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Wheat straw lignin fractionation and characterization as lignin-carbohydrate complexes

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ABSTRACT

Alkaline extracted and untreated wheat straw were ball-milled with liquid nitrogen cooling rendering them completely soluble in the solvent system dimethylsulfoxide—aqueous tetrabutylammonium hydroxide for subsequent fractionation into two lignin-carbohydrate complex fractions termed glucan-lignin and xylan-lignin according to their preferred association with glucan or arabinoxylan, respectively. This is the first description using this fractionation protocol for wheat straw. Eventually, acidolysis lignins were prepared from both lignin-carbohydrate complexes and structurally characterized using wet chemistry and NMR spectroscopy methods. Using the novel procedure we could reveal differences regarding wheat straw lignin association with polysaccharides, *p*-hydroxycinnamic acids and tricin as well as in their monomer composition. In glucan-lignin the lignin moiety was found to be linked mainly to glucan but also to branched arabinoxylan. Xylan-lignin, however, was rich in structures creating cross-links between lignin and linear arabinoxylan via ether-ester bridges by bi-functional ferulic acid. Intermolecular ether-ester-linkages by ferulic acid connecting the lignin moieties of the two LCC fractions glucan-lignin and xylan-lignin were proposed. Alkaline extraction of the straw resulted in a strikingly lower recovery of xylan-lignin in the subsequent fractionation which was attributed to cleavage of ester linkages between ferulic acid and arabinoxylan. Structural characteristics indicated glucan-lignin and xylan-lignin deriving from different morphological origins of the cell wall.

1. Introduction

Wood lignin is considered to be intimately associated with plant cell wall polysaccharides by covalent bonds of benzyl ether, benzyl ester and phenyl glycoside type linkages. In annual plants additionally *p*-hydroxycinnamic acid (*p*HCA) moieties like *p*-coumaric acid (*p*CA) and ferulic acid (FA) are incorporated into the cell wall compound connected to both lignin as well as polysaccharides (Iiyama et al., 1994). For wheat straw it was shown that *p*CA was mainly bound by ester-bonds to lignin side-chains while FA was found to be predominantly ether-bound to lignin but also ester-bound to both lignin and hemicelluloses (Kondo et al., 1992; Lam et al., 1994; Scalbert et al., 1986, 1985). FA ester-bound to arabinose side-chains of arabinoxylan is supposed to form characteristic cross-links between arabinoxylan chains via FA dimerization. Further, it was shown that this ester-bound FA was available for radical coupling reactions with lignin monomers giving evidence that FA-arabinoxylan structures could act as initiation sites for lignification resulting in cross-links between lignin and grass cell wall polysaccharides as so-called lignin-carbohydrate complexes (LCCs) (Ralph et al., 1995, 1998). Intra- and/or inter-molecular ester-ether bridges formed by FA also between lignin fragments of wheat straw were proposed by Sun et al. (2002). Beside the strongly associated lignin-carbohydrate complexes of the cell wall polymers also the presence of a non-core lignin fraction (around 20% of total lignin), easily soluble in alkali, was reported to exist in wheat straw (Buranov and Mazza, 2008). We recently described three major lignin fractions isolated from wheat straw using a protocol combining ball-milling and acidolysis (Zikeli et al., 2014). One fraction – extractable by neutral dioxane-water without prior ball-milling and therefore termed as Free lignin (FL) – was characterized as a low-molar-mass glucan-linked lignin, rich in *p*HCA and tricin groups and loosely trapped inside the lignocellulose network. A second fraction – Dioxane lignin (DL) – was extractable by the same solvent only after ball-milling and showed association with linear arabinoxylan while the third major fraction – supposedly connected to branched arabinoxylan – required acidolysis treatment after ball-milling for

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successful isolation of Acidolysis lignin (AL). As a fourth fraction a residual core glucan-lignin was characterized which was resistant to all isolation methods applied. FL and DL showed low Klason lignin (KL) contents and lignin characteristics indicating their native state as an LCC fraction deriving from middle lamella regions while AL was assumed to represent secondary wall lignin. In our current work a new approach for lignin wheat straw characterization was used to initially separate different LCC fractions and subsequently isolate the contained lignin as AL fractions for detailed structural analysis.

Recently, various approaches were conducted to improve existing lignin isolation techniques. Gu et al. (2015) completely dissolved wheat straw internodes in LiCl-DMSO for a subsequent isolation of a cellulolytic enzyme lignin (CEL). Successfully, the yield of CEL and its purity were improved through this procedure while lignin structure was changed only little. Wheat straw lignin was also fractionated using green solvents like mixtures of acetone and water in order to selectively extract lignin fractions of either low molar mass or high molar mass depending on the volume ratios of acetone and water (Boeriu et al., 2014). Further, wheat straw lignin originating from an ethanol-based organosolv process was characterized by Huijgen et al. (2014). The organosolv lignin fraction was isolated in high yield and purity, but the content of condensed phenolic hydroxyl groups reported was higher than in wheat straw lignin isolated under less harsh conditions indicating structural alterations. Alkaline pulping, indicated for grass lignin due to structural features like alkali-labile ester-bonds, was recently compared to microwave-assisted alkaline pulping and an organosolv process using formic acid and hydrogen peroxide by Rossberg et al. (2015). The used organosolv process provided lignin with high purity but in much lower yield than from alkaline pulping and also compared to the ethanol-based organosolv process mentioned above. The usage of microwaves reduced the pulping time while lignin purity and composition were similar to the reference alkali-lignin isolated.

