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Effect of pretreatment on arabinoxylan distribution in wheat bran

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ABSTRACT

Arabinoxylan is one of the potential key products of a wheat bran based biorefinery. To develop a suitable process for the isolation of arabinoxylans, the effect of different processing approaches needs to be determined. In this work, chemical analysis was supplemented by immunolocalization of arabinoxylan by confocal microscopy, which proved valuable in the assessment of cell-structural changes occurring upon different chemical and mechanical bran treatments. The influences of acid, lye and hydrogen peroxide treatment, ball-milling, extrusion, fermentation and treatment with esterase, xylanase and a combination thereof were investigated.

Extensive ball-milling showed the best selectivity for harvesting arabinoxylan. Chemical treatments gave the highest yields, but did so at the cost of selectivity. Fermentative and enzymatic treatments were hampered by coextraction of other compounds.

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1. Introduction

Wheat bran, the outer layer of the wheat kernel, is an abundant by-product of white flour production. Given its high content of nutritionally valuable and technologically desirable compounds, it is gaining interest as a raw material for biorefineries (Apprich et al., 2014). However, these components are either embedded in a complex and recalcitrant matrix or constitute this matrix themselves. This makes pretreatment imperative for down-stream processing. The choice of an appropriate pretreatment as well as suitable parameters is far from trivial and has to be adjusted according to the objective of the fractionation (Prückler et al., 2014).

Arabinoxylan is the most abundant valuable in wheat bran at about 32% of total dry matter (Maes & Delcour, 2002). As a dietary fiber substituted with hydroxycinnamates, such as ferulic and *p*-coumaric acid, arabinoxylan exhibits both nutritional and rheological benefits (Bauer, Harbaum-Piayda, Stockmann, & Schwarz, 2013; Berlanga-Reyes, Carvajal-Millan, Lizardi-Mendoza, Islas-Rubio, & Rascon-Chu, 2011; Hopkins et al., 2003). There is little consensus on the most advantageous methodology for selective extraction. Approaches range from chemical treatments, such as lye-based or oxidizer-assisted (Bataillon, Mathaly, Cardinali, & Duchiron, 1998; Maes & Delcour, 2001; Sun, Cui, Gu, & Zhang, 2011), over enzymatic treatments (Swennen, Courtin, Lindemans, & Delcour, 2006) to mechanical treatments, such as ball-milling (Van Craeyveld et al., 2009).

Few studies have been undertaken to compare the effectiveness of different treatments. Zhou et al. (2010) have compared peroxide-assisted alkaline extraction with enzymatic extraction and found the former to be more effective by 50%. However, they also found significant structural differences in the isolated products, which has to be taken into consideration for the intended application. Given the complex matrix of wheat bran, high yields usually only come at the cost of extensive treatment times or harsh conditions, which cause degradation of product and residual material, and challenges economic and ecological feasibility. Regardless whether the aim is to isolate arabinoxylan or to remove it in the purification of other target compounds, tracking its course is mandatory.

The aim of this study was to evaluate the efficacy of a wide spectrum of pretreatments with regard to their impact on arabinoxylan distribution and extractability. Evaluated pretreatments were acid, lye and hydrogen peroxide treatment, ball-milling, extrusion, fermentation and treatment with esterase, xylanase and a combination thereof. The effect of the pretreatments was assessed based on the carbohydrate content and distribution after

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thorough washing of the solid residue. An increase in extractability corresponds to a reduced amount of the solid sample. Three analytical approaches were chosen: quantification of the carbohydrate content by methanolysis, generating profiles of the carbohydrate distribution by enzymatic peeling and imaging of the arabinoxylan distribution by fluorescence labeling.

Carbohydrate content can be quantified after acidic cleavage of polysaccharides into monosaccharides. The favored method is methanolysis combined with GC–MS or GC–FID analysis, due to low degradation of the comparatively frail pentoses (Sundberg, Sundberg, Lillandt, & Holmbom, 1996; Willför et al., 2009). The composition of the carbohydrate fraction of the sample is gained and expressed as monosaccharide content. Being a mild method, methanolysis cannot decompose crystalline cellulose. Therefore, the glucose measured in this analysis is derived from residual starch, β-glucan and amorphous cellulose only. However, it cannot resolve arabinoxylan spatially which is a major requirement in the assessment of a pretreatment.

Hence, samples were also subject to an enzymatic peeling in order to obtain a cross-sectional profile of polysaccharide distribution (Sjöberg, Potthast, Rosenau, Kosma, & Sixta, 2005). The washed solids were treated with specific enzymes and the released monosaccharides in the supernatant were quantified. Thus, it was determined whether the effect of the pretreatment was only superficial or was affecting the whole sample.

