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# Structural features and assessment of prebiotic activity of refined arabinoxylooligosaccharides from wheat bran

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ABSTRACT

Wheat bran (WB) samples were subjected to two stage processing (aqueous extraction and hydrothermal treatment) to assess their potential as a raw material for the manufacture of xylan-derived prebiotics. The liquid phase from the second stage, containing hemicellulose-derived soluble arabinoxylooligosaccharides (AXOS), was refined by consecutive steps of membrane filtration and ion exchange. The purified AXOS were characterised by matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF-MS) and chromatographic techniques. The most complex saccharide identified was made up of 19 pentoses. Human faecal slurry cultures were used to assess the bifidogenic activity of AXOS and their effects on the production of Short Chain Fatty Acids (SCFA) and lactic acid. The results were compared with data obtained using fructooligosaccharides (FOS), which are accepted as the gold standard of a prebiotic ingredient. The stimulatory effects reached by AXOS upon the bifidobacterial population were of the same order as those obtained with FOS. Higher SCFA production was observed with AXOS in comparison with FOS.



## 1. Introduction

In the past few years, prebiotic oligosaccharides have been obtained from a number of biomass sources, including *Eucalyptus* wood (Gullón et al., 2011a), pine wood (Rivas, Gullón, Gullón, Alonso, & Parajó, 2012), rice husks (Gullón et al., 2011b), malting wastes (Gullón, González-Muñoz, & Parajó, 2011c), corn cobs (Garrote, Yáñez, Alonso, & Parajó, 2008), apple pomace (Gullón, Gullón, Sanz, Alonso, & Parajó, 2011), sugar beet pulp (Martínez, Gullón, Schols, Alonso, & Parajó, 2009), and orange peel wastes (Martínez, Yáñez, Alonso, & Parajó, 2010).

WB is available worldwide in enormous quantities and commercialised as a source of dietary fibre. WB also contains a number of other valuable components, such as phenolic compounds, starch, and proteins (Xie, Cui, Li, & Tsao, 2008); but its fermentability by colonic microbiota is usually poor (Rose, Patterson, & Hamaker, 2010).

Hydrothermal processing (also known as autohydrolysis), a technology using water as the sole fractionation reagent, is suitable for breaking down the hemicelluloses into

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oligosaccharides (Madhukumar & Muralikrishna, 2010). WB arabinoxylan (AX) includes water-unextractable (WU-AX) and water-extractable (WE-AX) fractions (Swennen, Courtin, Lindemans, & Delcour, 2006), which show linear backbones of (1 → 4)-linked β-D-xylopyranose units. The xylose units in the backbone can be unsubstituted, monosubstituted at C-(*O*)-3- or di-substituted with L-arabinofuranose at C-(*O*)-2 and/or C-(*O*)-3. The presence of other substituents such as glucuronic acids, D-xylose, D-galactose and/or hydroxycinnamic acids (mainly ferulic acid) has also been reported (Damen et al., 2012).

Prebiotic properties have been reported for AX obtained by enzymatic or chemical methods (Broekaert, Courtin, & Delcour, 2011a), a finding that could be valuable for the wheat industry (Rose & Inglett, 2010). Prebiotics are defined as substrates that may improve the host health by selectively stimulating the growth and/or metabolic activity of one or a limited number of beneficial bacteria in the colon (Roberfroid et al., 2010). The human gastrointestinal tract represents a complex ecosystem where the available carbohydrates influence the growth of the gut microbiota (Manisseri & Gudipati, 2010).

Fermentation of prebiotics by selected colonic bacteria results in the production of lactate and SCFA such as acetate, propionate and butyrate (Al-Sheraji et al., 2013; Sarbini et al., 2013). The intestinal generation of SCFA lowers pH, increases the bioavailability of calcium and magnesium, and inhibits the growth of potentially harmful bacteria. Among SCFA, butyrate is particularly important, as it provides energy for colonocytes, stimulates colon epithelial cells (increasing their absorptive capacity), and inhibits the growth of colonic carcinoma cells *in vitro* and *in vivo* (Van Craeyveld et al., 2008).

