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Production of structurally diverse wheat arabinoxylan hydrolyzates using combinations of xylanase and arabinofuranosidase

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ABSTRACT

Structurally different wheat arabinoxylan hydrolyzates (AXH) were generated using different combinations of *Cellvibrio japonicas* xylanase (CJX), *Aspergillus niger* xylanase (ANX), *Bifidobacterium adolescentis* arabinofuranosidase (BAF) and *Clostridium thermocellum* arabinofuranosidase (CAF). Between the two xylanases, ANX might be an enzyme of choice for the production of AXH with simple structural details while CJX might be selected for the production of AXH with more complex structural features. Addition of BAF followed by CAF is more effective in generating AXH with higher amount of unsubstituted xylose. CJX series resulted in lower molecular weights compared to ANX series. The information derived about the capabilities of the two xylanases and two arabinofuranosidase could provide important information in decision making regarding enzymes to be used to generate AXH with specific structural details. Such hydrolyzates could be useful as substrate for future research exploring the effect of fine structural details in AXH on their biological and physical properties.

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1. Introduction

Arabinoxylan (AX) is the predominant polysaccharide in the cell wall of wheat grain (Saulnier, Sado, Branlard, Charmet, & Guillon, 2007). It consists of a backbone of β-(1,4)-linked xylose residues, which are substituted with arabinose residues on the C(O)-2 and/or C(O)-3 position (Dornez, Gebruers, Delcour, & Courtin, 2009). Since AX is mainly composed of xylose and arabinose, it is commonly referred to as pentosans. Phenolic acids such as ferulic acid can be ester linked on the C(O)-5 position of arabinose. Due to the complex structure of AX it is evident that the degradation of the molecule requires a diverse array of enzymes with varying substrate specificities. Endo-β-(1,4)-D-xylanases (EC 3.2.1.8, xylanase) are the major enzymes involved in AX degradation. They cleave AX by internally hydrolyzing the 1,4-β-D-xylosidic linkage between xylose residues in the xylan backbone in a random manner (Collins, Gerday, & Feller, 2005; Dornez et al., 2009) giving rise to arabinoxylan hydrolyzates (AXH) of different degree of polymerization (DP). The enzyme α-L-arabinofuranosidases (EC 3.2.1.55) remove arabinose substituents from the xylan backbone (Dornez et al., 2009) yielding AX of different degree of arabinose substitution (DS). Thus, treatment of AX polysaccharide with various xylanase and arabinofuranosidase can yield AXH with variable DP and DS. Most of the glycoside hydrolases that are classified in Glycoside hydrolase family 10 (GH10) are endo-β-1,4-xylanases (e.g., endo-1,4-β-xylanase from *Cellvibrio japonicus*) (Pollet, Delcour, & Courtin, 2010). GH10 xylanases attack both linear substrates as well as substituted heteroxylans. They attack the xylosidic linkage next to a single or double substituted xylose toward the non-reducing end and require two unsubstituted xylose residues between branched residues (Fig. 1). Thus, it can hydrolyze AX with high degree of substitution (DS) into smaller fragments. GH11 xylanase (e.g., endo-1,4-β-xylanase M4 from *Aspergillus niger*) exclusively consist of true endo-β-1,4-xylanases that cleave internal β-1,4-xylosidic bonds and preferably cleave unsubstiuted regions of the backbone (Pollet et al., 2010). GH11 cannot attack the xylosidic linkage toward the non-reducing end next to a branched xylose and require three unsubstituted consecutive xylose residues for hydrolysis. Hence, GH11 xylanases have a low activity on heteroxylans with a high DS. Only C3-linked arabinose residues from double-substituted xylose residues are hydrolyzed by Arabinoxylan arabinofuranohydrolase from *Bifidobacterium adolescentis* (GH 43) (Van den Broek et al., 2005) while α-L-arabinofuranosidase from *Clostridium thermocellum* (GH51) catalyses the hydrolysis of "α-1,5-linked arabino-oligosaccharides and the α-1,3 arabinosyl side chain decorations of xylan with equal efficiency" (Taylor et al., 2006). Thus, it is also highly efficient in the removal of the α-1,3-linked arabinoside substitutions from