The effects of the pretreatments were further elucidated by imaging the arabinoxylan distribution by immunolocalization in combination with confocal microscopy. Fluorescent-labeled monoclonal antibodies raised against specific polysaccharide epitopes allow for the localization of cell wall components, such as arabinoxylan, on a microscopic scale (McCartney, Marcus, & Knox, 2005). This technique has been used to map the polysaccharide distribution in cell walls of different wood types and to gain insight into the formation of cell walls (Donaldson, 2009; Donaldson & Knox, 2012). So far, immunolocalization has found little application in wheat bran research. To our knowledge it has been employed exclusively to follow xylanase-mediated degradation of arabinoxylan (Beaugrand et al., 2004a; Beaugrand, Reis, Guillon, Debeire, & Chabbert, 2004b).

2. Materials and methods

2.1. Sample preparation

Samples are classified in three treatment categories: chemical, mechanical and fermentation/enzymatical treatment. Chemical treatments were acid treatment (sample ID 1.1), lye treatment (1.2) and hydrogen peroxide treatment (1.3). Mechanical treatments were ball milling for 5 min (2.1) and 60 min (2.2) and extrusion (2.3). Fermentation and enzymatic treatments were fermentation with *Lactobacillus plantarum* (3.1); treatment with esterase (3.2); xylanase (3.3); a combination of esterase and xylanase (3.4); and a combination of *Lactobacillus brevis*, esterase and xylanase (3.5).

2.1.1. Chemical treatments

10 g of wheat bran were stirred at 60 °C for 4 h in one of the following: 200 mL of 1 M sulfuric acid, 1 M sodium hydroxide or 2% hydrogen peroxide solution adjusted with sodium hydroxide to pH 11.5 (all Sigma-Aldrich, St. Louis, MO, USA). The resulting slurries were filtrated and the residues were washed three times with 100 mL of water. Samples were freeze-dried before further analysis.

2.1.2. Mechanical treatments

Wheat bran was extruded using a Bühler Twin Screw Extruder BCTL 42/20D model (Bühler Group, Uzwil, Switzerland) without preconditioner. SME (specific mechanical energy) input was 179 Wh/kg at a maximum temperature of 133 °C.

Wheat bran was ball-milled with a Retsch MM 2000 ball-mill (Retsch, Haan, Germany) using 25 mL containers at about 10% loading with a single 25 mm stainless steel ball at 20 Hz. Milling times were 5 and 60 min.

2.1.3. Fermentation and enzymatic treatments

Wheat bran was inoculated with 2% w/w of bacterial culture for fermentation or 1% w/w of enzyme. After addition of 50% v/w of physiological sodium chloride solution samples were kept at room temperature for five days and then dried at 40 °C. Samples were treated with esterase Sternzym FSR 22010 (SternEnzym, Ahrensburg, Germany) and xylanase Pentopan Mono BG (Novozyme, Bagsvaerd, Denmark). Bacterial cultures were *L. brevis* DSM 20054 (DSMZ GmbH, Braunschweig, Germany) and *L. plantarum* WCFS1. For different combinations applied to samples see Table 1.

2.2. Methanolysis

In order to remove mobilized arabinoxylan before methanolysis, 200 mg of bran sample were stirred in 10 mL water for 1 h, filtrated, washed twice with 10 mL of water and dried in vacuo at room temperature.

2.2.1. Procedure and derivatization

Methanolysis was performed according to Sundberg et al. (1996). For silylation, dried samples after methanolysis were left to equilibrate in 400 μL of pyridine for 1 h. 200 μL of BSTFA (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide) containing 10% TMCS (trimethylchlorosilane; both Sigma-Aldrich) were added and the samples kept at 70 °C for 2 h. Samples were diluted with 600 μL of ethyl acetate, filtrated through a 0.45 μm PTFE syringe filter and analyzed by GC–MS.

2.2.2. GC–MS

GC–MS was performed as follows. 0.2 μL of silylated sample was injected (260 °C, splitless) on a 30 m/0.25 mm HP-5 column (film thickness 0.25 μm) in an Agilent 6890N Series GC System with an Agilent 5973 Series Mass Selective Detector. The temperature program was 140 °C (1 min); 4 °C/min to 210 °C (0 min); 30 °C/min to 260 °C (5 min). The carrier gas was helium (0.9 ml/min, constant flow). Detector conditions were 70 eV with a scan range from 45 to 950 Da. Data was acquired and processed with MSD Chemstation E.2.01.1177 software from Agilent Technologies.

2.3. Enzymatic peeling

Procedure for enzymatic peeling was adopted from Sjöberg et al. (2005) with modifications to the enzyme mix to suit wheat bran polysaccharides.

2.3.1. Preparation of enzyme mix

The enzyme mix was composed of 0.5 g cellulase from *Trichoderma reesei* (Sigma-Aldrich), 3 g xylanase Pentopan Mono BG (Novozyme) and 2 mL of GH 43 α-L-arabinofuranosidase from *Bifidobacterium adolescentis* (Megazyme Internartional, Wicklow, Ireland). Cellulase activity is given as ≥1 U/mg, xylanase as 2.5 FXU/mg (farbe xylanase units) and α-L-arabinofuranosidase activity as 102 U/mg. The enzyme mix was filtrated through Whatman filter paper grade 4 and then desalted and concentrated by ultrafiltration to 1% of its original volume with a molecular weight cutoff at 1000 Da and then filled up to 50 mL with water.

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