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Structural characterization of stalk lignin from banana plant

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ABSTRACT

Dioxane lignins from two fractions of banana plant 'Dwarf Cavendish' stalk (floral stalk (DL_{FS}) and rachis (DL_R)) were structurally characterized by a set of spectroscopic (Ultraviolet (UV), FTIR, solid- and liquid-state NMR) and chemical degradation (permanganate (PO) and nitrobenzene oxidation (NO)) techniques. Despite both lignins are of HGS-type, strong structural differences were observed between them. Thus, DL_{FS} showed almost twice the abundance of H and G units and almost half of the abundance of S units when compared to DL_R. DL_R possessed significantly higher amount of β-O-4′ structures (0.32/C_6 against 0.12/C_6) and the molecular weight (5400 Da against 3750 Da) than those of DL_{FS}. About 72% of the condensed structures in DL_{FS} are of β-5 and 5–5′ types, whereas 4-O-5′-diaryl ether structures were the most abundant condensed structures in DL_R. Most of H units in both lignins are terminal phenolic coumarates linked to lignin substructures by ester bonds. Both lignins are structurally associated with suberin-like components in cell wall tissues. Structural features of stalk lignin were discussed in terms of possible restrictions for the kraft pulping of integral stem material.

1. Introduction

In the last years, a significant attention has been paid to the new sources of vegetable fibres, alternative to wood raw materials, for the pulp and paper applications and biocomposites. Banana plant, a monocotyledonous annual herbaceous plant, has been suggested as a suitable crop for these types of applications (Cordeiro et al., 2004, 2005; Faria et al., 2006).

Annually about 72.5 million tons of bananas are produced (FAO, 2006), being the *Cavendish* variety the most produced and exported, corresponding to about 1/3 of the world production. After harvesting of the single bunch of bananas, a considerable amount of agricultural residues are produced, where the foliage, pseudo-stem and rachis are the most important morphological parts in terms of produced volume and of fibre quality. Since the above plant materials are available in the banana producing regions during all the year, these can be an important industrial source of fibres and chemicals, thus constituting an additional economical profit to farmers (Reddy and Yang, 2005).

One of the macromolecular components that plays an important physiological role in plant and determine the chemical processing of cellulosic fibres is lignin (Terashima and Fukushima, 1993). Regarding the pulp and paper applications, the amount and the structure of lignin determine the pulping and bleaching responses of the plant material. The lower the lignin content and the proportion of condensed structures in lignin, the less energy and reagents consumption are normally needed for the chemical pulping and bleaching processes. In contrast, the presence of high lignin concentrations in the middle lamella of plant cells is advantageous, for example, for the fibreboard production. The lignin has excellent compatibility with the thermosetting resins commonly used in

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fibreboard manufacture and contributes itself as an adhesive between fibres (Donaldson et al., 2001).

As part of a global research project, aiming to find new applications for 'Dwarf Cavendish' (*Musa acuminata* Colla var. *cavendish*) vegetal residues, we have studied the chemical composition of the plant tissues from different morphological regions of this banana plant variety (Oliveira et al., 2005, 2006a,b, 2007). These studies revealed the significant variations in the chemical composition and the structure of components from different morphological parts. Thus, a cellulose content of 37.3% in leaf sheaths and only 15.7% in floral stalk was found; the starch content varied between 26.3% in floral stalk and 0.4% in petioles/midrib; the lignin content varied between 24.3% in leaf blades and 10.5% in rachis and the amount of lipophilic extractives was 5.8% in leaf blades and 1.2% in petioles/midrib. These findings allowed some propositions on the eventual utilization of different parts of banana plant and to formulate some restrictions for their integral processing. In particular, the successful kraft pulping of pseudo-stem was explained by a relatively low content (about 13%) and particular structure of lignin in leaf sheaths (high proportion of S units and low condensation degree) of the banana plant (Oliveira et al., 2007). At the same time, the preliminary results on the lignin structure in stalk, another counterpart of pseudo-stem, indicated its eventual negative effect on the total pulping efficiency and pulp quality, indicating that these two parts of pseudo-stem (leaf sheaths and stalk) should be separated before pulping (Oliveira et al., 2007). In this context, additional information on stalk lignin structure would be helpful to evaluate the risks of the integral stem pulping.

