AX from both aleurone and pericarp layers water-extractable as it significantly reduces their molecular mass, thus producing AXOS with a significantly higher average arabinose to xylose ratio than that of those obtained by enzymatic production.[6]

Psyllium (*Plantago ovata* Forsk) seed husk is an also promising starting material for AXOS production.[6,7] The structure of psyllium seed husk AX largely differs from that of wheat bran AX. Psyllium seed husk AX are highly branched polysaccharides with a main chain of densely

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substituted $\beta$-1,4- and $\beta$-1,3-linked xylopyranose residues. Single arabinofuranose and xylopyranose residues or short side chains consisting of these monosaccharides are attached at the C(*O*)-2 and/or C(*O*)-3 positions of the main-chain xylose residues.[8] In addition, the backbone of psyllium seed husk AX contains residues such as rhamnose (3−5%) and galacturonic acid (5−8%).[8,9] The structural features of psyllium seed husk AX thus allows producing AXOS with structures differing from those of wheat bran AXOS. Also here, ball milling is an effective way to increase the levels of water-extractable AX and to produce low molecular mass AX and AXOS from psyllium seed husk.[7]

Considering the large influence of carbohydrate structure on fermentation in the gut,[10,11] we hypothesized that fermentation of AXOS from wheat bran pericarp and psyllium seed husk origin would be significantly different from that of AXOS structures investigated so far. Knowledge on the differential AXOS fermentation profiles may be important for future studies investigating the prebiotic potential of these new AXOS sources. The main objective of this study was, therefore, to investigate the in vitro fermentabilities of several AXOS and low molecular mass AX preparations produced from ball milled wheat bran and PSH, and to compare them with those of enzymatically produced wheat bran AXOS. Carbohydrate consumption, enzyme activities, and short-chain fatty acid production were measured upon incubation of AXOS and AX with a colon compartment suspension of the Simulator of the Human Intestinal Microbial Ecosystem.[12]

## MATERIALS AND METHODS

**Materials.** Commercial wheat bran and milled psyllium seed husk (85% husk material, overall particle size <250 $\mu$m) were obtained from Dossche Mills & Bakeries (Deinze, Belgium) and Biofiber-Damino (Gesten, Denmark), respectively. Enzyme preparations from Novozymes (Bagsvaerd, Denmark) were heat-stable $\alpha$-amylase "Termamyl 120 L", bacterial protease "Neutrase 0.8 L", and a glycoside hydrolase family 10 xylanase from *Aspergillus aculeatus* "Shearzyme 500 L". Glycoside hydrolase family 11 *Bacillus subtilis* xylanase "Grindamyl H640" was from Danisco (Brabrand, Denmark), and Xylazyme AX tablets were from Megazyme (Bray, Ireland). Xylanase units were defined as described further. Units of amylase and protease activity were as defined by the suppliers. Chemicals and reagents were purchased from Sigma-Aldrich (Bornem, Belgium) and were of at least analytical grade.

**Production of AX and AXOS Preparations.** In what follows, the production of AX and AXOS preparations from either wheat bran or psyllium seed husk is described. The resulting preparations are coded as WB-xx-0.yy or PSH-xx-0.yy to indicate their source (WB, wheat bran; PSH, psyllium seed husk, avDP (xx) and arabinose to xylose ratio (0.yy)).

*Production of Destarched Deproteinized Wheat Bran and Wheat Pericarp Enriched Material.* A suspension of wheat bran in tap water (1:10 w/v) was treated with Termamyl 120 L (120 units/kg wheat bran, 90 min, 90 °C) and then with Neutrase 0.8 L (32 units/kg wheat bran, 4 h, 50 °C, pH 6.0) to hydrolyze starch and residual proteins, respectively. The suspension was then boiled for 20 min, filtered with a cellulose filter (Whatman International, Maidstone, UK), and the retentate was washed with water and lyophilized, yielding destarched deproteinized wheat bran. Wheat pericarp enriched material was produced from destarched deproteinized wheat bran as follows: destarched deproteinized wheat bran was suspended in deionized water (1:14 w/v) and incubated under continuous stirring with *B. subtilis* (1.4 xylanase units/g destarched deproteinized wheat bran) and *A. aculeatus* (21 xylanase units/g destarched deproteinized wheat bran) xylanases for 16 h at 50 °C. After inactivation of the enzymes by boiling (20 min) and filtration, the retentate was lyophilized.

*Production of Ball Milled Wheat Bran, Destarched Deproteinized Wheat Bran, or Wheat Pericarp Enriched Material.* Wheat bran, destarched deproteinized wheat bran, or wheat pericarp enriched material was ground (500 rpm, 24 h, 20% filling degree) in a laboratory Retsch (Haan, Germany) PM 100 planetary ball mill, equipped with a 250 mL zirconium milling jar with 6 zirconium balls (20 mm diameter).

*Production of Ball Milled Psyllium Seed Husk.* Psyllium seed husk was ground (500 rpm, 48 h, 30% filling degree) in the above Retsch PM 100 mill.

*Production of WB-74-0.61, WB-46-0.63, and WB-42-0.92.* Ball milled wheat bran, ball milled destarched deproteinized wheat bran, and ball milled wheat pericarp enriched material, respectively, were extracted with deionized water (2.0% w/v, 60 min, 6 °C) under continuous stirring. After centrifugation (10 000*g*, 20 min, 18 °C), the residue was re-extracted with deionized water (30 min, 6 °C) and centrifuged (10 000*g*, 20 min, 18 °C). Supernatants were combined and proteins removed with silica gel as described previously.[13] Minerals were removed by treatment (30 min, room temperature) with the mixed bed ion-exchange resin Ionac NM 60 (Sybron Chemicals, Birmingham, UK), as described by the manufacturer. The mixture of silica and AXOS was sieved (125 $\mu$m), and the resulting filtrate was lyophilized.

