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Extraction of water-soluble xylan from wheat bran and utilization of enzymatically produced xylooligosaccharides by *Lactobacillus*, *Bifidobacterium* and *Weissella* spp.

Peter Immerzeel [a], Peter Falck [b], Mats Galbe [c], Patrick Adlercreutz [b], Eva Nordberg Karlsson [b], Henrik Stålbrand [a,*]

[a] *Biochemistry and Structural Biology, Department of Chemistry, Lund University, P.O. Box 124, 22100 Lund, Sweden*
[b] *Biotechnology, Department of Chemistry, Lund University, P.O. Box 124, 22100 Lund, Sweden*
[c] *Department of Chemical Engineering, Lund University, P.O. Box 124, 22100 Lund, Sweden*

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ABSTRACT

Xylan was extracted from wheat bran after heat pretreatment in water using either an autoclave or a microwave oven. Xylooligosaccharides (XOS) were produced from the xylan using the thermostable xylanase *Rm*Xyn10A and the potential prebiotic properties of XOS were studied *in vitro* with different human gut bacteria: *Lactobacillus brevis* (DSMZ 1269), *Bifidobacterium adolescentis* (ATCC 15703) and two strains of recently isolated lactic acid bacteria from the species pair *Weissella cibaria/confusa*. The highest yield of (arabino)xylan with the heat pretreatment was obtained at 185 °C for 10 min. Higher temperature led to fewer arabinose substitutions present on the backbone which in turn resulted in a slightly more efficient enzymatic hydrolysis by *Rm*Xyn10A.

Using the produced XOS hydrolysate as carbon source, xylobiose uptake was confirmed for all bacterial species studied while xylotriose uptake could be confirmed for *B. adolescentis* and the *Weissella* strains. The negative control strain *Escherichia coli* (BL 21) did not use XOS as a carbon source. *L. brevis*, *B. adolescentis* and the *Weissella* spp. all showed growth on XOS, verified by increases in cell density, lactic acid and acetic acid production after 48 h incubation. Corresponding increases were not found using the non-hydrolysed xylan as carbon source.

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1. Introduction

Flour from wheat (*Triticum* spp.) is produced worldwide in large amounts by the agricultural processing industry and the byproduct wheat bran is currently used as animal feed. For the valorisation of wheat bran a way would be to fractionate this material into polysaccharide fractions for various applications. Of commercial interest are saccharide fractions that will help to support a healthy gut flora once combined in food products, such as functional foods. Saccharide fractions which are included as non-digestible food ingredients that support health promoting bacteria are named ‘prebiotics’ (Gibson & Roberfroid, 1995). Such prebiotic saccharides should, in general, be resistant to endogenous hydrolysis in the stomach and the small intestine and rather get metabolised in the colon by the gut flora and promote bacteria which are health beneficial. In the past extensive research has been conducted on fructooligosaccharides and galactooligosaccharides for application as prebiotics. Xylooligosaccharides (XOS) have been studied in less detail but it has been reported that XOS can have a prebiotic effect on *Bifidobacterium* and *Lactobacillus* species (Broekaert et al., 2011; Manisseri & Gudipati, 2010) which are considered to be among the most important beneficial microbes in the human gut (Kleerebezem & Vaughan, 2009). The health beneficial effects are in part related to the excretion of lactic acid and short chain fatty acids (SCFA) such as acetic acid, propionic acid and butyric acid by gut bacteria (Gibson, 1999; Louis & Flint, 2009; Servin, 2004). The degree of polymerisation (DP) and the type and degree of substituents of XOS may influence the rate of fermentation as shown by XOS from *Eucalyptus* wood during *in vitro* fermentation (Kabel, Kortenoeven, Schols, & Voragen, 2002).

The main components of wheat bran are: non-starch polysaccharides, starch, proteins and lignin (Zhang et al., 2011). Arabinoxylan hemicellulose is the major non-starch polysaccharide. Xylan from wheat bran is composed of a ß-D-(1,4)-linked xylopyranosyl backbone and can be substituted with α-L-arabinofuranosyl as the major side group on O2 and/or O3 (Scheller & Ulvskov, 2010).

