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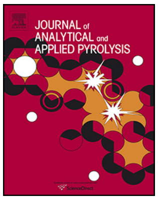

S/G ratio and lignin structure among Eucalyptus hybrids determined by Py-GC/MS and nitrobenzene oxidation

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ABSTRACT

The ratio among syringyl (S) and guaiacyl (G) lignin substructures is increasingly used as criteria in the selection of Eucalyptus species to pulping processes. The S/G distribution of several Brazilian Eucalyptus species and their crossings was determined by analytical pyrolysis-GC/MS (Py-GC/MS) and alkaline nitrobenzene oxidation directly from wood fibers. The ratio varied among the Eucalyptus hybrids. In Py-GC/MS, the S/G ratio is calculated by summing up the proportions of S and G type lignin pyrolysis products with various 1–3 carbon containing side chain structures. Py-GC/MS revealed that the distribution of the lignin side chain structures varied among the species. Similar trends were seen both in the G and in the S series. The result was verified with principal component analysis (PCA), which also revealed that similar side chains structures in the G and S units are correlated with each other. That PCA result confirmed that all G and S type degradation products should be included in the calculation of S/G ratios instead of selected products. S/G ratios determined by Py-GC/MS and alkaline nitrobenzene oxidation were close to each other, but not equal. Lower values were obtained by Py-GC/MS. The differences between the two methods are probably related the degradation mechanism of lignin with Py-GC/MS and nitrobenzene oxidation methods. Based on these results, it is not possible to say which of the methods is more reliable. However, both methods can well be used to compare the S/G ratios of lignin among samples.

1. Introduction

Lignin is a highly heterogeneous polymer composed of hydroxycinnamyl alcohols, including coniferyl, sinapyl and *p*-coumaryl alcohol building blocks. The structure of lignin varies depending on the plant species, cell type and environmental factors. Lignin units are linked by a variety of ether and carbon–carbon bonds that are usually classified into condensed linkages, such as biphenyl, biphenyl ethers and non-condensed linkages, including alkyl-aryl ether linkages (β-O-4) [1]. In hardwood species like Eucalyptus spp, lignin is mainly composed of guaiacyl (G) and syringyl (S) moieties. Several studies have shown that the structure of lignin, particularly S/G ratio, affects the processability of the wood, *e.g.* delignification [2–4]. Furthermore, the S/G ratio determined both with alkaline nitrobenzene and with permanganate oxidation methods has been found to correlate with the proportion of the condensed structures of lignin. The higher the S/G ratio less condensed structures has been determined from the wood [5].

Analytical pyrolysis combined with gas chromatography–mass spectrometry (GC/MS) has been increasingly used to determine lignin structure from wood fibers, because time consuming sample preparation is not needed. In addition, the phenolic lignin pyrolysis products have been identified and reported [6–8]. The technique has increasingly been used for the estimation of the S/G ratio of lignin [2,4,9–15]. Another method commonly used for the same purpose is the nitrobenzene oxidation method [9–17]. In the alkaline nitrobenzene oxidation, lignin is oxidatively cleaved to form aromatic carbonyl compounds, *i.e.* syringaldehyde and vanillin as main products [18]. Due to the lacking reactivity of nitrobenzene oxidation against condensed structures, the yield of the carbonyl compounds is approximately 50% of the lignin. Due to the high proportion of condensed guaiacyl units, only about 30% of them are converted to vanillin. On the contrary, the conversion of syringyl units to syringaldehyde may be as high as 90% due to the low proportion of condensation in this case. Therefore, the obtained S/G ratio by alkaline nitrobenzene oxidation is expected to be higher than the actual proportion of guaiacyl and syringyl units in lignins [1,17].