*Production of WB-40-0.34 and WB-4-0.22.* Destarched deproteinized wheat bran was incubated under continuous stirring for 10 h at 50 °C with *B. subtilis* xylanase (1.2 xylanase units/g destarched deproteinized wheat bran) or with *B. subtilis* (1.2 xylanase units/g destarched deproteinized wheat bran) and *A. aculeatus* xylanases (21 xylanase units/g destarched deproteinized wheat bran), respectively. After inactivation of the enzymes by boiling (30 min) and filtration, the solution was concentrated to 20% dry matter in a falling film evaporator and finally spray-dried. The resulting material was suspended in water and further purified as described for WB-74-0.61.

*Production of PSH-300-0.29.* Ball milled psyllium seed husk was extracted with deionized water (2.0% w/v, 60 min, 18 °C) under continuous stirring. After centrifugation (10 000*g*, 20 min, 18 °C), the residue was re-extracted with deionized water (30 min, 18 °C) and centrifuged (10 000*g*, 20 min, 18 °C). Supernatants were combined. Proteins were removed from the supernatant with silica gel.[13] The resulting supernatant was dialyzed against water (48 h, 6 °C, 1:20 v/v) and lyophilized.

*Production of PSH-200-0.27.* Ball milled psyllium seed husk was suspended in water (2.5% w/v). The pH was adjusted to 2.8 with 1.0 M HCl, and the suspension was incubated for 16 h at 80 °C under continuous stirring. After being cooled to room temperature and neutralized to pH 7.0, the suspension was centrifuged (10 000*g*, 20 min, 18 °C). Ethanol (95%) was slowly added to the supernatant to a concentration of 85%. The mixture was then stirred for 30 min at room temperature and kept overnight at 6 °C. The precipitate was recovered by centrifugation (10 000*g*, 20 min, 6 °C), solubilization in deionized water, and lyophilization.

*Production of PSH-88-0.16 and PSH-72-0.14.* These samples were prepared similarly to PSH-200-0.27, except that the temperature−pH conditions for acid hydrolysis were different. Incubation at 90 °C and pH 2.8 resulted in PSH-88-0.16, while incubation at 90 °C and pH 2.6 resulted in PSH-72-0.14.

**Characterization of the AX and AXOS Preparations.** Moisture contents were analyzed according to AACC International Method 44-19.[14] Protein contents were estimated colorimetrically with the Folin−Ciocalteu reagent,[15] using bovine serum albumin as standard. Total, reducing end, and monomeric sugar contents were determined by gas chromatography analysis of alditol acetates as described earlier.[6] The AX content was calculated as 0.88 times the sum of the contents of the monosaccharides arabinose and xylose.[5] Combination of data for total and reducing end monosaccharides allowed one to calculate

the arabinose to xylose ratio and avDP of the AXOS preparations as follows:

$$\text{arabinose to xylose ratio} = \frac{\text{total arabinose concentration} - \text{monomeric arabinose concentration}}{\text{total xylose concentration} - \text{monomeric xylose concentration}}$$

$$\text{avDP} = \frac{(\text{total xylose} - \text{monomeric xylose}) + (\text{total arabinose} - \text{monomeric arabinose})}{\text{reducing end xylose} - \text{monomeric xylose}}$$

Because the main-chain xylose residues of psyllium seed husk AX can carry single arabinose and xylose residues as well as short side chains consisting of these monosaccharides, while wheat bran AX are substituted with monomeric arabinose, arabinose to xylose ratios and avDP of psyllium seed husk AX and AXOS and wheat bran AXOS reflect different structural properties. Only for wheat bran AXOS does the arabinose to xylose ratio reflect the degree of branching.

**In Vitro Fermentation.** Batch experiments were performed by sampling 15 mL from the descending colon compartment of the Simulator of the Human Intestinal Microbial Ecosystem. This simulator is a multicompartment dynamic model of the human digestive tract representing stomach, small intestine, and large intestine conditions. The reactor was inoculated with a microbial community, isolated from a fecal sample from a human volunteer and following a stabilization period of 3 weeks; the three colon compartments (ascending, transverse, descending) particularly harbor a microbial community of which the composition and the metabolic activity are representative of that in the in vivo situation.[12] No additional nutrients were provided as we wanted AX and AXOS to be the primary C-source in the medium. This way, the potency of in vitro cultured gut microbes to metabolize the different AXOS structures was evaluated.

Following sampling of 15 mL of the descending colon suspension, this microbial suspension was subsequently incubated with 1% (150 mg) of AX or AXOS under anaerobic conditions (flushing of the headspace with $N_2$ for 30 min) for 48 h at 37 °C. At 6, 24, and 48 h, samples were taken from the incubated mixture with a needle protruding the butyl rubber stoppers that seal off the incubation bottles. Upon sampling, the mixture was flushed to ensure anaerobic conditions. Samples were immediately stored at −20 °C until further analysis.