* Corresponding author. Tel.: +46 462228202.
E-mail address: henrik.stalbrand@biochemistry.lu.se (H. Stålbrand).

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The amount of arabinose associated to the xylose backbone can be expressed as the Ara/Xyl ratio and for wheat bran values of 0.47 and 0.56 have been reported (Kabel, Carvalheiro, et al., 2002; Saulnier, Sado, Branlard, Charmet, & Guillon, 2007). Wheat bran is composed of various component tissues and the chemical fine structure of arabinoxylan varies in each layer (Saulnier et al., 2007). Hemicellulose extraction procedures from various plant tissues affect the structure and degree of substitution of the hemicellulose as shown for xylan (Schooneveld-Bergmans, Hopman, Beldman, & Voragen, 1998). This modification may affect both the enzymatic hydrolysis and ultimately utilisation of xylooligosaccharides by probiotic bacteria. In a study of arabinoxylooligosaccharides (AXOS) uptake by three different *Bifidobacteria* strains it was shown that two strains were able to utilise AXOS although with different strategies (Pastell, Westermann, Meyer, Tuomainen, & Tenkanen, 2009). After enzymatic removal of the arabinose substituents *Bifidobacterium adolescentis* metabolised the XOS whereas *Bifidobacterium longum* metabolised the released arabinose. In addition the study showed that AXOS with doubly substituted D-Xyl*p* were fermented with a lower efficiency compared to AXOS consisting of singly substituted D-Xyl*p*. Besides the pattern of arabinose substitution on the xylan backbone, other substituents may also adversely affect the degradability and microbial use, e.g. as shown for rye arabinoxylans (Glitsø, Jensen, & Knudsen, 2000).

Several different pretreatments for xylan extraction from wheat bran have been reported such as application of steam (Schooneveld-Bergmans et al., 1998), extraction with chemicals such as barium and calcium hydroxide (Bergmans, Beldman, Gruppen, & Voragen, 1996) or a combination of steam and chemicals (Maes & Delcour, 2001). In different studies xylan has been purified and characterised (Kabel, Carvalheiro, et al., 2002; Theander & Westerlund, 1986) and enzymatically hydrolysed for the production of XOS (Swennen, Courtin, Lindemans, & Delcour, 2006; Zhang et al., 2011). Enzymatic xylan hydrolysis has also been combined with tests of the prebiotic properties of the hydrolysate (Madhukumar & Muralikrishna, 2010; Manisseri & Gudipati, 2010). The aim of this study was to prepare variations of xylan from wheat bran and to gain more insight into the prebiotic properties of these xylan extracts and enzymatic hydrolysis products from the xylan. The studies referred to above have either focused on xylan extraction, purification, characterisation or enzymatic hydrolysis combined with growth of probiotic bacteria. In our study we have been able to integrate all these aspects from wheat bran heat pretreatment and xylan extraction and enzymatic treatment to probiotic growth in one workflow. This allowed us to investigate the effects of different pretreatment procedures on xylan yield, the efficiency of enzymatic hydrolysis of xylan including analysis of the product size and structure. It furthermore allowed us to study the effect of the xylo-saccharide preparations effect on bacterial growth performance and production of lactic acid and short chain fatty acids. Here we used the potentially probiotic bacteria *Lactobacillus brevis* and *B. adolescentis*. Since we recently showed that some *Weissella* spp. are able to metabolise XOS produced from birch wood (Patel et al., 2013) we also included two of these strains in the study.

