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Morphological characteristics and composition of lipophilic extractives and lignin in Brazilian woods from different eucalypt hybrids

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ABSTRACT

The morphological and chemical characteristics of the woods from several eucalypt hybrids from the Brazilian Genolyptus program were studied. The hybrids selected for this study were *Eucalyptus grandis* × *E. urophylla* (IP), *E. urophylla* × *E. urophylla* (U1 × U2), *E. grandis* × [*E. urophylla* × *E. globulus*] (G1 × UGL), and [*E. dunnii* × *E. grandis*] × *E. urophylla* (DG × U2). The analyses of the lipophilic extractives indicated a similar composition in all eucalypt hybrids, which were dominated by sitosterol, sitosterol esters and sitosteryl 3β-D-glucopyranoside. These compounds are responsible for pitch deposition during kraft pulping of eucalypt wood. Some quantitative differences were found in the abundances of different lipid classes, the wood from U1 × U2 having the lowest amounts of these pitch-forming compounds. The chemical composition and structure of lignins were characterized by Py-GC/MS and 2D-NMR that confirmed the predominance of syringyl over guaiacyl units and only showed traces of *p*-hydroxyphenyl units in all the woods, with the highest S/G ratio for G1 × UGL. The 2D-NMR spectra gave additional information about the inter-unit linkages in the lignin polymer. All the lignins showed a predominance of β-O-4′ ether linkages (75–79% of total side-chains), followed by β–β′ resinol-type linkages (9–11%) and lower amounts of β-5′ phenylcoumaran-type, β-1′ spirodienone-type linkages or β-1′ open substructures. The lignin from the hybrid G1 × UGL presented also the highest proportion of β-O-4′ linkages, and therefore, it is foreseen that the wood from this hybrid will be more easily delignifiable than the other selected Brazilian eucalypt hybrids. In complement to these chemical analyses, the morphological characterization of fibers, vessels and fines revealed that hybrid eucalypt clone DG × U2 presented the most interesting properties for the manufacture of paper pulps and biofuels.

1. Introduction

Eucalypt is a fast growing tree whose wood is the main raw material for paper pulp production in Southwest Europe, Brazil, South Africa, and other countries. Eucalypt is the largest single global source of market pulp and its use for pulp production has greatly increased during last decades, the world production attaining nearly 20 million tons/year, which is about 60% of the total hardwood pulp produced (Trabado and Wilstermann, 2008). An additional capacity over 10 million tons/year is expected in the next 5 years. Different eucalypt species are used for pulp and papermaking, including *Eucalyptus globulus*, *E. nitens*, *E. maidenii*, *E. dunni*, *E. grandis*, *E. urophylla* and *E. saligna*, and single, double and triple crossings hybrids among these species. The major interest in eucalypt wood comes from its low production cost in certain regions, due mainly to high forest productivity and high pulp yield, and the outstanding quality of their fibers.

Biomass production costs are low in Brazil compared to other parts of the world, due to proper climate, large available areas for cultivation, advanced forest and agricultural technologies and excellent adaptation of certain crops in the tropical climate. Thus, Brazil presents great potential for the growing of eucalypt plantations that are highly productive, and reaching up to 60 m^3/ha/year in some cases (SBS, 2007). Eucalypt plantations in Brazil represent over 3.8 million hectares. Among the planted eucalypts, 55% corresponds to *E. grandis*, 17% to *E. saligna*, 11% to hybrids, 9% to *E. urophylla*, 2% to *E. viminalis* and 6% to other species (STCP, 2007).

The interest in the use of eucalypt wood for paper pulp production has promoted the research of the chemical characteristics of different species (del Río et al., 2005; Evtuguin et al., 2001;

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González-Vila et al., 1999; Gutiérrez et al., 1999; Capanema et al., 2005; Ibarra et al., 2007; Rencoret et al., 2007, 2008, 2011) aiming to improve the industrial use of this interesting raw material for pulp and paper manufacturing. However, only limited studies have been published regarding the chemical characteristics of eucalypt woods grown in tropical areas, such as the so-called *E. urograndis*, a hybrid derived from the single crossing between *E. grandis* and *E. urophylla*, that is one of the main eucalypt hybrids used for pulping in Brazil (Freire et al., 2006b; Silvério et al., 2007).