**Metabolic Activity Analysis.** Before analysis, samples were centrifuged (3000*g*, 4 °C, 15 min). Residual sugar contents in fermentation samples were determined by gas chromatography analysis as described previously.[6] For analysis of short-chain fatty acids, the following were added to vials containing 1.00 mL of fermentation sample: 0.50 mL of 9.2 M sulfuric acid, 0.40 mL of 0.75% (v/v) 2-methylhexanoic acid (internal standard), 0.40 g of NaCl, and 2.00 mL of diethyl ether. Following shaking during 2 min, the vials were centrifuged (3000*g*, 3 min), and the diethyl ether phases containing the organic acids were analyzed with gas chromatography as described earlier.[16]

Extracellular xylanase, arabinofuranosidase, and xylanase activities were assayed. Xylanase activities were determined using an adaptation of the Xylazyme AX method described in the Megazyme T-XYZ200 03/06 data booklet. An aliquot (50 μL) was added to 450 μL of McIlvaine buffer pH 6.5, prepared by mixing appropriate amounts of 0.10 M citric acid and 0.20 M disodium phosphate. A Xylazyme AX tablet was added after a preincubation of 10 min at 37 °C. After 4 h incubation, the reaction was stopped by adding 5.0 mL of 2.0% (w/v) Tris solution followed by vigorous vortex mixing. The solutions were filtered, and the extinction at 590 nm was measured with an Ultraspec III UV/vis spectrophotometer (Pharmacia Biotech, Uppsala, Sweden) against a control, prepared by adding a Xylazyme AX tablet only after inactivation of the sample with Tris solution. Activities were expressed in xylanase units. One xylanase unit is the amount of enzyme required to yield an extinction at 590 nm of 1.0 under the conditions of the assay.

Arabinofuranosidase and xylosidase activities were determined using *p*-nitrophenyl-α-L-arabinofuranoside and *p*-nitrophenyl-β-D-xylopyranoside. Aliquots (50 μL) of *p*-nitrophenyl-α-L-arabinofuranoside or *p*-nitrophenyl-β-D-xylopyranoside solutions (5.0 mM) in the above McIlvaine buffer pH 6.5 were added to a 50 μL sample and incubated at 37 °C in a microtiter plate. The reaction was stopped by adding 100 μL of Tris solution (2.0% w/v). In a control assay, *p*-nitrophenyl-β-D-xylopyranoside or *p*-nitrophenyl-α-L-arabinofuranoside solution was only added after addition of Tris solution. The release of *p*-nitrophenol from the *p*-nitrophenol-glycosides was determined colorimetrically at 415 nm with a microtiter plate reader model 680 (Biorad Laboratories, Nazareth, Belgium). Calibration curves, made with *p*-nitrophenol (0−0.05 mM), allowed activity quantitation.

Suspension from the descending colon compartment of the Simulator of the Human Intestinal Microbial Ecosystem without added AXOS was analyzed as a control (time 0 h).

**Statistics.** A two-way repeated measurements ANOVA was used to investigate the effect of AXOS substrate and incubation time on carbohydrate consumption, enzyme activities, and short-chain fatty acid production. Statistical Analysis System software (SAS Institute, Cary, NC) was used.

## RESULTS

**Composition and Structural Features of Isolated AX and AXOS.** AX and AXOS preparations with different structural features were obtained from wheat bran and psyllium seed husk. WB-74-0.61, WB-46-0.63, and WB-42-0.92, all produced from ball milled wheat bran, had lower AXOS contents (42−66%) than the other preparations, whereas enzymatically produced wheat bran AXOS preparations (WB-40-0.34 and WB-4-0.22) had high AXOS contents (77−81%) (Table 1). The low AX content of WB-74-0.61 (42%) corresponded to a high content of glucose (22%), probably originating from starch and β-glucan, which were coextracted upon aqueous extraction of ball milled wheat bran. As a starch removing step was included in the production procedures of the other wheat bran AXOS preparations, they contained less glucose, and, consequently, more AXOS. Arabinose to xylose ratios of enzymatically produced wheat bran AXOS were low (0.22 and 0.34), while AXOS produced from ball milled wheat bran had higher arabinose to xylose ratios (0.61−0.92). The avDP of the wheat bran AXOS preparations varied from 4 to 74.

All four psyllium seed husk preparations had a high AX content (75−83%). The arabinose to xylose ratios and avDP values of the AX and AXOS material in these preparations varied from 0.14 to 0.29 and from 72 to 300, respectively. The most drastic hydrolysis conditions (90 °C, pH 2.6) resulted in AXOS with a low arabinose to xylose ratio (0.14) and avDP (72), while less drastic conditions (80 °C, pH 2.8) resulted in a higher arabinose to xylose ratio (0.27) and avDP (200).

**Table 1. AX, Glucose, Galactose, and Protein Content and Structural Properties of AX/AXOS in the Different Wheat Bran (WB) or Psyllium Seed Husk (PSH) Derived AX or AXOS Preparations**

| | AX (% dm)[a,c] | arabinose to xylose ratio | avDP[b] | glucose (% dm) | galactose (% dm) | protein (% dm) |
|---|---|---|---|---|---|---|
| WB-74-0.61 | 42 | 0.61 | 74 | 22 | 2 | 11 |
| WB-46-0.63 | 66 | 0.63 | 46 | 7 | 2 | 9 |
| WB-42-0.92 | 61 | 0.92 | 42 | 8 | 3 | 20 |
| WB-40-0.34 | 77 | 0.34 | 40 | 1 | 1 | 1 |
| WB-4-0.22 | 81 | 0.22 | 4 | 12 | 1 | 3 |
| PSH-300-0.29 | 60 | 0.29 | 300 | 1 | 3 | 2 |
| PSH-200-0.27 | 83 | 0.27 | 200 | 1 | 5 | 6 |
| PSH-88-0.16 | 80 | 0.16 | 88 | 1 | 4 | 7 |
| PSH-72-0.14 | 78 | 0.14 | 72 | 1 | 4 | 7 |

[a]dm, dry matter. [b]Average degree of polymerization. [c]Average values of triplicate measurements are shown. Error on these measurements is <3%.