In pyrolysis, lignin is thermally degraded to a mixture of phenols by heating samples in an inert atmosphere. The formed products retain the structural information of the original polymer. However, it has been difficult to find direct relationship among the phenolic pyrolysis degradation products and the original lignin structure. It is proposed that the phenols formed result from the cleavage of

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the β-O-4 and some condensed linkages such as β-5 [19–22]. However, the yield of the pyrolysis products is expected to be highly dependent on the number of condensed structures present in lignin [9,10,12,23]. Thus, also Py-GC/MS overestimates the syringyl units in lignin similarly to nitrobenzene oxidation. A correction equation has been developed in order to compensate for the effect of condensation and thus reduce the yield of S units with relation to G units [10]. The yield of the monomeric pyrolysis degradation products for the different Japanese hardwood species has been reported to vary from 12.2 to 26.1% of Klason lignin [14]. For hardwood type synthetic dehydrogenation polymers the pyrolysis yield varied from 10 to 20% [12]. The pyrolysis yields were thus much lower than that reported for the nitrobenzene oxidation yields of vanillin and syringaldehyde [12,14]. S/G ratios by Py-GC/MS and alkaline nitrobenzene oxidation have been compared [9,11–14]. The ratios for wood samples are close to each other, but not equal [11,13,14]. In several studies poor chromatographic separation of carbohydrate derivatives from lignin derivatives has caused problems in integration of lignin derivatives [11,13,14]. To overcome this problem, selected lignin pyrolysis degradation products have been used for the S/G ratio calculation by Py-GC/MS [11,13]. That, however, may lead to false information about the S/G ratios. Good correlation among Py-GC/MS and nitrobenzene oxidation methods has been obtained for the isolated lignins [12]. In isolated lignin samples, the cellulosic fiber matrix is not present, which in wood samples may affect the result. The problem related to overlapping carbohydrate derivatives is also overcome. However, the isolation process may change the lignin structure and also the isolation increases the analysis time.

Eucalyptus wood is fast growing plant material that is increasingly used for pulp production in temperate zones. S/G ratio of lignin is one of the most important parameters used to evaluate the performance of Eucalyptus wood. Thus, reliable and fast methods for the determination of S/G ratios of lignin directly from wood samples without any need of lignin isolation are needed. In this study, a large number of wood samples from several Eucalyptus species including *E. grandis*, *E. urophylla*, *E. globulus*, *E. dunnii*, *E. camaldulensis* and their crossings were subjected to Py-GC/MS analysis. The aim of the study was to determine possible differences in the structure of lignin and S/G ratio among Eucalyptus hybrids by Py-GC/MS. Especially the effect of the change of species in Eucalyptus crossings on lignin structure was studied. In addition, Py-GC/MS was compared with alkaline nitrobenzene oxidation, aiming at clarifying the potential of the two methods for the estimation of the S/G ratio in lignin. The aim was also to understand the reasons behind the observed differences among the S/G ratios obtained with Py-GC/MS and alkaline nitrobenzene oxidation.

2. Material and methods

2.1. Sample preparation

Eighteen 7-year-old Eucalyptus hybrids, including a number of double/triple/fourth crossings among *E. grandis*, *E. urophylla*, *E. globulus*, *E. dunnii* and *E. camaldulensis* species obtained from Brazil were used as raw materials of this study. Detailed list of the Eucalyptus hybrids is presented in Table 1.

For the chemical analyses, wood chips were prepared with a laboratory chipper (a Chogokukikai model). The chips were well mixed (260 m^3 rotary mixers) and screened according to SCAN-CN 40:94 procedures [24]. For the S/G ratio determination with alkaline nitrobenzene oxidation, the chips were grinded in a Wiley type mill (40 mesh screen) to 40–60 mesh and extractives were removed (mixture of ethanol/toluene 1:2 followed first by ethanol and secondly by hot water) according to TAPPI T264cm-97 standard procedure [25]. Lignin composition and S/G ratio was measured by Py-GC/MS for the Wiley milled chips without extractives removal.

Table 1
Samples.

