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Impact of hydrothermal pre-treatment to chemical composition, enzymatic digestibility and spatial distribution of cell wall polymers

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HIGHLIGHTS

- The hydrothermal pretreatment causes solubilization and relocalization of xylan.
- Xylan, phenolic acids and diferulates solubilize in temperature dependent manner.
- Altered autofluorescence profile indicates structural changes in lignin.
- These changes lead to improved enzymatic digestibility of the solid carbohydrates.

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ABSTRACT

The effect of hydrothermal pretreatment on chemical composition, microscopic structure and enzymatic digestibility of wheat straw was studied. Wheat straw was pretreated with increasing severity to obtain series of samples with altered chemistry and structure. The hydrothermal pretreatment caused solubilisation of arabinoxylan and phenolic acids and their dimers in a temperature dependent manner with minor effects on the cellulose and Klason lignin content. In the cell wall level, the pretreatment intensified staining of cellulose and relocalised xylan in the cell walls. The distribution, properties and content of the cell wall phenolic compounds was altered as observed with phloroglucinol and autofluorescence imaging. In the enzymatic hydrolysis, the highest yields were obtained from the samples with a low xylan and diferulate content. On the cell wall structural level, the sample with the highest digestibility was observed to have intensified cellulose staining, possibly reflecting the increased accessibility of cellulose.

1. Introduction

Utilisation of plant biomass as an alternative for fossil carbon sources in fuel and chemical production is one of the greatest challenges in the current world with diminishing amounts of fossil carbon and impact of their usage on carbon dioxide emissions. Utilisation of the non-food plant biomass, such as agricultural wastes is often preferred in order to avoid conflicts between food and chemical production. Typically these materials are structurally inhomogeneous plant cell wall fragments originating from different plant tissues and cell types and contain cellulose, hemicelluloses and lignin as major components.

Wheat straw is one of the potential agricultural residues relevant for utilization in biorefineries. Botanically, wheat straw consists of stems, leaves and often traces of other organs mixed up in harvest. Chemically, wheat straw is mainly composed of plant cell wall structural polymers: cellulose (31.5–48.6% dm), hemicelluloses (22.6–38.8% dm) and lignin (5.3–19%) with ca 3.5–8.7% of protein and 4.2–7.5% ash (Lee et al., 2007) thus containing substantial amount of convertible carbohydrates. Cellulose in wheat straw is mainly cellulose I allomorph with relatively low crystallinity (40%) (Liu et al., 2005), while hemicellulose fraction is mainly composed of arabino/glucuronoxylans (e.g. Sun et al., 2005). The monosaccharides in the wheat straw hemicelluloses are substituted with acetyl and especially phenolic acids groups, which are implicated to be involved in the crosslinking of the hemicelluloses and lignins (Buanafina, 2009). The lignin of grasses is composed of syringyl, guaiacyl and *p*-hydroxycoumaryl units with significant incorporation of phenolic acids. The major components in the inorganic (ash) fraction of wheat straw are silica (silicon dioxide) and potassium and calcium oxides (Dodson et al., 2011).

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In nature, plant biomass carbohydrates are degraded by various micro-organisms capable of producing carbohydrate active enzymes (http://www.cazy.org/). The enzymatic hydrolysis of structural carbohydrates, cellulose and structurally heterogeneous hemicelluloses, is carried out by various glycosidic hydrolases (cellulases, hemicellulases) and carbohydrate active esterases as well as oxidative enzymes. For natural lignocellulosic materials, the enzymatic hydrolysis rates and yields are too low for industrial purposes due to the recalcitrant structure of plant cell walls, therefore in industrial applications biomass needs to be modified and the cell wall structure opened in an economically efficient process. For this, various pretreatment methods have been developed for enhancing the enzymatic hydrolysis of lignocelluloses (Galbe and Zacchi, 2007). Commonly used techniques apply high temperatures with or without suitable catalysts (hydrothermal, steam explosion), which result in partial solubilisation and increased enzymatic digestibility (Ballesteros et al., 2006; Thomsen et al., 2008).

The solid fraction of lignocellulose after the hydrothermal pretreatment consists of residues of cell walls and tissues, which are the main targets of the enzymatic hydrolysis. In the present work the relation between chemical composition, microscopic structure and enzymatic digestibility of solid fraction of hydrothermally pretreated wheat straw was examined in order to understand the chemical and structural features that promote the enzymatic digestibility. This was carried out by pretreating the wheat straw in increasing severity to produce series of samples with gradually altered structure and chemistry. The samples were analysed for the major chemical components and the microscopic structure and evaluated for enzymatic digestibility using commercial enzyme preparations.