**In Vitro Fermentation of AX and AXOS.** The in vitro fermentability of all wheat bran AXOS and psyllium seed husk AX and AXOS preparations was assessed by incubating each sample with descending colon suspension from the Simulator of the Human Intestinal Microbial Ecosystem reactor.

*Carbohydrate Consumption.* Measurement of arabinose and xylose concentrations during incubation showed that all AX and AXOS preparations were partially fermented after 48 h. The degree of carbohydrate fermentation increased with incubation time (Figure 1). The enzymatically produced wheat bran AXOS were significantly better fermentable (52−53%) than the AXOS produced from ball milled wheat bran (28−43%). WB-74-0.61 and WB-46-0.63 were fermented to a higher extent (40−43%) than pericarp AXOS (WB-42-0.92, 28%). A significant portion (32−50%) of the psyllium seed husk AX and AXOS material was fermented after 48 h. The highest degrees of fermentation were found in PSH-72-0.14 (50%) and PSH-88-0.16 (49%). PSH-300-0.29 was the least fermentable preparation. For all preparations tested, arabinose to xylose ratios of the unfermented fraction were higher when incubating longer (Figure 2). This was more pronounced for wheat bran AXOS than for the psyllium seed husk AX and AXOS preparations.

*Enzyme Activities.* After 6 h incubation, xylanase activities (Figure 3A) had increased to 2−4 times the value at time 0 h in all samples but WB-74-0.61. Values for the psyllium seed husk AX and AXOS preparations were on average 2.1 times higher than those for the wheat bran AXOS preparations. For most preparations, xylanase activity slightly decreased upon further incubation, except for PSH-300-0.29, WB-46-0.63, and WB-42-0.92, which showed increased xylanase activities. After 48 h incubation, the highest xylanase activities were measured in PSH-300-0.29 and WB-42-0.92.

Arabinofuranosidase activities (Figure 3B) also increased during the initial incubation phase for all samples except WB-74-0.61. After 6 h incubation, the psyllium seed husk AX and AXOS preparations showed activities that were on average 3.5 times higher than activities in the wheat bran AXOS preparations, and 7−8 times higher than the value at the start of the experiment. After 48 h incubation, arabinofuranosidase activities decreased to levels similar (psyllium seed husk AX and AXOS) or significantly ($p < 0.01$) lower (wheat bran AXOS) than the values at the start of the experiment.

Xylosidase activities (Figure 3C) showed trends similar to those for arabinofuranosidase activities, although their readings were substantially lower.

*Short-Chain Fatty Acid Production.* Acetic acid concentrations strongly increased for all preparations upon incubation with human colon suspension, the increase being the strongest in the first 24 h of incubation (Figure 4A). After 24 h, values were 1.9−2.6 (42−57 mM) times higher than that of the control (22 mM). Acetic acid concentrations were significantly ($p < 0.01$) higher for three out of four psyllium seed husk AXOS preparations than for the other preparations. Values after 48 h incubation were slightly lower (38−51 mM) than those after 24 h (42−57 mM). At that time point, fermentation of all psyllium seed husk AX or AXOS preparations resulted in significantly ($p < 0.01$) higher concentrations of acetic acid than fermentation of the wheat bran AXOS preparations, except for WB-4-0.22.

Concentrations of propionic acid also increased for all preparations with incubation time (Figure 4B). Values after 6, 24, and 48 h incubation differed significantly ($p < 0.01$) from that at the start. Propionic acid concentrations were the highest after 24 h incubation, with values being 1.9−2.8 times higher (27−39 mM) than that of the control (14 mM). Concentrations after 48 h incubation (26−38 mM) were comparable to

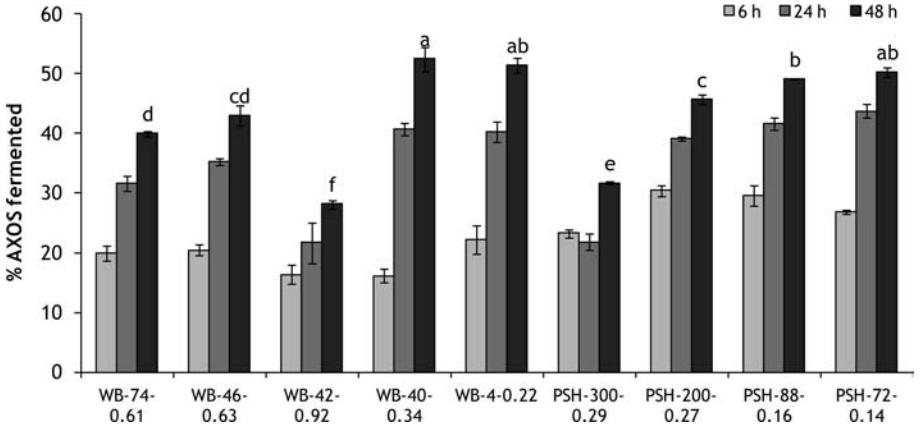


**Figure 1.** Consumption of AX or AXOS [expressed as % of AX in the preparation] after 6, 24, and 48 h incubation (37 °C, anaerobic) with human colon suspension. Values (48 h fermentation) without common letter differ significantly ($p < 0.05$). Average values of triplicate measurements are shown. Error bars represent standard deviations.