In this paper, we report the morphological and chemical characteristics of the woods from different eucalypt hybrids grown in Brazil and coming from the Brazilian Network of Eucalyptus Genome Research, the so-called Genolyptus program (Grattaplagia, 2003, 2004), a nationwide initiative which involved integrated advances in genomic resources, molecular breeding and wood phenotyping technologies. Double and triple crossings were selected for this study. A special emphasis will be put in the lipid and lignin composition since these two fractions play an important role during pulping, bleaching and papermaking. The content and chemical structure of wood components, in particular the lignin content and its composition in terms of its *p*-hydroxyphenyl (**H**), guaiacyl (**G**) and syringyl (**S**) moieties and the different inter-unit linkages are important parameters in pulp production in view of delignification rates, chemical consumption and pulp yields, as well as in subsequent bleaching. Higher S/G ratios imply higher delignification rates, less alkali consumption and therefore higher pulp yield (González-Vila et al., 1999; del Río et al., 2005). On the other hand, extractives, especially the lipophilic compounds, are also important for pulp and paper production (Back and Allen, 2000). Extractives are often released from the fibers during pulping and can form colloidal pitch and cause production troubles as deposits. In the manufacture of alkaline pulps, a large part of the lipids is removed from the wood during cooking. However, some substances survive this process and can be found as pulp extractives. If they form the so-called pitch deposits, the consequences are serious, including reduced product quality and higher operating costs due to production stops for cleaning the equipment (del Río et al., 1998, 2000; Gutiérrez et al., 2001a, 2001b; Silvestre et al., 1999). The increasing trend in recirculating water in pulp mills aggravates these problems.

Finally, the morphological characteristics of the wood components, which strongly influence the quality of the pulp, and of the final paper sheet, were also addressed. Longer, flexible and fibrillated fibers are preferred for reaching the required paper strengths, but shorter fibers and fines improve the optical properties. For hardwoods, and therefore for eucalypts, the vessels are wood components that can generate lack of resistance into the sheet, dust generation during sheet converting and printing defaults (picking and speckles). Lower vessel contents into the pulp would be preferable, and more particularly the earlywood vessels.

2. Materials and methods

2.1. Samples

The eucalypt hybrid woods selected for this study were supplied by the University of Viçosa and consist of five-year-old trees of the following double or triple crossings: (i) *E. grandis* × *E. urophylla* (IP); (ii) *E. urophylla* × *E. urophylla* (U1 × U2); (iii) *E. grandis* × [*E. urophylla* × *E. globulus*] (G1 × UGL); and (iv) [*E. dunnii* × *E. grandis*] × *E. urophylla* (DG × U2).

The wood samples were air dried. The dried samples were milled using a knife mill (Janke and Kunkel, Analysenmühle), and successively extracted with acetone in a Soxhlet apparatus for 8 h and with hot water (3 h at 100 °C). The acetone extracts were evaporated to dryness, and resuspended in chloroform for chromatographic analysis of the lipophilic fraction. The water soluble material was also evaporated to dryness and weighted. Two replicates were used for each sample. Klason lignin was estimated according to T222 om-88 (Tappi, 2004). Milled-wood lignin (MWL) was extracted from finely ball-milled (15 h) plant material, free of extractives and hot water soluble material, using dioxane-water (9:1, v/v), followed by evaporation of the solvent, and purified as described (Björkman, 1956). The final yields ranged from 15% to 20% of the original Klason lignin content.

2.2. GC and GC/MS analyses

The GC analyses of lipids were performed in an Agilent 6890N Network GC system equipped with a split–splitless injector and a flame ionization detector (FID). The injector and the detector temperatures were set at 300 °C and 350 °C respectively. Samples were injected in the splitless mode. Helium was used as the carrier gas. The capillary column used was a high temperature, polyimide coated fused silica tubing DB5-HT (5 m × 0.25 mm I.D., 0.1 μm film thickness; J&W Scientific). The oven was temperature-programmed from 100 °C (1 min) to 350 °C (3 min) at 15 °C min^{-1}. Peaks were quantified by area, and a mixture of standards (octadecane, palmitic acid, sitosterol, cholesteryl oleate, and sitosteryl 3β-D-glucopyranoside) was used to elaborate calibration curves. The data from the two replicates were averaged.

