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Production of arabinoxylan-oligosaccharide mixtures of varying composition from rye bran by a combination of process conditions and type of xylanase

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HIGHLIGHTS

- Heat pretreatment increased the content of water-soluble AX.
- Best process conditions resulted in a product containing 62% AX.
- A family 10 xylanase produced the highest yield of short oligosaccharides.

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ABSTRACT

The aim was to study arabinoxylan-oligosaccharide production from rye bran using heat pretreatment and enzymatic hydrolysis. Due to the potential application in foods, the purity of arabinoxylan was also assessed. Rye bran was heat pretreated to improve xylanase-catalyzed hydrolysis of arabinoxylan into arabinoxylan-oligosaccharides. Enzymatic removal of starch and proteins before or after heat pretreatment increased the purity, although at lower yield. The most attractive process resulted in 62% (w/w) arabinoxylan content after ethanol precipitation. Using xylanases from two glycoside hydrolase families (*Rm*Xyn10A from GH10 and Pentopan Mono BG from GH11), different mixtures of unsubstituted and arabinose-substituted xylooligosaccharides were produced. GH10 gave a higher yield of short oligosaccharides (60% w/w) with xylobiose as the main product; xylobiose and xylotriose were the main products with GH11 (40% w/w). Thus, heat pretreatment combined with enzymatic hydrolysis can be used to produce arabinoxylan-oligosaccharides from rye bran that are potentially useful in functional foods.

1. Introduction

Rye (*Secale cereale*) is an important cereal for the Nordic and central European economies. The grain is used to make different flours but some fractions are still underutilized and could be processed to obtain high-value products. Rye bran, a residue from rye flour production, could have added value because of its high content of the hemicellulose, arabinoxylan (AX). AX from rye bran has a potential value as a food ingredient, either as a dietary fiber or converted into prebiotics (Broekaert et al., 2011). Rye bran AX comprises a backbone of (1 → 4)-linked β-D-xylopyranosyl residues with single and/or double L-arabinofuranosyl (Ara*f*) residues linked to it through (1 → 2) and/or (1 → 3) α-bonds. The backbone of AX can also contain (4-*O*-methyl)-(1 → 2)-linked α-D-glucuronic acid residues. Acetyl groups and short sugar oligomers can also be linked to the xylose backbone. In addition, arabinose can be linked to hydroxycinnamic acids (Izydorczyk and Biliaderis, 1995; Vinkx and Delcour, 1996).

The mixture of oligosaccharides derived from hydrolysis of cereal AX are called arabinoxylan-oligosaccharides (A)XOS and comprises xylooligosaccharides (XOS) and arabinoxylooligosaccharides (AXOS) (Broekaert et al., 2011). XOS stimulate some *Lactobacillus* and *Bifidobacterium* spp. (Crittenden et al., 2002; Falck et al., 2013), while AXOS stimulate mainly *Bifidobacterium* spp. (Falck et al., 2013; Pastell et al., 2009; Rivière et al., 2014). Short AXOS with a degree of polymerization (DP) of 5 or less were best at increasing bifidobacteria when structurally different AXOS were investigated in a rat model (Van Craeyveld et al., 2008).

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With regard to (A)XOS production, several studies have focused on wheat bran but relatively few on rye bran (Broekaert et al., 2011), one of which was a study on rye bran milling (Van Craeyveld et al., 2009). Commercially available rye AX has been enzymatically hydrolyzed into (A)XOS with prebiotic potential (Rantanen et al., 2007), including promoting growth of bifidobacteria in vitro (Pastell et al., 2009).

Endo-1,4-β-xylanases (EC 3.2.1.8) from glycoside hydrolase (GH) family 10 and 11 are the most studied in AX degradation (Collins et al., 2005). The success of enzymatic degradation of the AX backbone depends on the recalcitrance of the cell wall matrix and the degree of substitution (DS) of Ara*f*. Studies have shown that it is possible to use xylanases from family 10 and 11 to degrade parts of wheat bran AX into (A)XOS (Beaugrand et al., 2004; Maes et al., 2004; Swennen et al., 2006). GH11 enzymes were more efficient against water-unextractable AX compared with GH10. A possible explanation is that the family 11 xylanases, which are generally smaller in size compared with family 10, can more easily penetrate the cell wall matrix (Paës et al., 2012). In several known cases, family 11 xylanases cannot fully hydrolyze AX from parts of the bran with high DS because of its narrow substrate-binding cleft. Especially narrow are parts interacting with the thumb-loop structural element important for the specific action among GH11 xylanases (Pollet et al., 2009). This limits the hydrolysis to areas of the bran with less substituted AX. Family 10 xylanases, on the other hand, are the preferred xylanases for degrading AX, in general, they have a more open substrate-binding cleft and can accommodate C3-linked arabinose decorations in the −2 subsite (Pell et al., 2004).