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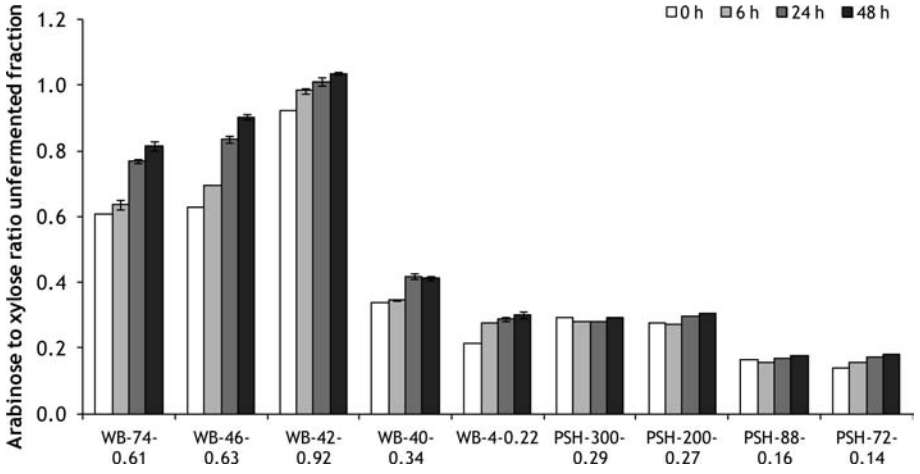


**Figure 2.** Arabinose to xylose ratios of unfermented carbohydrate fractions after 0, 6, 24, and 48 h incubation (37 °C, anaerobic) of AX or AXOS with human colon suspension. Average values of triplicate measurements are shown. Error bars represent standard deviations.

those of 24 h. After 24 and 48 h incubation, three out of four psyllium seed husk samples showed significantly ($p < 0.01$) higher concentrations than the other preparations.

After 6 h of incubation, butyric acid concentrations differed significantly ($p < 0.01$) from that at the start of the experiment for WB-74-0.61, WB-46-0.63, PSH-300-0.29, PSH-200-0.27, and PSH-88-0.16 (Figure 4C). Butyric acid concentrations increased upon further incubation, all values differing significantly ($p < 0.01$) from that of the control after 24 and 48 h incubation. Values after 24 and 48 h incubation were comparable (2.1−3.2 mM) and 1.3−2.0 times higher than the control (1.7 mM), respectively. Fermentation of PSH-300-0.29, PSH-200-0.27, and PSH-88-0.16 resulted in significantly ($p < 0.01$) higher butyric acid concentrations than fermentation of the other preparations after 24 and 48 h incubation.

For all preparations except WB-40-0.34, concentrations of branched short-chain fatty acids (sum of isobutyric and isovaleric acids) slightly increased during the first 24 h of incubation and decreased again during the last 24 h (Figure 4D). Concentrations after 48 h did not differ significantly ($p < 0.01$) from that at the start of the experiment, except for WB-46-0.63.

## DISCUSSION

**AX and AXOS Samples.** Using wheat bran and psyllium seed husk and different key processing steps, ball milling, and/or enzymatic or acid hydrolysis of AX, a range of AX and AXOS samples with different avDP and arabinose to xylose ratios were produced. To the best of our knowledge, no preparation with an arabinose to xylose ratio as high as that of WB-42-0.92 has been produced earlier. It was the result of enzymatic hydrolysis of wheat bran followed by ball milling. Its high arabinose to xylose ratio is due to pericarp AX that are rendered water-extractable upon ball milling.[6] Small differences in hydrolysis conditions (80 °C, pH 2.8 versus 90 °C, pH 2.6) can generate structurally different degradation products (PSH-200-0.27 versus PSH-72-0.14), as previously demonstrated.[17]

**Fermentations.** All preparations produced from wheat bran or psyllium seed husk were partially fermentable, but the extent of fermentation strongly differed. Among the wheat bran AXOS preparations tested, even the preparation with the highest arabinose to xylose ratio, WB-42-0.92, was partially fermented (28%). For all wheat bran AXOS preparations, fermentation of the lower arabinose to xylose ratio materials proceeded more readily than that of the more substituted molecules, resulting in arabinose to xylose ratios of unfermented fractions that were clearly higher as incubation proceeded. These observations are consistent with the finding that rye aleurone AX (arabinose to xylose ratio = 0.42) are fermented more readily and to a greater extent in pigs than pericarp AX (arabinose to xylose ratio = 1.04).[11] Disubstituted xylose residues are particularly poorly degradable by bacterial enzymes.[11] Most xylanases preferably act on unsubstituted xylose regions, the presence of numerous substituents impeding the formation of enzyme−substrate intermediates.[18] In addition, most arabinofuranosidases only release arabinoses from singly substituted xylose residues, and only a few specialized enzymes act on doubly substituted xylose residues.[19] Against this background, it is rather surprising that 28% of the saccharides in the WB-42-0.92 preparation were fermented, while rye pericarp AX with an arabinose to xylose ratio of 1.04 were not fermented at all in pigs.[11] The partial fermentability of wheat pericarp-derived AXOS observed in the present study may be explained by the low avDP (42) of the AXOS components, in contrast to the high molecular mass of the pericarp AX used in a previous study.[11]

The significantly higher fermentability (52−53%) of the enzymatically produced wheat bran AXOS preparations than of the preparations produced through ball mill treatment (28−43%, arabinose to xylose ratios 0.61−0.92) is most probably due to the lower arabinose to xylose ratios of the former (0.22−0.34), which are similar to those previously described for wheat bran derived AXOS.[5] Xylanases solubilize lowly substituted AX from the aleurone layer and nucellar epidermis, while the more complex AX in the outer pericarp are not degraded.[20] The observed fermentability of the enzymatically produced samples, however, was lower than expected from literature. Incubation of low avDP (2−11) AXOS with a human faecal inoculum indeed resulted in fermentation of the majority of the oligosaccharides within 24 h.[21] The lower values observed here are probably due to differences in experimental conditions between our and a previous study.[21] The latter created an ideal fermentation environment by adding peptone and several micronutrients to the samples.