The GC/MS analysis were performed on a Varian 3800 gas chromatograph coupled with an ion-trap detector (Varian 4000) equipped with a high-temperature capillary column (DB5-HT, 15 m × 0.25 mm i.d., 0.1 μm film thickness; J&W Scientific). Helium was used as carrier gas at a rate of 2 mL min^{-1}. The oven was heated from 120 °C (1 min) to 380 °C (5 min) at 10 °C min^{-1}. The temperature of the injector during the injection was 120 °C, and 0.1 min after injection was programmed to 380 °C at a rate of 200 °C min^{-1} and held for 10 min. The temperature of the transfer line was set at 300 °C. The lipophilic extractives were analyzed both underivatized and as trimethylsilyl derivatives formed by reaction with bis(trimethylsilyl)trifluoroacetamide (BSTFA). Compounds were identified by comparing their mass spectra with mass spectra in the Wiley and NIST libraries, by mass fragmentography and, when possible, by comparison with authentic standards.

2.3. Gel permeation chromatography

GPC was performed on a Shimadzu LC-20A LC system (Shimadzu, Kyoto, Japan) equipped with a photodiode array (PDA) detector (SPD-M20A; Shimadzu) using the following conditions: column, TSK gel α-M + α-2500 (Tosoh, Tokyo, Japan); eluent, 0.1 M LiBr in dimethylformamide (DMF); flow rate, 0.5 mL min^{-1}; temperature, 40 °C; sample detection, PDA response at 280 nm. The data acquisition and computation used LCsolution version 1.25 software (Shimadzu). The molecular weight calibration was via polystyrene standards.

2.4. Py-GC/MS

Pyrolysis of MWL (approximately 100 μg) was performed with a 2020 micro-furnace pyrolyzer (Frontier Laboratories Ltd.) connected to an Agilent 6890 GC/MS system equipped with a HP 5MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and an Agilent 5973 mass selective detector (EI at 70 eV). The pyrolysis was performed at 500 °C. The oven temperature was programmed from 50 °C (1 min) to 100 °C at 30 °C min^{-1} and then to 300 °C (10 min) at 10 °C min^{-1}. He was the carrier gas (1 mL min^{-1}). The compounds were identified by comparing their mass spectra

Table 1
Content (%) of different components of the woods of the different eucalypt hybrids (as dry, ash-free basis).

Component	IP	U1 × U2	G1 × UGL	DG × U2
Acetone extractives (lipophilics)	0.6 (0.2)	2.1 (0.2)	2.2 (0.3)	0.9 (0.2)
Water soluble material	1.4	1.7	1.8	1.5
Klason lignin*	24.2	24.3	24.1	24.5
Acid-soluble lignin	3.0	3.4	3.4	3.1

* Corrected for proteins and ash.

with those of the Wiley and NIST libraries and reported in the literature (Faix et al., 1990; Ralph and Hatfield, 1991). Peak molar areas were calculated for the lignin-degradation products, the summed areas were normalized, and the data for two repetitive analyses were averaged and expressed as percentages.

2.5. NMR spectroscopy

2D-NMR spectra were recorded at 25 °C in a Bruker AVANCE 600 MHz, equipped with a cryogenically-cooled z-gradient triple resonance probe. Forty milligrams of MWL were dissolved in 0.75 mL of dimethylsulfoxide (DMSO)-d_6, and HSQC (heteronuclear single quantum correlation) spectra were recorded. The spectral widths were 5000 and 13,200 Hz for the ^{1}H and ^{13}C dimensions, respectively. The number of collected complex points was 2048 for ^{1}H dimension, with a recycle delay of 5 s. The number of transients was 64, and 256 time increments were always recorded in ^{13}C dimension. The $^1J_{CH}$ used was 140 Hz. The *J*-coupling evolution delay was set to 3.2 ms. Squared cosine-bell apodization function was applied in both dimensions. Prior to Fourier transformation, the data matrixes were zero filled up to 1024 points in the ^{13}C dimension. The central solvent peak was used as an internal reference (δ_C 39.5; δ_H 2.49). HSQC cross-signals were assigned by comparing with the literature. The cross-signal intensity depends on the particular $^1J_{CH}$ value, as well on the T_2 relaxation time; therefore, a direct intensity analysis of the different signals is impossible. Thus, integration was performed separately for the different regions of the spectra, which contain signals corresponding to chemically-analogous C–H pairs, with similar $^1J_{CH}$ coupling values. In the aliphatic oxygenated region, inter-unit linkages were estimated from C_α–H_α correlations, except for structures **E** and **F** described below where C_β–H_β and C_γ–H_γ correlations were used, respectively, and the relative abundance of side-chains involved in different substructures and terminal structures were calculated (with respect to total side-chains), as well as the percentage of each inter-unit linkage type with respect to the linkage total. In the aromatic region, C–H correlations from S and G units were used to estimate the S/G ratio.