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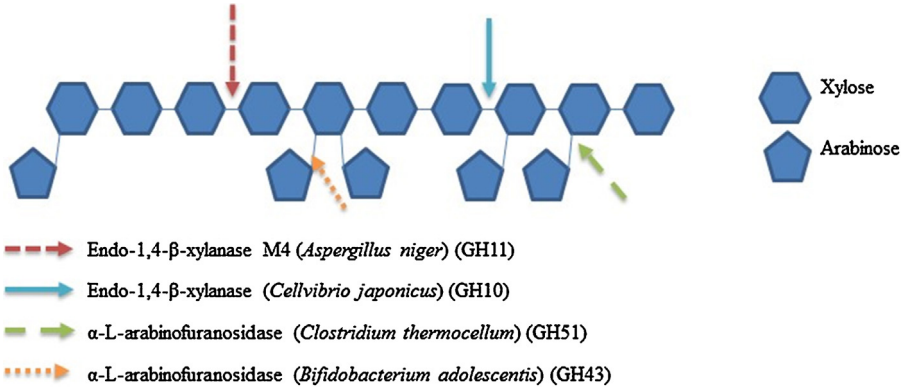

Fig. 1. Simplified illustration of hydrolysis specificities of two different xylanase and two different arabinofuranosidase along the arabinoxylan molecule. Endo-1,4-β-xylanase from *Cellvibrio japonicus* (GH10) attack the glycosidic linkage next to a single or double substituted xylose toward the non-reducing end and require two unsubstituted xylose residues between branched residues (Pollet et al., 2010). As a result, GH10 xylanases can even degrade AX with high degree of substitution into smaller fragments. GH11 xylanase (e.g. endo-1,4-β-xylanase M4 from *Aspergillus niger*) (GH11) exclusively consist of true endo-β-1,4-xylanases that cleave internal β-1,4-xylosidic bonds (Pollet et al., 2010) and preferably cleave unsubstiuted regions of the backbone. It cannot attack the xylosidic linkage toward the non-reducing end next to a branched xylose and require three unsubstituted consecutive xylose residues for hydrolysis. Hence, GH11 xylanases have a low activity on heteroxylans with a high DS. α-L-arabinofuranosidase from *Bifidobacterium adolescentis* (GH 43) releases only C3-linked arabinose residues from double-substituted xylose residues (Van den Broek et al., 2005). α-L-arabinofuranosidase from *Clostridium thermocellum* (GH51) catalyzes the hydrolysis of C3-linked arabinosyl side chain of xylan (Taylor et al., 2006).

wheat arabinoxylan itself (Taylor et al., 2006). There is a growing awareness for the production of dietary fibers and prebiotics with health benefits. The production of structurally defined fibers from plant polysaccharides such as wheat AX is highly encouraged. Thus, based on the enzymatic specificities, we wanted to investigate how these enzymes could be utilized to produce hydrolysis products of desired characteristics. Similar approaches to produced enzymatically tailored AX hydrolyzates from corn AX had been previously reported (Xu, 2012). However, there are significant structural differences between wheat AX and corn AX (Rose, Patterson, & Hamaker, 2010). Alkaline extractable AX from wheat bran has large proportion of unsubstituted xylose regions compared to corn and rice bran AX (Rose et al., 2010). Thus, the objective of this research was to produce structurally different arabinoxylan hydrolyzates (AXH) derived from wheat AX by means of enzymatic treatments. The insight we get about the capabilities of the enzymes used in the current study might benefit many industries (Beg, Kapoor, Mahajan, & Hoondal, 2001; Clarke, Rixon, Ciruela, Gilbert, & Hazlewood, 1997; Falck et al., 2013; Spagna, Andreani, Salatelli, Romagnoli, & Pifferi, 1998) in their decisions about use of xylanases and arabinofuranosidases to achieve desired end products.