No.	Eucalyptus hybrids	Short code
1	*E. urophylla* (IP) × *E. grandis* (IP)	IP
2	*E. urophylla* (Flores IP) × *E. urophylla* (Timor)	U1×U2
3	*E. urophylla* (Flores IP) × *E. camaldulensis* (VM2)	U1×C2
4	*E. urophylla* (Flores IP) × [*E. urophylla* (R) × *E. globulus* (R)]	U1×UGL
5	*E. grandis* (Coffs Harbour) × [*E. urophylla* (R) × *E. globulus* (R)]	G1×UGL
6	*E. dunni* (R) × [*E. urophylla* (R) × *E. globulus* (R)]	D1×UGL
7	*E. camaldulensis* (VM1) × [*E. urophylla* (R) × *E. globulus* (R)]	C1×UGL
8	[*E. dunnii* (R) × *E. grandis* (R)] × [*E. urophylla* (R) × *E. globulus* (R)]	DG×UGL
9	[*E. dunnii* (R) × *E. grandis* (R)] × *E. urophylla* (Timor)	DG×U2
10	[*E. dunnii* (R) × *E. grandis* (R)] × *E. camaldulensis* (VM1)	DG×C1
11	[*E. dunnii* (R) × *E. grandis* (R)] × *E. globulus* (R) (Dad, pollen)	DG×GL2
12	*E. dunni* (KR) × *E. globulus* (R) (Dad, pollen)	D2×GL2
13	*E. grandis* (Coffs Harbour) × *E. globulus* (R) (Dad, pollen)	G1×GL2
14	*E. urophylla* (Timor) × *E. globulus* (R) (Mom, stigma)	U2×GL1
15	*E. urophylla* (Timor) × *E. camaldulensis* (VM1)	U2×C1
16	*E. camaldulensis* (VM1) × *E. camaldulensis* (VM1)	C1×C2
17	*E. dunni* (R) × *E. dunni* (KR)	D1×D2
18	*VCP – E. grandis* (VCP) × *E. urophylla* (VCP)	VCP

2.2. Determination of the S/G ratio by nitrobenzene oxidation/HPLC

The determination of the S/G ratio of each extractive-free wood sample was obtained by the nitrobenzene oxidation according to Lin and Dence [17]. Approximately 0.2 g (oven dryed) of each wood sample together with NaOH aqueous solution (7 mL; 2 mol L^{-1}) and nitrobenzene (0.5 mL) was loaded into a stainless steel reactor and heated up to 170 °C for 2.5 h, with analyses repeated twice. The oxidized material was then extracted with chloroform (6× 30 mL). After the first extraction, HCl was added (2.5 mL; 4 mol L^{-1}) to the aqueous phase. The combined organic phases were evaporated. The sample was transferred to a 50 mL volumetric flask and the volume completed with acetonitrile/water solution (1:1, v/v). Following, the solution was filtered in a regenerated cellulose membrane, pore size 0.45 μm, analyzed by high performance liquid chromatography (HPLC) in a Shimadzu CBM-10A apparatus equipped with a UV-VIS detector SPD-10AV, operating at 280 nm wavelength, LC-10AD pump and Rheodyne injector with a 20 mL loop and a Lichrosorb RP-18 (250 mm × 4 mm, 5 μm) reverse phase analytical column. The mobile phase was composed of acetonitrile/water (1:6, v/v) and the pH was adjusted to 2.6 with trifluoroacetic acid (TFA) buffer. The column temperature was kept at 40 °C, and mobile phase flow of 1.5 mL/min was used. Vanillin and syringaldehyde standards (Aldrich, Milwaukee, WI, USA) were used for quantification of derivatives of guaiacyl and syringyl units, respectively. Calibration curves using vanillin and syringaldehyde standards were obtained in the concentrations of 0.375, 0.75, 1.125, and 1.5 mmol L^{-1} for vanillin, and 0.825, 1.65, 2.475, and 3.3 mmol L^{-1} for syringaldehyde. The solutions were prepared in an acetonitrile/water mixture (1:1, v/v), at pH 2.6. The equation used for vanillin and syringaldehyde were $y = 5{,}669{,}520x - 84{,}175$ ($R^2 = 0.9993$, %RSD = 1.35) and $y = 3{,}056{,}620x + 75{,}181$ ($R^2 = 0.9996$, %RSD = 2.05), respectively. The partial least square method was used to fit the data.