2. Materials and methods

2.1. Material and isolation of hemicellulose

Wheat bran was obtained from Lantmännen Reppe AB (Lidköping, Sweden) and stored at −20 °C upon further processing. For the extraction of hemicellulose wheat bran was milled (0.5 Mesh). Heat pretreatment on 1 g wheat bran (90.2 g dry content/100 g total content) were performed with an autoclave at 121 °C for 1, 15 or 24 h and 15 g wheat bran (90.2 g dry content/100 g total content) with a microwave oven (Milestone MLS-1200 Mega Microwave Workstation, Sorisole, Italy) at 150 °C, 185 °C or 195 °C. The dry content of the wheat bran (90.2 g dry content/100 g total content) was adjusted to 20 g dry content/100 g total content with Milli-Q water before the pretreatments. The microwave oven was programmed for a two minute heat increase from room temperature to the final temperature which was then kept constant for 10 or 60 min (Roos, Persson, Krawczyk, Zacchi, & Stålbrand, 2009). The cooling of the micro wave Teflon treatment vessel took place in a water bath with streaming tap water. In order to compare the different pretreatments a severity factor was determined for each pretreatment. This severity factor Log (R_0) is calculated according the following formula (Overend, Chornet, & Gascoine, 1987): Log (R_0) = Log ($t \cdot \exp(T - T_{ref}/14.75)$) where T_{ref} is the reference temperature, which is set to 100 °C, T is the temperature at which the reaction takes place in degrees Celsius and t is the duration of the treatment in min. After the pretreatment the water soluble saccharides were extracted by adding Milli-Q water to the sample (total volume 15 mL for the autoclaved material and 200 mL for the material from the microwave oven) and 5 min continuous stirring. The suspension was centrifuged (3200 × *g* for 5 min) and the supernatant was filtered with filter paper (No. 1003) (Munktell & Filtrak, Bärenstein, Germany) and freeze dried. Carbohydrate analysis was performed on the dried extracts. For the hydrolysis and removal of starch α-amylase and amyloglucosidase were applied under the conditions provided by the manufacturer (Megazyme, Wicklow, Ireland). In order to separate high molecular weight hemicellulose from the low molecular weight starch degradation products ethanol was added to the extract to a final ratio of 4:1 ethanol:water. The pellet was washed once more with the same ethanol concentration and freeze dried. Carbohydrate analysis was performed on the dried pellet.

2.2. Enzymatic hydrolysis of xylan

The xylan from the de-starched extract was hydrolysed by the thermostable endoxylanase *Rm*Xyn10A from *Rhodothermus marinus* (Nordberg Karlsson, Bartonek-Roxå, & Holst, 1997). The extract was suspended in 0.05 mol/L phosphate buffer pH 6.8 and hydrolysed at 50 °C (6.3 U/100 mg xylan). After the incubation the solution was boiled for 10 min and the partially hydrolysed xylan as well as the unhydrolysed xylan were transferred to Hungate anaerobic culture tubes and flushed with dry nitrogen to ensure oxygen free conditions followed by autoclavation (121 °C) for 20 min. Quantification of the formed hydrolysis products was achieved by High-Performance Anion-Exchange Chromatography.

2.3. In vitro fermentation experiments

L. brevis (DSMZ 1269), *B. adolescentis* (ATCC 15703), *Weissella* spp. (strain 92 and AVI) (Patel et al., 2013), and *Escherichia coli* (BL 21) were used to study the uptake of xylooligosaccharides. *L. brevis* and *Weissella* spp. were grown in 15 mL tubes with MRS medium (de Man, Rogosa and Sharpe) without shaking. The tubes were placed in a closed container having an anaerobic atmosphere created by a moisturised reaction agent Anaerocult (Merck, Germany). *B. adolescentis* was grown in Hungate anaerobic culture tubes with *Bifidobacterium* medium containing cysteine-HCl (0.5 g/L) without shaking. Oxygen free growth conditions were maintained by flushing the tubes with nitrogen gas before closing and by degassing the buffer and carbon sources with nitrogen gas. The *E. coli* culture was grown in polystyrene culture tubes with a loose cap that allowed aeration (Simport T406-33) (Simport Scientific, Beloeil, Canada) and the tubes were shaken at 350 rpm

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