Industrial Crops and Products 55 (2014) 63–69

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Extractability and digestibility of plant cell wall polysaccharides during hydrothermal and enzymatic degradation of wheat straw (*Triticum aestivum* L.)

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ARTICLE INFO

Article history:
Received 29 October 2013
Received in revised form 31 January 2014
Accepted 2 February 2014
Available online 2 March 2014

Keywords:
Wheat straw
Biochemical conversion
Intermolecular organisation
CoMPP
Enzymatic digestibility

ABSTRACT

Fuels and chemicals derived through biochemical conversion of agricultural by-products such as wheat straw (*Triticum aestivum* L.) is an area currently under intense research. In this study, separate leaves and stems were hydrothermally pretreated and enzymatically hydrolysed and analysed chemically and by comprehensive microarray polymer profiling (CoMPP). This way, the effects of each degradation step to the intermolecular organisation of specific polysaccharides in the cell walls were elucidated. After pretreatment, the degree of polymerisation (DP) of released xylo-oligosaccharides in both samples was up to about 20, but mostly around 3–8, and notably more acetylated in stems. Arabinoxylan (AX) and mixed-linkage glucan (MLG) became water-extractable while xylan, xyloglucan (XG), mannan and glucan remained only alkali-extractable. All polysaccharides became partly digestible after pretreatment however, regardless their extractability in water or only alkali. Based on the results, AX and MLG appear to be loosely bound in the cell wall matrix while the other polysaccharides are bound more tightly and shielded from enzymatic attack by AX and MLG until pretreatment. The gradual solubilisation and digestion of the polysaccharides during pretreatment and hydrolysis correlate well with previous models of the polysaccharides' structural organisation in the cell wall.

1. Introduction

More basic understanding of the structural organisation and intermolecular dynamics during biochemical conversion wheat straw (*Triticum aestivum* L.) into fuels and other high-value chemical products is still needed to improve the cost-effectiveness and economic viability of large-scale production. In wheat stems, half the tissue volume consists of parenchyma cells called the cortex; the other half consists mostly of tightly bundled sclerenchyma fibres, providing mechanical support for the vascular bundles (tracheids and sieve-tube elements) and the plant as a whole. The main tissue in leaves is mesophyll consisting of loosely packed parenchyma cells, like the cortex. Parenchyma cells in wheat straw have non-lignified primary walls while both sclerenchyma fibres and tracheids have lignified secondary cell walls (Alberts et al., 2004; Carpita, 1996; Rost et al., 1998a,b). When incubated with commercially available enzyme mixes or rumen microorganisms, parenchyma cells in various grasses were found to be the most digestible cell type followed by sieve-tube elements (Akin, 2008; Hansen et al., 2011).

The main chemical components in wheat straw cells are cellulose, hemicellulose and lignin besides silica (SiO_2) and other minerals. In smaller amounts are also pectins, cutin and epicuticular waxes, proteins, glycoproteins, *p*-hydroxycinnamic acids, lipids and nucleic acids (Epstein, 1999; Raven et al., 1999; Sun et al., 2000).

Cellulose consists of β-1,4-D-glucan chains bundled together in microfibrils (MF) that form either crystalline or so-called 'amorphous' structures (Newman and Hemmingson, 1995). In the primary type II cell walls of Poaceae (grasses, e.g. wheat) the main hemicelluloses are arabinoxylan (AX), xyloglucan (XG),

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http://dx.doi.org/10.1016/j.indcrop.2014.02.002

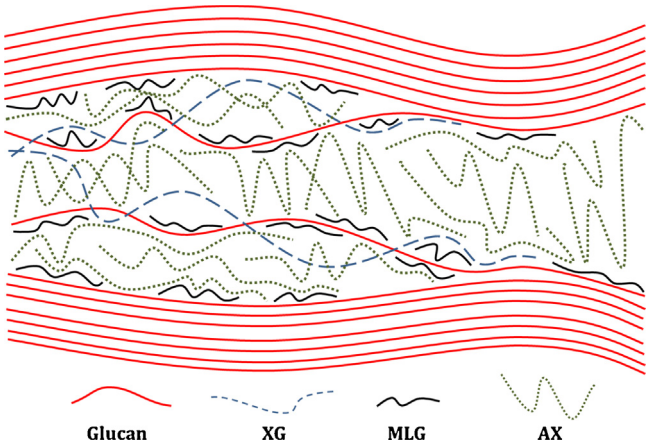

Fig. 1. Schematic representation of the primary type II cell wall in grasses as proposed by Buckeridge et al. (2004) and Park and Cosgrove (2012). XG and relatively unsubstituted AX tether the disaligned glucan chains while MLG and more substituted AX fill the interspace between the MFs.

mixed-linkage glucan (MLG) while xylan and glucomannan are predominantly found in the secondary cell walls (Albersheim et al., 2011a). A schematic representation of the primary type II cell wall is presented in Fig. 1, based on earlier publications (Buckeridge et al., 2004; Park and Cosgrove, 2012). An equally detailed representation of the secondary cell wall does, to the best of the authors' knowledge, not yet exist (see below).

AX has a β-1,4-linked xylan backbone and constitutes 30–40% of the primary cell walls in grasses. It principally has α-L-arabinosyl and to lesser extents acetyl substituents which prevent the xylan sequences from associating with other polysaccharides via hydrogen bonds (Albersheim et al., 2011a; Andrewartha et al., 1979; Izydorczyk et al., 2000). In more lignified cell types, 4-*O*-methyl-α-D-glucuronic acids (4-*O*-MeGlcA) are found together with ferulic (and coumaric) acids attached to arabinosyl (Albersheim et al., 2011c; Carpita, 1996; Trethewey et al., 2005).

