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Extraction of soluble arabinoxylan from enzymatically pretreated wheat bran and production of short xylo-oligosaccharides and arabinoxylo-oligosaccharides from arabinoxylan by glycoside hydrolase family 10 and 11 endoxylanases

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ABSTRACT

The enzymatic, ecofriendly pretreatment of wheat bran with α-amylase from *Bacillus amyloliquifaciens* or *B. licheniformis* at 90 °C for 1.5 h followed by Neutrase at 50 °C for 4 h, aqueous liquefaction at 121 °C for 15 h and ethanol precipitation enabled the production of soluble arabinoxylan (AX) with purity of 70.9% and 68.4% (w/w) respectively. Process alternatives tried, to simplify the process and curtail the cost resulted in AX products with different purities, yields and arabinose to xylose ratio (A/X). Among the two glycoside hydrolase (GH) family endoxylanases evaluated, GH10 family hydrolysed soluble AX more efficiently with xylanase from *Geobacillus stearothermophilus* T-6 (*Gs*Xyn10A) producing maximum amount of quantifiable short xylo-oligosaccharides (XOS) and arabinoxylo-oligosaccharides (AXOS) (53% w/w) followed by the catalytic module of *Rhodothermus marinus* Xyn10A (*Rm*Xyn10A-CM) with 37% (w/w) conversion. The GH11 family endoxylanases, from *Thermomyces lanuginosus* (Pentopan Mono BG™) and *Neocallimastix patriciarum* (*Np*Xyn11A) gave conversions of 21% and 22% (w/w) of the soluble AX, respectively (major AXOS products were not quantified). In addition to the XOS formed such as X_2, X_3 and X_4, the AXOS products identified were A^3X and A^2XX in the case of *Gs*Xyn10A and *Rm*Xyn10A-CM while Pentopan Mono BG and *Np*Xyn11A produced XA^3XX as the major AXOS product.

1. Introduction

In the past, a variety of physico-chemical methods have been used for the isolation of hemicellulose from plant biomass, for the development of value added products. The major constraint in the extraction process is the recalcitrance of the biomass arising from the closely knit physical and chemical association of hemicellulose with the phenylpropanoid network of lignin, cellulose and other cell wall components. Steam explosion (Xing et al., 2014), microwave extraction (Panthapulakkal et al., 2013), alkaline extraction after delignification with ammonia, sodium chlorite or hydrogen peroxide (Ebringerova and Heinze, 2000), autohydrolysis (Falck et al., 2014) and ultrasound (Yuan et al., 2010) are some of the methods used to extract arabinoxylan, an important dietary fiber from hemicellulose. Alkaline extraction is widely used for the isolation of arabinoxylan, but results in the deacetylation and removal of ferulic acid, an antioxidant (Schooneveld-Bergmans et al., 1998) especially at higher temperatures and the alkaline extracts need to be neutralized and desalted before it can be used in food products, while microwave treatment and steam explosion usually require costly experimental setup.

The structure of arabinoxylan, is highly diverse in plant tissues and the backbone is composed of β-1,4-linked xylopyranose residues with α-L-arabinofuranose units substituted at O-2 and/or O-3 or unsubstituted (Lequart et al., 1999). They may also have α-1,2-linked glucuronic or methyl-glucuronic acid residues as substituents (Ma et al., 2012) and ferulic acid may be found esterified to the O-5 position of arabinose units (Mathew and Abraham, 2004). When feruloylated arabinoxylans are consumed as components of dietary fiber, esterases in the human intestine are able to hydrolyze the ester linkage releasing ferulic acid, which provide protection against colorectal cancer (Ferguson et al., 2005). The antioxidant capacity of AXOS fraction is influenced by the content of esterified ferulic acid, A/X ratio and the degree of polymerisation (DP) (Malunga and Beta, 2015).