2.6. Morphological characterization of the fibers and vessels

The wood samples were treated with a solution of 3.4% sodium chlorite in acetic acid buffer (pH 4.9) at 100 °C under stirring until the separation of the wood components from their matrix. The obtained fibers and vessels suspension was washed with tap water and filtered before analysis with the MorFi and CyberMetrics analyzers. The MorFi analyzer was designed and developed by CTP for the morphological characterization of fibers, vessels and fines (Eymin-Petot-Tourtollet et al., 2003). The analysis was carried out on a pulp suspension passing through a specific cell illuminated by a laser beam and connected to a high-resolution CCD camera. This analysis allowed reliable statistical measurement of thousands of fibers, vessels and fines to determine the main morphological and dimension characteristics of the pulp components. The CyberBond analyzer allowed to measure the relative bonded area index (RBA) of the fibers, an important characteristics controlling the inter-fiber bonding potential (Das et al., 2003). After deposition on a glass slide, the fibers were analyzed by the way of a microscope lens coupled to a CCD camera. The RBA index measurement was based on the Clarke's method and the image analysis allowed calculating automatically the index (Das et al., 2003). Cross sections of the eucalypt wood samples were directly examined with a light microscope to observe the distribution of fibers and vessels.

3. Results and discussion

3.1. Chemical characteristics of the Brazilian woods from different eucalypt hybrids

The content of acetone extractives, water soluble material, Klason lignin and acid soluble lignin in the Brazilian woods from the different eucalypt hybrids is listed in Table 1. The Klason lignin content was very similar for all of them, in the range from 24.1 to 24.5%. The total acetone extractives content of the woods from the different eucalypt hybrids ranges from 0.6 to 2.2% of dry material. However, the content of lipophilic extractives, estimated as the fraction of the acetone extracts that can be redissolved in chloroform, is much lower and very similar for all the woods, ranging from 0.2 to 0.3% of the total dry material. This low lipophilic content is similar to that found in woods from different eucalypt species (Rencoret et al., 2007).

3.2. Lipid composition of the Brazilian woods from different eucalypt hybrids

Although the lipid content is low and similar in all the selected hybrid eucalypt woods, it is not only the content but the composition that strongly affects the pitch deposition during pulping and papermaking (Back and Allen, 2000). Therefore, the detailed chemical composition of the lipophilic extractives in the different woods was investigated. The underivatized and silylated lipophilic extracts from the hybrid eucalypt woods were analyzed by GC and GC/MS using short- and medium-length high temperature capillary columns, respectively, with thin films, according to the method previously described (Gutiérrez et al., 1998). The GC/MS chromatogram of the lipid extracts (as trimethylsilyl ether derivatives) from a selected eucalypt hybrid (IP) is shown in Fig. 1. The identities and abundances of the main lipophilic compounds identified in the selected Brazilian eucalypt hybrids are detailed in Table 2, and their structures are depicted in Fig. 2.

The most predominant lipophilic compounds present in all the eucalypt woods selected for this study were steroids, including sterols, sterol glycosides and sterol esters with lower amounts of steroid ketones and steroid hydrocarbons. Other important lipophilic compounds found were series of fatty acids, glycerides (including mono-, di- and triglycerides) and minor amounts of squalene, tocopherols (in free and esterified form) and a series of alkyl ferulates. This composition is similar to that reported in the woods of other eucalypt species (Gutiérrez et al., 1999; Rencoret et al., 2007).