A wide range of fractionation techniques exist to extract lignin from woody or non-woody biomass, but most of them aim for a complete separation of the lignin and the carbohydrate portion due to industrial interests. However, when studying native lignocellulosic structure, a different approach is needed with the focus to leave native bonds between lignin and carbohydrates intact for further analysis. Lawoko et al. (2005) developed a method for the isolation of individual LCC fractions from ball-milled spruce wood and pulp in quantitative yield according to their association with different polysaccharides. Enzymatic treatment was used followed by urea and aqueous alkali treatments. As the two major LCC fractions one glucomannan-linked lignin and one xylan-linked lignin were identified and the respective lignin parts showed structural differences with a linear β-*O*-4′-linked lignin type in the xylan-lignin fraction. Lu and Ralph (2003) used dimethylsulfoxide (DMSO) together with the strong base tetrabutylammonium fluoride (TBAF) to fully dissolve finely ground softwood and hardwood samples for subsequent structural analysis by 2D NMR spectroscopy. Li et al. (2011) used a similar solvent system, DMSO with aqueous tetrabutylammonium hydroxide (TBAH), to achieve complete dissolution of Eucalypt wood and pulp after an initial ball-milling step in order to degrade crystalline cellulose. Following their protocol two structurally different LCC fractions could be isolated. The major fraction (LCC1) was identified as a glucan-lignin complex enriched in G units while the second fraction (LCC2) was found to be a xylan-lignin with strongly dominating S units. This protocol was further elaborated by Du et al. (2013) in order to also fractionate softwood introducing of a step using barium hydroxide to precipitate a mannan-enriched LCC fraction.

In the present study the solvent system DMSO/TBAH was used for the first time to completely dissolve ball-milled wheat straw and eventually obtain two separate LCC fractions – one associated with glucan and the other associated with xylan – both from untreated (US) and alkaline-pretreated wheat straw (AS). Physical alteration of the intimate cell wall structure through ball-milling was needed to ensure the accessibility of the applied solvent system for complete dissolution of the straw samples. In order to prevent lignin structure alteration during milling, cooling with liquid nitrogen was applied. Samples of the specific lignin fractions contained in these LCCs were then isolated by acidolysis-assisted dioxane-water extraction. By degradation of the associated polysaccharides under acidic conditions lignin can be solubilized in dioxane-water and isolated in high purity (Gellerstedt et al., 1994). Obtained Acidolysis lignin (AL) fractions were structurally characterized in detail using one- and two-dimensional NMR spectroscopy as well as carbohydrate and Klason lignin analysis. Lignin dissolved in the alkaline pretreatment liquor was recovered and structurally characterized as well. Compositions and structures of LCCs and their respective lignin fractions derived from US and AS were compared aiming for deeper understanding of the structure of the native lignocellulosic network in wheat straw.

2. Experimental

2.1. Materials

Air-dried wheat straw (*Tritium aestivum* L.) from Fa. Manz GmbH, (Parndorf, Austria) was cut in an Ultra Centrifugal Mill (Zm 200, Retsch GmbH, Haan, Germany) using a 40 mesh sieve. The milled straw was then acetone-extracted for 6 h in a Büchi Extraction System B-811 and extractives were determined gravimetrically. After drying at 40 °C in a heating oven (Model ED115, Binder GmbH, Germany) the extracted milled straw (US) was treated in a vibratory ball-mill under liquid nitrogen cooling (CryoMill, Retsch GmbH, Haan, Germany). The milling cycles of 1 min were interrupted by cooling cycles of 0.5 min. The 30 min milling time required for complete dissolution of the cell wall material in DMSO/aqueous TBAH was determined in pre-tests.

A representative portion of the wheat straw was pretreated in alkaline alcohol/water using 40% ethanol and 8 wt% NaOH at 70 °C for 18 h. Alkaline-pretreated wheat straw (AS) was submitted to ball-milling as described above for US. The alkaline pretreatment liquor was further used for the isolation of Alkali lignin (AlkL) (see Section 2.3). In pre-tests the required time for complete dissolution of AS in the used solvent system was determined as 23 min of ball-milling.

2.2. LCC extractions

LCC fractions were isolated according to Li et al. (2011) with slight modifications. 4.7 g of the ball-milled powders of US and AS were slowly added to a mixture of 50 ml DMSO and 50 ml aqueous TBAH (50:50 vol%) under mechanical stirring. After complete addition stirring was continued for 4 h at room temperature. For precipitation of LCC1 the solution was slowly added in small portions into 700 ml of distilled water and the precipitate was separated by centrifugation after a resting time of 48 h and washed once with distilled water. Separated crude LCC1 was then suspended in 700 ml distilled water and the pH value was adjusted to 5.0–5.5 with hydrochloric acid to neutralize residual TBAH base and complete precipitation of LCC1. LCC1 was then separated by centrifugation, washed with distilled water and lyophilized. The pooled supernatants from the centrifugation steps were subjected to ultrafiltration using a PALL Omega 1 kDA membrane in order to isolate LCC2 in the retentate. The retentate was repeatedly diluted with water to remove dissolved salts and subsequently lyophilized to obtain LCC2.