The potential of new prebiotic candidates is usually evaluated by comparison with commercial FOS, as the latter are well known to fulfil the necessary requirements by means of *in vitro* and *in vivo* studies performed with animals and humans (Roberfroid, 2007). Due to this and to their bifidogenic effects, FOS are nowadays accepted as the gold standard for comparison purposes (Bosscher, 2009).

AXOS have been produced from WB by xylanase treatments (Swennen et al., 2006). Autohydrolysis is a potential alternative to enzymatic hydrolysis (Rose & Inglett, 2010), but this technology leads not only to hemicellulose dissolution, but also to starch extraction. As starch and starch-derived saccharides do not cause prebiotic effects, their removal from WB before autohydrolysis results in increased concentration of AXOS. For this purpose, a mild aqueous extraction is suitable for removing both starch and other undesired non-saccharide components from WB.

Refining of AXOS present in the autohydrolysis media can be carried out by membrane processing (Vegas et al., 2008), which enables the selective removal of monosaccharides and other undesired compounds (for example, derived from extractives and lignin). Ultrafiltration enables the fractionation of hemicellulose-derived saccharides according to their molar mass, whereas nanofiltration may be useful for concentrating solutions and/or for additional purification by removing undesired low molar mass compounds (Gullón et al., 2010).

In this work, commercial WB was used as a feedstock to obtain refined AXOS by consecutive stages of aqueous extraction, autohydrolysis, membrane separation and ion exchange. The structural characterisation of the refined product was performed by High Performance Size Exclusion Chromatography (HPSEC), MALDI-TOF-MS and High Performance Anionic Exchange Chromatography- Pulsed Amperometric Detector (HPAEC-PAD); whereas their prebiotic potential was assessed by fermentation with faecal inocula. Substrate consumption, generation of organic acids and the preferential growth of bifidobacteria were assessed in the fermentation assays.

## 2. Materials and methods

### 2.1. *Raw material*

Commercial WB from Santiveri (Barcelona, Spain) was purchased in a local store in Ourense (Spain). Samples from various packages were homogenised in a single lot before analysis and processing.

### 2.2. *Water extraction and autohydrolysis*

WB samples were subjected to the processing scheme shown in Fig. 1. The first aqueous treatment, performed to remove water-soluble extractives and part of the starch, was performed at a mass ratio of 10 g water/g oven dry solids in a 0.6 L stirred, stainless steel reactor, (model 4842 from Parr Instrument Company, Moline, Illinois, USA). The reactor was heated up to achieve 100 °C for 1 h (Parajó, 2012), and then the medium was immediately cooled and filtered. The extracted solid was washed with hot water, air-dried and subjected to an hydrothermal stage at 155 °C for 1 h, conditions reported as optimal for hemicellulose solubilisation (Parajó, 2012). Once the reaction time elapsed, the reactor was cooled and the liquid phase was recovered by filtration, analysed and processed as described in the next sections.

### 2.3. *Processing of autohydrolysis liquors*

#### 2.3.1. *Membrane processing*

The experimental setup employed in membrane processing consisted of a 6 L feed tank (in which temperature was controlled by flushing tap water through a refrigeration coil), a diaphragm pump, two pressure gauges (at the membrane inlet and outlet) to measure the transmembrane pressure (TMP), a needle valve located after the membrane to achieve the desired TMP, and a flowmeter to measure the recycle flow. A regenerated cellulose spiral membrane (Millipore TFF6, Billerica, MA, USA), with a 0.54 $m^2$ filtration area, 1000 Da molecular weight cut-off and 4 bar maximum working pressure, was used in the experiments. The operating pressure was fixed in 3.5 bar, as enhanced oligosaccharide retention was observed under these conditions in preliminary experiments (data not shown). Stream A (see Fig. 1) was subjected to discontinuous diafiltration (DD), which involved addition of deionized water up to reach a solvent to sample mass ratio of 1 kg/kg and further concentrate the resulting solution. The