Free sterols were among the major compound class in the extracts of all eucalypt woods (ranging from 412 to 902 mg/kg

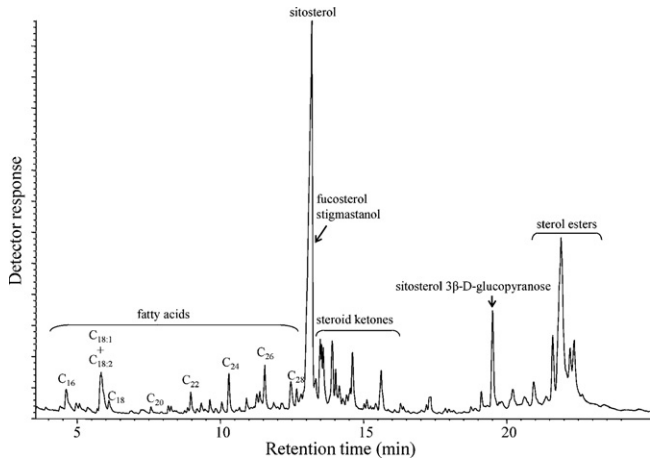

Fig. 1. Chromatogram of the lipophilic extractives (as TMSi derivatives) from a selected eucalypt hybrid (IP) wood.

wood), sitosterol (I; Fig. 2) and stigmastanol (II) being the main sterols present in all eucalypt woods, with lower amounts of campesterol (III), stigmasterol (IV), fucosterol (V), cycloartenol (VI), 24-methylenecycloartanol (VII) and 7-oxositosterol (VIII). The wood from U1 × U2 presents the lowest content of free sterols, which are among the main compounds responsible for pitch deposition during kraft cooking of eucalypt wood (del Río et al., 1998, 2000; Silvestre et al., 1999), while the rest of the eucalypt hybrids selected for this study present higher amounts of free sterols, and therefore will be more prone to have pitch deposition problems. Significant amounts (from 97 to 178 mg/kg wood) of sterol glycosides, principally sitosteryl 3β-D-glucopyranoside (IX), were found in the extracts of all the eucalypt woods. Sterol glycosides were first reported in *E. globulus* wood by Gutiérrez and del Río (2001) together with the corresponding acyl steryl glycoside, principally sitosteryl (6′-O-palmitoyl)-3β-D-glucopyranoside. Sitosteryl 3β-D-glucopyranoside was especially abundant in the wood of the DG × U2 hybrid while the U1 × U2 wood presents the lowest content. Sterol esters were also present in high amounts among the lipophilic extractives of the different eucalypt hybrid woods, accounting from 346 to 433 mg/kg wood, the major abundances

Table 2
Composition of lipophilic extractives (mg/kg wood) from the woods of the different eucalypt hybrids.

Compound	IP	U1 × U2	G1 × UGL	DG × U2
Fatty acids	289.7	139.8	310.2	208.0
n-Pentadecanoic acid	2.0	1.1	1.9	1.1
n-Hexadecanoic acid	43.9	35.8	54.9	40.9
n-Heptadecanoic acid	4.2	2.5	4.1	4.3
9,12-Octadecadienoic acid	49.8	28.6	54.1	40.1
9-Octadecenoic acid	27.0	15.5	50.0	25.7
n-Octadecanoic acid	19.9	10.8	16.5	15.9
n-Nonadecanoic acid	1.7	0.8	1.4	1.1
n-Eicosanoic acid	6.8	3.7	8.9	6.2
n-Heneicosanoic acid	6.3	1.6	5.4	5.1
n-Docosanoic acid	16.5	4.2	13.6	8.9
n-Tricosanoic acid	4.2	3.8	5.8	9.6
n-Tetracosanoic acid	38.0	11.6	35.7	22.1
n-Pentacosanoic acid	13.0	3.6	8.1	9.6
n-Hexacosanoic acid	40.0	11.0	23.8	13.2
n-Heptacosanoic acid	3.6	1.3	3.7	0.9
n-Octacosanoic acid	12.8	3.9	22.3	3.3
Steroid hydrocarbons	35.7	16.4	19.3	27.8
Stigmasta-4,22-diene	3.1	1.8	2.0	2.4
Stigmasta-3,5-diene	25.0	11.2	13.5	24.3
Stigmasta-3,5,7-triene	7.6	3.4	3.8	1.1
Other hydrocarbons	23.3	4.8	13.6	12.8
Squalene	23.3	4.8	13.6	12.8
Sterols	901.5	411.8	731.1	895.4
Campesterol	10.7	4.3	6.5	8.9
Stigmasterol	15.3	2.5	3.8	2.4
Sitosterol	640.9	300.9	520.1	684.6
Stigmastanol	199.7	87.4	169.9	161.6
Fucosterol	23.5	14.3	14.6	30.9
Cycloartenol	4.1	1.2	13.6	3.8
24-Methylenecycloartanol	3.4	0.3	1.4	1.9
7-Oxositosterol	3.9	0.9	1.2	1.3
Tocopherols	2.1	1.2	6.9	3.7
α-Tocopherol	1.7	0.9	5.4	3.0
β-Tocopherol	0.4	0.3	1.5	0.7
Steroid ketones	44.7	19.8	60.7	26.5
Stigmasta-3,5-dien-7-one	1.7	1.2	5.8	3.2
Stigmast-4-en-3-one	33.8	13.5	40.0	16.4
Stigmastan-3-one	0.9	0.6	3.4	1.2
Stigmast-4-en-3,6-dione	1.2	0.6	2.8	0.8
Stigmasta-3,6-dione	7.1	3.9	8.7	4.9
Monoglycerides	157.4	130.5	201.9	123.0
2,3-Dihydroxypropyl tetradecanoate	0.1	0.1	0.1	0.1
2,3-Dihydroxypropyl hexadecanoate	2.6	2.8	2.5	2.1
2,3-Dihydroxypropyl octadecanoate	7.3	6.7	8.3	7.6