AX or AXOS degradation requires the cooperative action of xylan degrading enzymes like xylanases, arabinofuranosidases, and xylosidases.[18] Enzyme activities generally increased for all wheat

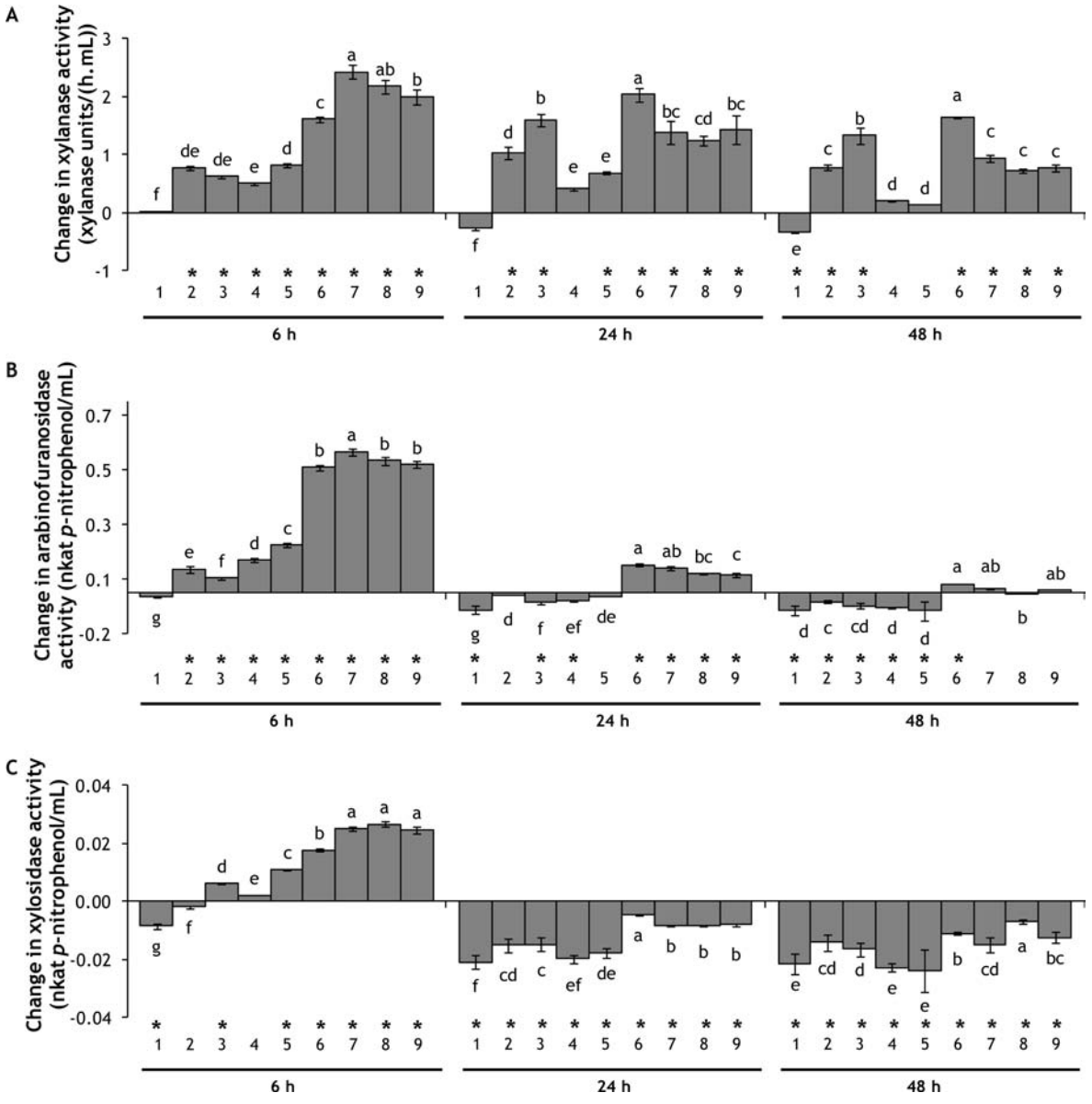


**Figure 3.** Change in (A) xylanase (xylanase units/(h·mL)), (B) arabinofuranosidase (nkat *p*-nitrophenol/mL), and (C) xylosidase (nkat *p*-nitrophenol/mL) activity after 6, 24, and 48 h incubation of AX or AXOS preparations with human colon suspension as compared to control time 0 h. 1 = WB-74-0.61; 2 = WB-46-0.63; 3 = WB-42-0.92; 4 = WB-40-0.34; 5 = WB-4-0.22; 6 = PSH-300-0.29; 7 = PSH-200-0.27; 8 = PSH-88-0.16; 9 = PSH-72-0.14. Data are expressed as means ± standard deviations. Values from the same time point differ significantly ($p < 0.01$) when not sharing a common letter. Values with an asterisk differ significantly ($p < 0.01$) from the control (time 0 h; 0.80 xylanase units/(h·mL), 0.08 nkat *p*-nitrophenol/mL, and 0.03 nkat *p*-nitrophenol/mL for (A), (B), and (C), respectively).