2. Methods

2.1. Wheat straws and enzyme preparations

The natural wheat straw was collected from local farms in Estonia. The commercial enzyme preparations Celluclast 1.5 L (49 filter paper units (FPU)/ml) and Novozym 188 (5760 nkat/ml) were purchased from Novozymes (Denmark).

2.2. Hydrothermal pretreatment

The hydrothermal pretreatment was carried out in a batch mode using fixed bed flow-through reactor (solid phase was not stirred, liquid phase was circulated through biomass material bed). Ca 20 g (DM) of milled wheat straw was suspended to water in a ration 1:10 (DM) and incubated at 180, 190 and 200 °C for 10, 15 and 20 min using a straw: water ratio 1:10 (DM) without additional chemical catalysts. After pretreatment, the solid fractions were separated and stored at −20 °C.

2.3. Preparation of alcohol insoluble residues (AIR) from wheat straw samples

AIRs were prepared from pretreated material following determination of dry matter (DM) according to a modified method of Waldron and Selvendran (1990). Approximately 100 g of pretreated samples together with the controls were extracted with hot ethanol (250 ml, 60 °C, 5 min) to remove any alcohol soluble phenolics and filtered through a 30 mm GF/C filter (Whatman, Maidstone, UK) overlaid with a 30 mm nylon mesh (75 μm). The AIRs were washed twice in acetone and air-dried at room temperature.

2.4. Dry matter content

Approximately 100 mg of each sample and the control wheat straw were weighed in an Infra red balance (Mettler PM200) heated to 100 °C. The dry matter (DM) content was measured for 40 min or until a stable weight was obtained.

2.5. Carbohydrate analysis

Sugars were released from the fractions by hydrolysis with H_2SO_4 (72% w/w) for 3 h, followed by dilution to 1 mol L^{-1} and hydrolysis at 100 °C for 2.5 h (Saemen hydrolysis) (Saeman, 1945). Hydrolyzed monosaccharides were reduced and then analysed as their alditol acetates by GC (Blakeney et al., 1983) using 2-deoxyglucose (200 μL, 1 mg/mL) as an internal standard. Alditol acetates were quantified by gas chromatography (Perkin Elmer, P.E. Auto system XL Gas Chromatograph) using P.E. Nelson Turbochrom software, version 4.1 to process data. The sample (1 μl) was injected onto an RESTEK rtx-225 (15 m × 320 μm) column under helium gas. The flow rate was 2 ml/min with an injection temperature of 200 °C and the run time was 35 min. A flame ionisation detector (FID) was used for detection (Perkin Elmer, model 235C). Uronic acid content was determined colorimetrically by the method of Blumenkranz and Asboe-Hansen (1973) after dispersal in 200 μl of 72% H_2SO_4 as above, dilution to 1 M and hydrolysis for 1 h at 100 °C.

2.6. Klason lignin analysis

Klason lignin was quantified gravimetrically by a modified method of Browning (1967). Alcohol insoluble residues (AIRs) (100 mg) were dispersed in 1.5 ml of 72% H_2SO_4 and incubated at 30 °C for 1 h shaking frequently. The samples were further incubated for 2.5 h after diluting with 10.5 ml water in a temperature controlled oven set at 100 °C. The residues were recovered by filtration through pre-weighed sintered glass funnels (10 mm diameter, Fisher Scientific) under vacuum. The insoluble material was washed with warm water until the residue was free of acid. The glass filters were dried at 50 °C in a temperature-controlled oven overnight or until a constant weight was obtained and Klason lignin calculated gravimetrically as a percentage of the starting material.

2.7. Analysis of phenolic acids

The total alkali-extractable hydroxycinnamate content of the alcohol insoluble residues (AIRs) was determined by saponification of 5 mg of sample in 4 M sodium hydroxide (1 ml; purged with nitrogen) for 17 h in the dark (shaking, room temperature). The samples were centrifuged (1000 rpm, 5 min) and the extract was taken for further analysis. *Trans*-cinnamic acid (10 μg/50 μl) was added as an internal standard and the solution adjusted to pH 2 with 6 M hydrochloric acid. Phenolic acids were extracted in 3 × 3 ml of ethyl acetate and the combined extracts evaporated to dryness (40 °C; under nitrogen). The dry extract was redissolved in aqueous methanol (MeOH/H2O, 50/50, v/v, 0.5 ml), filtered (0.22 μm fluoropore membrane) and injected onto a LUNA C18 reverse-phase HPLC column (Phenonomex, Macclesfield, UK). Ferulic and *p*-coumaric acid levels were quantified against standard curves and quantified according to the method of Waldron et al. (1996).

2.8. Microscopy

The wheat straw samples were examined with a Zeiss SteREO Discovery.V8 stereomicroscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) and imaged using an Olympus DP-25 single chip colour CCD camera (Olympus Life Science Europa GmbH, Hamburg, Germany) and the Cell^P imaging software (Olympus).