Table 2 (*Continued*)

Compound	IP	U1 × U2	G1 × UGL	DG × U2
2,3-Dihydroxypropyl eicosanoate	0.2	0.3	1.5	0.5
2,3-Dihydroxypropyl docosanoate	5.1	7.8	12.4	12.7
2,3-Dihydroxypropyl tricosanoate	0.2	0.3	0.0	0.1
2,3-Dihydroxypropyl tetracosanoate	16.9	13.1	22.4	18.1
2,3-Dihydroxypropyl pentacosanoate	3.7	2.2	2.9	2.1
2,3-Dihydroxypropyl hexacosanoate	48.6	30.6	29.2	39.0
2,3-Dihydroxypropyl heptadecanoate	7.4	5.3	6.5	4.1
2,3-Dihydroxypropyl octacosanoate	59.7	56.3	102.8	31.8
2,3-Dihydroxypropyl nonacosanoate	3.2	2.8	5.7	2.8
2,3-Dihydroxypropyl triacontanoate	2.4	2.2	7.6	2.0
Diglycerides	23.4	10.4	14.2	15.7
Dipalmitin	8.5	1.9	2.5	1.9
Palmitoylstearin	12.8	6.6	8.5	11.4
Distearin	2.1	1.9	3.2	2.4
n-Alkylferulates	17.0	9.3	51.6	11.8
trans-Docosanylferulate	0.9	0.7	9.0	1.6
trans-Tricosanylferulate	0.2	0.1	0.5	0.2
trans-Tetracosanylferulate	3.7	2.0	18.1	3.5
trans-Pentacosanylferulate	0.5	0.2	1.1	0.3
trans-Hexacosanylferulate	5.5	3.5	18.9	3.2
trans-Heptacosanylferulate	0.7	0.1	0.6	0.4
trans-Octacosanylferulate	5.5	2.7	3.4	2.6
Sterol glycosides	130.0	96.5	128.9	177.7
Sitosteryl-3β-D-glucopyranoside	130.0	96.5	128.9	177.7
Tocopherol esters	3.3	4.3	9.2	9.0
β-Tocopherol ester	0.8	0.6	2.4	3.9
α-Tocopherol esters	2.5	3.7	6.8	5.1
Sterol esters	384.6	432.6	375.9	346.4
Sitosteryl esters	238.2	274.7	263.8	274.6
Stigmastanol esters	40.1	46.3	48.9	33.2
Other sterol esters	106.3	111.6	63.2	38.6
Triglycerides	11.1	4.8	10.3	9.5

observed for U1 × U2 wood and the lowest for DG × U2 wood. The sterol esters corresponded mainly to the sitosterol ester series, being sitosteryl linoleate (X) the major sterol ester present in all the eucalypt wood extracts. Other steroid compounds, such as steroid ketones and steroid hydrocarbons, were also important components in the lipophilic extractives from the different eucalypt woods. Steroid ketones accounted for 20–61 mg/kg wood, being mainly constituted by stigmastan-3-one (XI), stigmasta-3,5-dien-7-one (XII), stigmast-4-en-3-one (XIII) and stigmasta-3,6-dione (XIV), with smaller amounts of stigmast-4-en-3,6-dione. Different steroid hydrocarbons (di- and triunsaturated) were also identified, although in low amounts (16–36 mg/kg wood), stigmasta-3,5-diene being the most predominant.