bran AXOS preparations during incubation, except for WB-74-0.61, where activities did not exceed the values measured at time 0. The high concentration of glucose present in this preparation (22%) probably suppresses the production of AX degrading enzymes and may initially limit direct AXOS metabolism.[2] Despite the low enzyme activities, WB-74-0.61 was fermented to a similar extent as WB-46-0.63, a preparation containing AXOS with a comparable arabinose to xylose ratio and avDP, but much less glucose than WB-74-0.61. Most likely, initial enzyme activities present in the fermentation samples were sufficient for AXOS breakdown. Also, the experimental setup that was chosen in this study allows for AXOS metabolism to occur as a long incubation time, 48 h, was chosen.

Acetic and propionic acid concentrations produced by fermentation of wheat bran AXOS differed significantly from the control for all time points measured, whereas butyric acid concentrations started to increase only after 24 h incubation. The postponed increase of butyric acid is not surprising, as this short-chain fatty acid is typically produced through cross-feeding from primary fermentation products such as lactic and acetic acids.[22] Individual short-chain fatty acid concentrations were comparable for all wheat bran AXOS preparations, although the highest increases in acetic and propionic concentrations were obtained with the AXOS preparation with the lowest arabinose to xylose ratio (0.22) and avDP (4). A preparation with a comparable arabinose to xylose ratio (0.34) but higher avDP (40) was best at suppressing the production of branched short-chain fatty acids.

Despite the complex structure of psyllium seed husk derived AX samples, incubation of the preparations resulted in fermentation degrees of up to 50% after 48 h. As psyllium


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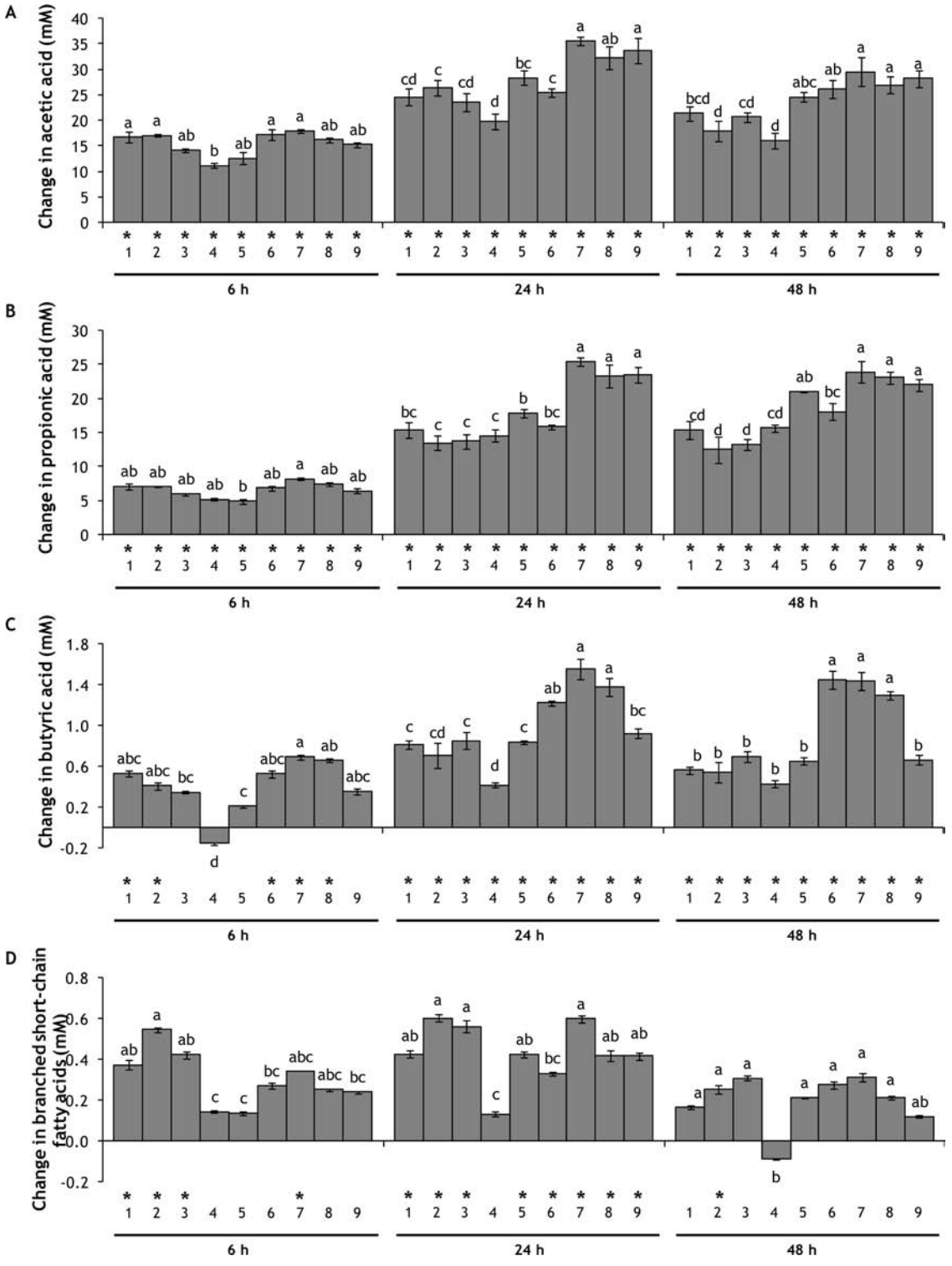