However, the use of larger xylanases from family 10 has limitations because they have a slow penetration of the cell wall and are less efficient in hydrolyzing insoluble AX, and thus require a pretreatment step. This could be accomplished by alkaline extraction, which has been used successfully to isolate AX from wheat bran (Bergmans et al., 1996; Maes and Delcour, 2001). Heat pretreatment or autohydrolysis can also be used and has an advantage over other chemical extraction methods, such as alkaline extraction, because it does not require neutralization. Heat pretreatment is performed in a pressure vessel using water or steam and has been applied in many cases to isolate AX or make (A)XOS from different lignocellulose materials, such as barley husks (Roos et al., 2009). Wheat bran materials (Immerzeel et al., 2014; Schooneveld-Bergmans and Hopman, 1998) have also been used. The temperature and time have an impact on the DP and DS of the extracted AX or (A)XOS, which means that these parameters can be set to obtain the most desired properties (Roos et al., 2009). Combining a mild heat pretreatment with a xylanase treatment is attractive because it allows a more controlled hydrolysis of the water-solubilized AX. A harsher pretreatment might result in a high content of monosaccharides and loss of arabinose substituents as seen in a study using wheat bran to extract xylan (Immerzeel et al., 2014). A mild heat pretreatment also limits the formation of byproducts such as furfurals, the formation of which is very temperature dependent (Kataoka et al., 2008). Use of thermostable xylanases for (A)XOS production is beneficial, as the temperature from the heat pretreatment can be maintained in the following hydrolysis step. Immerzeel et al. (2014) showed that a family 10 xylanase from the marine bacterium *Rhodothermus marinus* (*Rm*Xyn10A) was able to hydrolyze AX from heat pretreated wheat bran at a high temperature, resulting in a mixture of XOS and AXOS.

The composition and purity of (A)XOS-containing extracts are influenced by the process design, and it is important to study the sequence of steps used to improve the purity of extracts, especially for food applications. It is important to limit the other main components, starch and proteins, in the bran material before the AX is used for hydrolysis. Comparing the efficiency of different families of xylanases on heat pretreated bran is also important because family 10 xylanases are usually less efficient on insoluble AX compared with family 11 xylanases. Being able to enzymatically hydrolyze parts of the bran where there is limited activity by xylanases remains a challenge in converting brans into (A)XOS and motivates a pretreatment.

Previous studies on AX from rye bran have investigated physical treatments of rye bran such as milling (Van Craeyveld et al., 2009) or hot-water extraction to solubilize AX (Karppinen et al., 2001) or a combination of shear treatment and xylanase treatment to extract AX (Figueroa-Espinoza et al., 2004). Heat treatment in an autoclave has been shown to increase the availability of AX for xylanases (Andersson et al., 2003) with the aim of solubilizing AX. The aim of the current investigation was to study the whole process of (A)XOS production with rye bran as the starting material using heat pretreatment and enzymatic hydrolysis. Due to the potential application in functional foods, the purity of AX was also assessed.

2. Methods

2.1. Raw materials, chemicals and enzymes

Rye bran was received from a local mill (Lilla Harrie Valskvarn AB, Farina, Kävlinge, Sweden) and milled in a Wiley mill to pass a 0.5-mm mesh. All chemicals were of analytical grade unless otherwise stated. α-Amylase (BAN 240L, Sigma, St Louis, MO) and amyloglucosidase (Megazyme, Wicklow, Ireland) were used to degrade starch. A protease (Neutrase 0.8L, Sigma) was used to degrade proteins. Thermostable xylanase *Rm*Xyn10A, catalytic domain, from GH10 was prepared as described elsewhere (Falck et al., 2013). Commercial xylanase from GH11, Pentopan Mono BG (Novozymes, Bagsvaerd, Denmark) was solubilized in 20 mM sodium phosphate buffer (SPB, pH 7.4) to a final concentration of 10 mg/mL. Two α-L-arabinofuranosidases were purchased from Megazyme: one GH43 enzyme from *Bifidobacterium* sp. (E-AFAM2) and one GH51 enzyme (E-ABFCJ) from *Cellvibrio japonicus*, previously *Pseudomonas cellulosa*.

2.2. Removal of starch and proteins from bran

Three different treatments were applied to rye bran to remove unwanted compounds before the heat pretreatment: washing, amylase treatment, and amylase treatment followed by a protease treatment (Fig. 1). For the amylase treatment, rye bran was suspended in 100 mM SPB (pH 6.0) in 50-mL plastic tubes, 0.12 U/g of α-amylase was added and the tubes were incubated in a water bath at 90 °C for 90 min. For the protease treatment, 0.035 U/g of Neutrase was added and the tubes were placed in an incubator at 50 °C with shaking for 4 h. Washing was done by suspending the rye bran in 100 mM SPB (pH 6.0) in 50-mL plastic tubes and only heated as for the amylase and protease treatments. All samples were centrifuged at 3900×*g* for 20 min and then washed once with 30 mL of Milli-Q water. The supernatants and wash fractions were stored at −18 °C until freeze drying, after which they were pooled. The pellets containing the remaining bran material were used for the heat pretreatment.

2.3. Heat pretreatment of bran

The pellets containing bran and untreated rye bran were transferred into 100-mL bottles with water to a dry weight content of 10%, adjusted with Milli-Q water, and then autoclaved (Thermo Scientific, Waltham, MA) at 121 °C for 15 h. After autoclaving, the samples were transferred into 50-mL plastic tubes and centrifuged