Free fatty acids were also important constituents of the different eucalypt wood extractives. The series of free fatty acids accounted for 140–290 mg/kg wood, and ranged from C_{15} to C_{28} with a strong even-over-odd carbon atom number predominance and the dominant component being the saturated palmitic (XV) and stearic acids together with the unsaturated oleic (XVI) and linoleic (XVII) acids. Fatty acids were also found as glycerides, including mono-, di- and triglycerides. Monoglycerides (XVIII) were present in all eucalypt hybrids in relatively high amounts (from 123 to 202 mg/kg wood), in the range C_{14}–C_{30}, and with a predominance of the even carbon atom number homologs (C_{24}, C_{26} and C_{28} were the most abundant). The series of diglycerides (10–23 mg/kg) and triglycerides (XIX; 5–11 mg/kg wood) were present in lower amounts. A series of *n*-alkyl ferulates (XX) was also found among the lipophilic extracts in relatively low amounts (9–52 mg/kg wood). Characterization of intact individual compounds was achieved based on the mass spectra obtained by GC/MS of the underivatized and their TMS ether derivatives already published (del Río et al., 2004). The series of *n*-alkyl *trans*-ferulates occurred in the range from C_{22} to C_{28}, with a predominance of the even carbon atom number homologs. Alkyl ferulates were also previously reported in the woods of different eucalypt species (Freire et al., 2002; Rencoret et al., 2007). Finally, minor amounts of other compounds such as the isoprenoid hydrocarbon squalene (XXI; 5–23 mg/kg wood), tocopherols (1–7 mg/kg wood) and tocopherol esters (3–9 mg/kg wood), were also found in all eucalypt extracts. Free and esterified tocopherols included both α-tocopherol (XXII) and β-tocopherol (XXIII), with predominance of α-tocopherol.

The different lipids classes have different behavior during cooking and bleaching (Gutiérrez et al., 2001a, 2001b; Freire et al., 2005, 2006a, 2006b; Marques et al., 2010). The glycerides (including mono-, di- and triglycerides), are completely hydrolyzed during alkaline cooking and the fatty acids dissolved. Sterol esters, however, largely survive alkaline cooking (del Río et al., 1998, 2000; Gutiérrez et al., 2001a, 2001b). At sufficiently high pH (as in kraft pulping), the acids dissociate and form fatty acid soaps and can thus dissolve in water to quite a high extent. By contrast, neutrals such as steroid hydrocarbons and ketones, and specially free and conjugated sterols do not form soluble soaps under the alkaline pulping conditions and therefore survive cooking. These compounds have a very low solubility in water and are difficult to remove, and therefore can be at the origin of pitch deposition. The high amounts of these neutral compounds in most of these woods, and particularly the high abundances of free and conjugated sterols, which have a high propensity to form pitch deposits (del Río et al., 1998, 2000; Silvestre et al., 1999; Gutiérrez and del Río, 2001; Gutiérrez et al., 2001a, 2001b) would point to a pitch deposition tendency of the lipophilics from these woods. Among them, the wood from U1 × U2 has the lowest content of these detrimental compounds