**Figure 4.** Change in (A) acetic acid, (B) propionic acid, (C) butyric acid, and (D) branched short-chain fatty acids (sum of isobutyric and isovaleric acid) concentrations after 6, 24, and 48 h incubation (37 °C, anaerobic) of AX or AXOS preparations with human colon suspension as compared to control time 0 h. 1 = WB-74-0.61; 2 = WB-46-0.63; 3 = WB-42-0.92; 4 = WB-40-0.34; 5 = WB-4-0.22; 6 = PSH-300-0.29; 7 = PSH-200-0.27; 8 = PSH-88-0.16; 9 = PSH-72-0.14. Data are expressed in millimolar as means ± standard deviations. Values from the same time point differ significantly ($p < 0.01$) when not sharing a common letter. Values with an asterisk differ significantly ($p < 0.01$) from the control (time 0 h; 21.7, 13.9, 1.7, and 37.3 mM for (A), (B), (C), and (D), respectively).

seed husk AX and AXOS fermentation still went on during the later incubation phase (24−48 h), fermentation levels exceeding 50% can be expected upon extended incubation. In earlier work, only 43% of total psyllium seed husk carbohydrates were fermented in vitro after 72 h.[9] From this, we may tentatively conclude that ball mill treatment of psyllium seed husk increases the rate and/or extent of fermentation. The treatment makes psyllium seed husk AX more accessible for enzymatic degradation by rendering the AX water-extractable and breaking covalent bonds. Whether ball milling effectively increases


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fermentability of psyllium seed husk AX and AXOS beyond the previously observed 54% of its carbohydrates upon consumption by humans[23] must be investigated with in vivo trials.

The arabinose to xylose ratio of the unfermented fraction was only slightly higher upon incubation of psyllium seed husk AX and AXOS preparations than that of the starting material, indicating that arabinose and xylose in these samples were fermented to a similar extent. This is possibly due to the unique character of psyllium seed husk AX molecules, in which arabinose and xylose residues are linked into a complex and strongly branched structure.[8]

Despite, or perhaps due to, its strong resistance to enzymatic degradation,[9] higher concentrations of AX degrading enzymes were produced upon incubation of psyllium seed husk AX or AXOS than upon incubation of wheat bran AXOS. Arabinofuranosidase and xylosidase activities were highest after 6 h incubation and decreased to much lower levels after 24 and 48 h. Xylanase activities were also highest after 6 h, but decreased only slightly upon further incubation. Most likely, first, arabinofuranosidases and xylosidases act on arabinose and xylose containing side chains, rendering the xylan backbone less substituted, and, consequently, more accessible for further degradation by xylanases.

Fermentation of the psyllium seed husk preparations gave rise to increased short-chain fatty acid concentrations. Fermentation of PSH-200-0.27, PSH-88-0.16, and PSH-72-0.14 resulted in the highest acetic and propionic acid concentrations, while fermentation of PSH-300-0.29, PSH-200-0.27, and PSH-88-0.16 produced the highest concentrations of butyric acid. Psyllium seed husk fermentation increases mainly acetic[23] and propionic acid concentrations[23] upon consumption by humans, but also leads to high butyric acid concentrations in vitro.[24]

The complexity of wheat bran AXOS and psyllium seed husk AX and AXOS does not prevent them from being fermented. All test preparations, differing largely in arabinose to xylose ratio and avDP, were partly fermented. The extent of fermentation was structure-dependent and decreased with increasing structural complexity, that is, higher arabinose to xylose ratio and avDP, of the AXOS. The lower fermentability of more complex AXOS structures may imply that they persist for a longer period of time during colon passage. As a result, their fermentation may partially take place in the more distal parts of the colon, where they can help suppress protein fermentation. Reduced protein fermentation in the colon is desired, because amino acid degradation pathways in bacteria result in the production of potentially toxic catabolites including ammonia, phenols, and thiols.[2] The increase in unbranched short-chain fatty acid concentrations upon incubation of all preparations suggests potential beneficial health effects. Unbranched short-chain fatty acids improve mineral absorption[22,25,26] and inhibit the growth of potentially harmful bacteria.[27] While acetic acid is the primary precursor in the production of body cholesterol, propionic acid directly inhibits cholesterogenesis and hepatic gluconeogenesis, resulting in reduced blood cholesterol levels.[22] Butyric acid especially inhibits the growth of colonic carcinoma cells.[28]

Despite their high arabinose to xylose ratio, AXOS derived from pericarp-enriched wheat bran were partly fermented, offering perspectives for the upgrading of the low-value wheat pericarp layer. Also, psyllium seed husk, a strong water-holding and, therefore, difficult to process material, can be turned into an easy to handle and interesting source of physiologically active AX or AXOS.

Further research is needed to investigate the possible competition between fermentation of wheat bran and psyllium seed husk AXOS and proteins. This can be done in a more extended semicontinuous batch experiment simulating the conditions of the different colon vessels, using phenol and *p*-cresol as protein fermentation markers.[29] Additional research is needed to investigate the possible changes in bacterial population upon incubation with the AXOS preparations. A long-term in vitro experiment, however, suggested that enzymatically produced wheat bran-derived AXOS mainly change the metabolic activity by "switching on" the production of AXOS-degrading enzymes, without significantly affecting the microbial community composition.[30]

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## ABBREVIATIONS USED

AX, arabinoxylan; AXOS, arabinoxylan oligosaccharides; avDP, average degree of polymerization

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