MLG consists of mixed β-(1,3)-(1,4)-linked glucosyl units and is the predominant non-cellulosic polysaccharide in grasses (Carpita and McCann, 2000). Roughly 90% consists of cellotriosyl (DP3) and cellotetrasyl (DP4) in a coil conformation (Buliga et al., 1986). The remaining 10% consists of sequences of up to 10 β-1,4-D-glucosyl units (Wood et al., 1994) which may associate with other polysaccharides via hydrogen bonds (Izydorczyk et al., 1998).

XG has a β-1,4-D-glucan backbone with α-D-xylosyl substituents and constitutes less than 2% of the primary cell walls in grasses. Nearly all substituents are single terminal xylosyl units (Albersheim et al., 2011a; Carpita, 1996). XG adheres strongly to disordered glucan chains via hydrogen bonds, thereby bridging adjacent MFs (Park and Cosgrove, 2012).

Xylan is the dominant hemicellulose in secondary cell walls together with glucomannan. Here, xylan is heavily acetylated while glucomannan has a linear β-1,4-D-linked backbone of glucan and mannan in 1:1–1:2 ratios (Albersheim et al., 2011a).

Hydrothermal pretreatment solubilises the hemicelluloses (Mosier et al., 2005; Palmqvist and Hahn-Hagerdal, 2000) and induces an acidic environment which partially cleaves other glycosidic bonds (Pedersen and Meyer, 2010) and internal β-aryl ether bonds in lignin (Li et al., 2007; Yelle et al., 2013). Unfortunately, sufficiently high temperatures also degrade arabinose and xylose to furfural (Pan et al., 2006b; Saha et al., 2005). Enzymatic hydrolysis requires accessibility of the polysaccharides either through exposure, solubilisation or loosening of the cell wall. The release of acetyl and other substituents plays a crucial role as xylanases are otherwise sterically hindered in binding to their substrate (Albersheim et al., 2011a; Kabel et al., 2007). The effectiveness of the hydrolysis is of course greatly influenced by the specificity and catalytic activity of the enzyme mix.

In this study, we investigated the gradual solubilisation of carbohydrates from leave blades and intermodal stems as they were hydrothermally pretreated and subsequently enzymatically hydrolysed. As controls, non-treated samples were also hydrolysed and non-treated and unwashed pretreated samples incubated without enzymes to account for thermal and mechanical effects. By performing quantitative chemical analyses in combination with CoMPP we related the degradation of the polysaccharides to their structural organisation in the cell wall as already described in previous models. The experimental setup is presented in Fig. 2.

2. Materials and methods

2.1. Pretreatment

The wheat straw was collected manually on the field at Tystofte, Denmark in 2006 and stored at ambient conditions. Composition analyses on non-treated and pretreated leaves and stems were performed in triplicate according to the procedure described by The National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008).

During pretreatment, 3 g of separated wheat leaves without sheaths and stems without nodes were added 80 mL of demineralised water in blue cap bottles. The bottles were placed in a custom-made high-pressure reactor and hydrothermally pretreated for 10 min at 190 ± 5 °C. The heat up time was 45 ± 5 min. Upon cooling, the pretreatment liquid was drained off and each sample washed three times in demineralised water. The washing water was added to the pretreatment liquid and added demineralised water up to a total volume of 250 mL. Finally, the liquids from each sample were filtered through 0.45 μm filters and 950 μL mixed with 50 μL of internal fucose standard solution.

2.2. Enzymatic hydrolysis

Prior to enzymatic hydrolysis the dry matter (DM) content for leaves and stems was measured in triplicates, equalling 93 and 94% when non-treated and 7 and 11% after pretreatment, respectively. Afterwards, triplicates of 0.5 g DM from each sample were placed in 20 mL plastic bottles and the DM content adjusted to 7% by the combined buffer (50 mM sodium citrate, pH 5.0) and enzyme solution (10 mg EP pr. g glucan CellicCTec2). Control samples incubated without enzymes were diluted with buffer solution only and the volume adjusted accordingly. During hydrolysis, samples were tumbled at 50 °C for one day and subsequently immersed in boiling water for 10 min. Afterwards, all liquid fractions were diluted 1000 times with MQ water, filtered through 0.45 μm filters and 950 μL mixed with 50 μL of internal fucose standard solution.

2.3. HPAEC

The filtered and diluted liquid fractions after pretreatment and enzymatic hydrolysis of leaves and stems in Sections 2.1 and 2.2 were analysed for released monosaccharides on a ICS5000 HPAEC-PAD with a 2 mm × 250 mm Dionex CarboPac® 130 PA1 analytical column (Thermo Fisher Scientific Inc., Sunnyvale, CA) connected to a 2 mm × 50 mm guard column at 0.25 mL/min at isocratic conditions (14 mM NaOH). Glucose concentrations were multiplied with 0.9 and xylose and arabinose with 0.88 to account for hydration during depolymerisation.

The contents of released monosaccharides were corrected for the amount of free monosaccharides present in the enzyme mix. For this, 2.5 mL triplicates of pure buffer solution were added 1.8 and 1.9 μL of 16 mg EP/mL Cellic CTec2. Afterwards, the samples were