XOS of prebiotic potential and of high demand as functional food additive can be produced from AX by mild acid hydrolysis (Vazquez et al., 2001) or enzymatic hydrolysis (Zhu et al., 2006). XOS have

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prebiotic effects when consumed as part of the diet as they are not hydrolysed or absorbed in the gastrointestinal tract and those with low degree of polymerization (DP 2–3) promote significant proliferation of beneficial microorganisms such as bifidobacteria and lactobacilli in human intestine (Hsu et al., 2004; Christensen et al., 2014; Pan et al., 2009). AXOS, particularly those with low DP including the AXOS-5-0.27 (average DP of 5 and A/X 0.27) and AXOS-3-0.26 (average DP of 3 and A/X 0.26) substrates, have shown significant bifidogenic properties in rats (Van Craeyveld et al., 2008).

Diverse types of xylanases have been identified from bacteria, fungi, yeasts and plants, and they are generally classified into GH family 5, 8, 10, 11, 30, and 43 according to their protein sequence similarities by CAZy database, of which the major ones belong to GH10 and 11 (Paës et al., 2012). In the current study, we have used wheat bran as a natural resource for the production of soluble AX by autohydrolysis and subsequently converted the AX in to arabinoxylan derived oligosaccharides or (A)XOS by the action of GH10 and GH11 endoxylanases.

2. Materials and methods

2.1. Materials

The endo-1,4-β-xylanases used in the current study include Pentopan Mono BG from *Thermomyces lanuginosus* (GH11) obtained from Novozymes A/S (Bagsvaerd, Denmark), *Np*Xyn11A from *Neocallimastix patriciarum* (GH11), *Gs*Xyn10A (GH10) from *Geobacillus stearothermophilus* (previously *Bacillus stearothermophilus*) purchased from Megazyme (Wicklow, Ireland) and *Rm*Xyn10A-CM (GH10), the catalytic module of the thermostable endoxylanase from *Rhodothermus marinus* (Abou-Hachem et al., 2003) which was produced and purified in house. The α-amylase from *Bacillus amyloliquefaciens* (designated as BaA) and protease from *Bacillus amyloliquefaciens* (Neutrase 0.8L) were obtained from Sigma-Aldrich (St Louis, MO) while the α-amylase from *Bacillus licheniformis* (designated BlA) or Termamyl 120L was obtained from Novozymes. The monosugar standards xylose, arabinose, glucose, galactose and fructose were also purchased from Sigma-Aldrich. The xylo-oligosaccharide (X_2, X_3, X_4, X_5, X_6) and arabinoxylo-oligosaccharide standard compounds (A^2XX (2^3-α-L-Araf-(1-4)-β-D-xylotriose), A^3X (3^2-α-L-Araf-(1-4)-β-D-xylobiose), XA^3XX (3^3-α-L-Araf-(1-4)-β-D-xylotetraose), XA^2XX (2^3-α-L-Araf-(1-4)-β-D-xylotetraose) and $A^{2+3}XX$ (2^3,3^3-di-α-L-Araf-(1-4)-β-D-xylotriose) were obtained from Megazyme (Wicklow, Ireland). Wheat bran was received from Nord Mills, Sweden.

2.2. Methods

2.2.1. Production of xylanase RmXyn10A-CM

The catalytic module of the xylanase Xyn10A from *R. marinus* was produced by batch cultivation in *E. coli* BL 21 (Abou-Hachem et al., 2003) and the cell pellets obtained by centrifugation were suspended in phosphate buffer (20 mM, pH 7.5) and disrupted using a french press (Glen-Mills, New Jersey) at 16000 psi internal cell pressure. The lysed cells were centrifuged at 15000 × *g* for 20 min. The supernatant was then heat treated at 70 °C for 30 min and centrifuged again at 15000 × *g* for 10 min to obtain the partially purified thermostable xylanase. The His-tagged catalytic module *Rm*Xyn10A-CM was further purified using a precharged Ni Sepharose 6 fast flow column (His Trap FF, GE Healthcare Life Sciences) by immobilised metal ion affinity chromatography (IMAC) as described earlier (Falck et al., 2013) and the purity of the eluted protein was analysed by SDS-PAGE.