In the present work, lignins from the internal (floral stalk) and external (rachis) stalk parts of 'Dwarf Cavendish' were characterized by a set of wet chemistry and spectroscopic (Ultraviolet (UV), FTIR and NMR) methods aiming to provide new structural information on these lignins and to justify the pseudo-stem fractionation before the pulping applications.

2. Materials and methods

2.1. Preparation of plant material

Floral stalk and rachis from mature banana plants 'Dwarf Cavendish' were collected in Madeira Island (Portugal) (Oliveira et al., 2007). The air-dried materials were milled in a Retsch AS200 and sieved to 40–60 mesh fractions. This fraction was submitted to successive extractions with ethanol/toluene (2:1, v/v) for 4 h followed by water for 6 h. The extractives contents were 3.2% and 9.6% (o.d. material) in floral stalk and 3.4% and 14.7% (o.d. material) in rachis, respectively (Oliveira et al., 2007).

2.2. Isolation of lignins

Lignins from floral stalk and rachis were isolated by acidolysis from alkali pre-extracted sawdust in a nitrogen atmosphere by the dioxane method as described previously (Oliveira et al., 2006a). Briefly, the sample was submitted to treatment with 0.3% NaOH solution during 1 h under reflux, exhaustively washed with distilled water and dried. Then, the alkali pre-extracted sawdust was submitted to three sequential extractions (30 min each) with a dioxane–water (9:1, v/v) solution containing 0.2 M HCl under reflux in a nitrogen atmosphere. After the dioxane–water wash, each portion of extract was concentrated separately, the resulting fractions were combined, and lignin was precipitated in cold water. The lignin was centrifuged, washed with water until pH 6 and freeze-dried. The lignin yields were about 37% and 33%, based on the Klason lignin in the alkali pre-extracted plant, for floral stalk and rachis, respectively.

Dioxane lignins were purified by two sequential purification steps: an extraction step with dioxane:methanol followed by an exhaustive chloroform extraction. These purified lignins (designated DL_{FS} and DL_R, for floral stalk and rachis lignin, respectively) were submitted to structural characterization analysis, representing about 22% and 20% of lignin in the alkali pre-extracted plant (based on Klason lignin) for floral stalk and rachis, respectively.

2.3. Chemical analysis

The alkaline nitrobenzene oxidation (NO) as well as the permanganate oxidations (PO) of *in situ* and isolated lignins were performed as described previously (Gellerstedt, 1992; Chen, 1992). The analysis of methoxyl groups was performed by Zeisel method (Zakis, 1994). The residual neutral sugars in the lignin sample were analyzed by GC as described previously (Blakeney et al., 1983). The elemental composition was determined on a LECO CHNS-932 instrument.

2.4. Size exclusion chromatography

Lignins (0.5%, w/w) were dissolved in 0.1 M LiCl dimethylformamide (DMF) and analyzed by Size Exclusion Chromatography (SEC) using a PL-GPC 110 chromatograph equipped with a pre-column Plgel 5 μm and two Plgel 5 μm Mixed D 300 mm × 7.5 mm columns at 70 °C. A solution of 0.1 M LiCl in DMF, used as an eluent, was pumped at a flow rate of 0.9 mL/min. The SEC columns were calibrated using lignin preparations previously characterized by ESI/MS (Evtuguin et al., 2001).

2.5. UV, FTIR and ^{13}C CP-MAS NMR

Ultraviolet (UV) spectra were recorded in 2-methoxyethanol solutions on a JASCO V-560 UV/vis spectrophotometer using 1 cm thick cell. Infrared (FTIR) spectra were recorded on a Mattson 7000 FTIR spectrometer in KBr pellets (1/250 mg). The spectra resolution was 4 cm^{-1} and 132 scans were averaged. ^{13}C solid-state NMR spectra were recorded at 100.6 MHz (9.4 T) on a Bruker Avance 400 spectrometer, with a 7-mm double bearing Bruker rotor, spun in air at 5.0 kHz. The Cross Polarization-Magic Angle Spinning (CP-MAS) spectra were recorded with a 5 s recycle delay and a 2 ms contact time. About 10,000 scans were collected. In all experiments the ^{1}H and ^{13}C 90° pulses were ca. 4 μs.