2. Materials and methods

2.1. Materials

Wheat samples were of variety Glenn grown in Casselton, ND in 2011. Heat stable α-amylase from *Bacillus licheniformis* (Termamyl® 120, 1186 units/mg protein; 19.8 mg protein/mL; A-3403-1MU) and protease from *Bacillus amyloliquefaciens* (P-1236-50 ML) were purchased from Sigma-Aldrich Inc. (Saint Louis, MO). Endo-1,4-β-xylanase (*C. japonicus*) (EC 3.2.1.8; CAZY Family: GH10; Cat. No: E-XYNACJ), endo-1,4-β-xylanase M4 (*A. niger*)(EC 3.2.1.8; CAZY Family: GH11; Cat. No: E-XYAN4), α-L-arabinofuranosidase (*C. thermocellum*) EC 3.2.1.55; CAZy family: GH51; Cat. No: E-ABFCT) and α-L-arabinofuranosidase (novel specificity) (*B. adolescentis*) (EC 3.2.1.55; CAZy Family: GH43; Cat. No: E-AFAM2) were purchased from Megazyme International Ireland, Wicklow, Ireland. All the other chemicals were of analytical grade.

2.2. Procedures for arabinoxylan hydrolyzate preparation

Wheat variety Glenn was used to extract arabinoxylans from wheat bran. The whole grain was milled in a Bühler MLU-202 mill (Bühler Industries Inc., Uzwil, Switzerland) and bran fraction was collected. The bran was then sieved on No 35 (500 micrometer mesh) sieve (100 g each for 4 min). This was done to further remove any flour remaining with the bran. The sieved bran was stored at 4 °C until further treatment. The bran was then ground using a hammer laboratory mill 3100 equipped with a 0.8 mm screen (Perten Instruments North America, Inc.) The ground bran was stored at 4 °C.

Ground wheat bran (500 g) was extracted with hexane (2 L) for 2 h on a shaker (Orbit shaker (Lab-Line instrumnts Inc. Melrose Park, IL, USA) at room temperature. The material was then filtered through Whatman No 1 filter paper using vacuum and dried under the hood for two days until no hexane smell was detected. The resulting material was called partially defatted bran (PDB) and was stored at 4 °C until further treatment.

Preparation of destarched deprotinised bran was carried out according to the method described by Rose et al. (2010) with some modifications. Partially defatted bran (250 g) was mixed with deionized water (2 L) and pH was adjusted to pH 7.0 using 1 M NaOH. The solution was then boiled for 20 min. to inactivate the endogenous enzymes. Then 250 μL of heat stable α-amylase from *B. licheniformis* was added. Starch was hydrolyzed at 90–95 °C for 2 h, and then cooled in an ice bath to 50 °C. The pH was adjusted to 6.0 using 1 M HCl, and 10 mL of protease was added and protein was hydrolyzed at 50 °C for 4 h with shaking (200 strokes/min) in a water bath (Type: 89032, VWR International, PA, USA). Next, the enzymes were inactivated by boiling the mixture for 30 min and were cooled in an ice bath to room temperature and pH was adjusted to 7.0. The slurry was centrifuged at 3000*g* for 15 min. The residue was termed destarched, deprotenized wheat bran (DSDPB) and stored in freezer (−4 °C) until further purification.

Alkaline hydrogen peroxide extraction of the DSDPB was carried out as previously described by Rose et al. (2010) with some modifications. Destarched deprotinized bran (half of the slurry: ~100 g) was suspended in 1 L of 1 M sodium hydroxide using a conical flask of 4 L, allowing space for foam generation during hydrogen peroxide addition. Under constant mixing at 60 °C, 42 mL of 30% hydrogen peroxide was slowly added to the mixture and was stirred for 4 h at 60 °C using magnetic stir bars. The resulting slurry was centrifuged