2.2.2. Enzyme activity assays

The xylanase activity was measured based on the determination of the reducing sugar by the dinitrosalicylic acid method. The endoxylanase activity was assayed using birch wood xylan (0.5% w/v) as substrate, in 100 mM sodium phosphate buffer of pH 6, 6.5 and 7.5 for the different enzymes following the method of Bailey et al. (1992) with modifications. One unit is the amount of enzyme required to release 1 mg of xylose/min at 40 °C and optimum pH. The method of Bernfeld (1955) was used for determining the activity of α-amylases, BaA and BlA. One unit is the amount of enzyme required to release 1 mg of maltose/min at ambient temperature and pH 6 and 6.9 respectively.

2.2.3. Extraction of soluble AX rich fraction from wheat bran

2.2.3.1. Pretreatment with α-amylases. Wheat bran was sieved through 25 mesh (710 μ). The sieved wheat bran (10% w/v) suspended in 100 mM sodium phosphate buffer containing 100 ppm calcium was treated separately with α-amylases (95.3 U/g of bran) from two different sources, *Bacillus amyloliquefaciens* (BaA) and *Bacillus licheniformis* (BlA) at 90 °C for 1.5 h and 140 rpm and checked for their efficiency in hydrolysing the starch components in bran. The buffer pH was 6.0 in the case of BaA and 6.9 in the case of BlA. The treated samples were centrifuged at 3893 × *g* for 10 min and the settled bran was washed with milli Q water (30 ml × 2). The supernatants, rich in starch hydrolysis products were pooled together and precipitated with ethanol at a final concentration of 80% (v/v) under stirring for 5 min at ambient temperature and allowed to settle at 4 °C for 1 h. The same procedure was used for ethanol precipitation

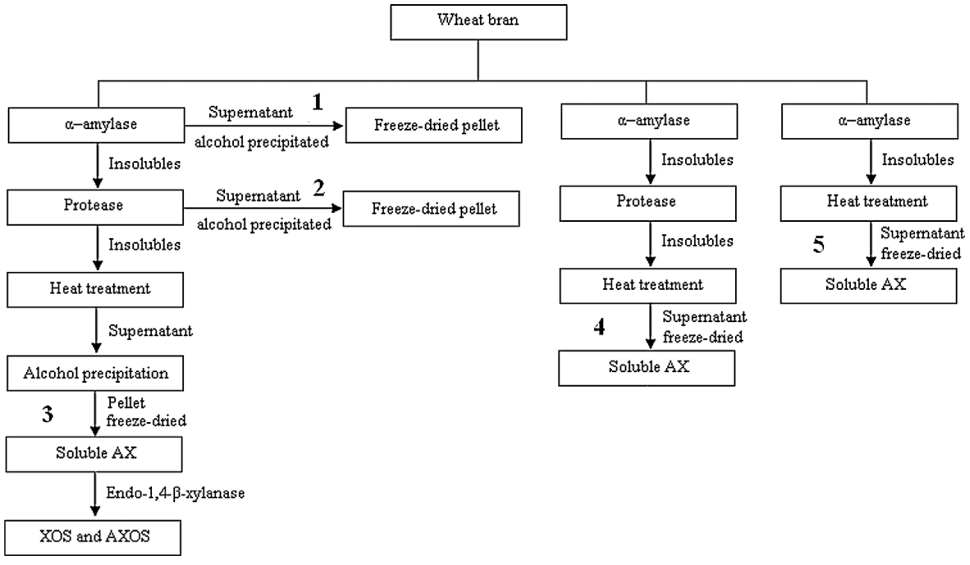

Fig. 1. Schematic representation of the major process pathways followed in the production of soluble AX and enzymatic hydrolysis of AX.