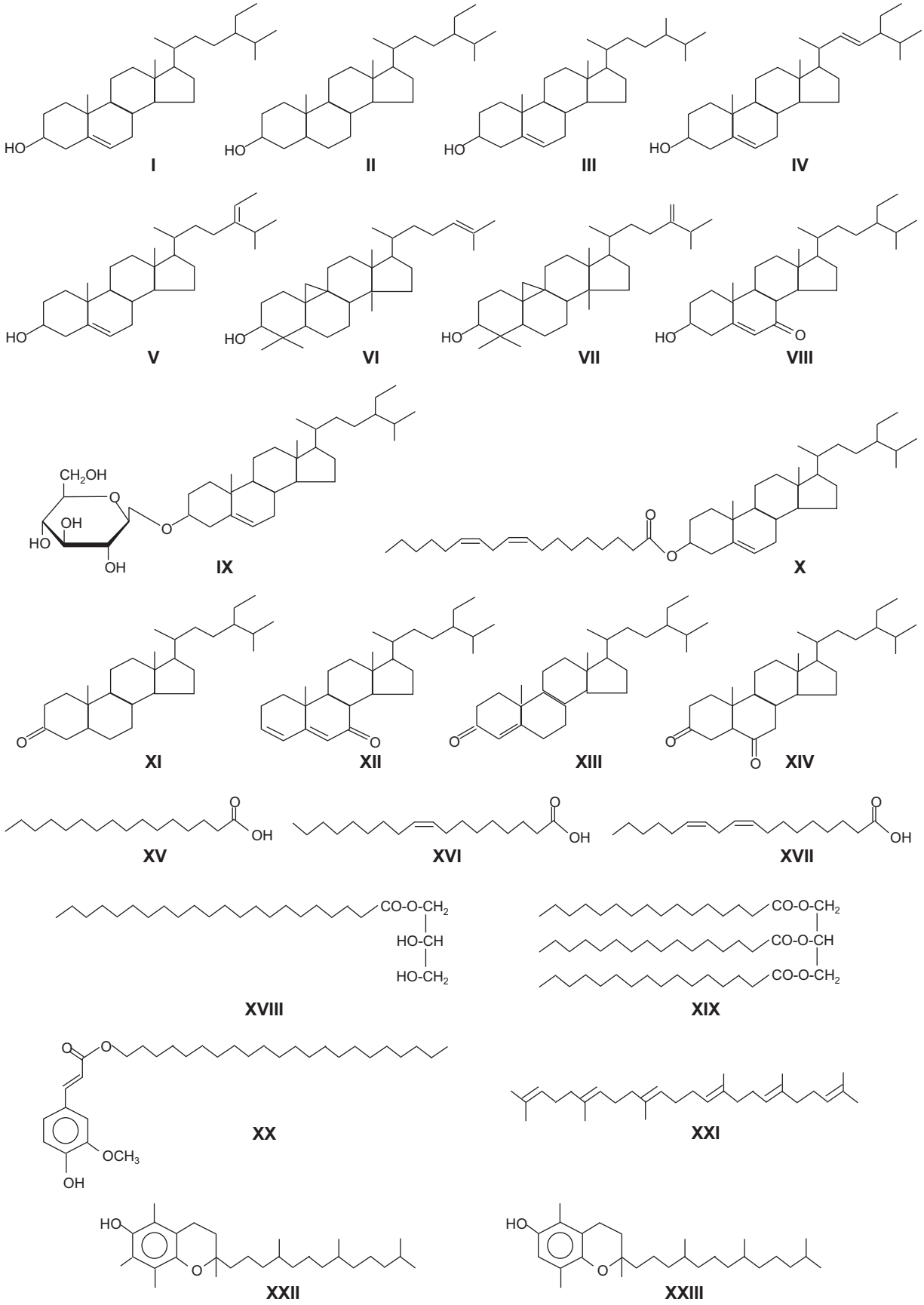

Fig. 2. Structures of the main compounds identified in the hybrid eucalypt woods and referred in the text. (I) Sitosterol, (II) stigmastanol, (III) campesterol, (IV) stigmasterol, (V) fucosterol, (VI) cycloartenol, (VII) 24-methylenecycloartanol, (VIII) 7-oxositosterol, (IX) sitosteryl 3β-D-glucopyranoside, (X) sitosteryl linoleate, (XI) stigmastan-3-one, (XII) stigmasta-3,5-dien-7-one, (XIII) stigmast-4-en-3-one, (XIV) stigmasta-3,6-dione, (XV) palmitic acid, (XVI) oleic acid, (XVII) linoleic acid, (XVIII) docosanoic acid, 2,3-dihydroxypropyl ester, (XIX) tripalmitin, (XX) *trans*-docosanyl ferulate, (XXI) squalene, (XXII) α-tocopherol, (XXIII) β-tocopherol.

Table 3
Weight-average (M_w) and number-average (M_n) molecular weights (g mol^{-1}), and polydispersity (M_w/M_n) of the MWL from the woods of the different eucalypt hybrids selected in this study.

	IP	U1 × U2	G1 × UGL	DG × U2
M_w	15,000	12,900	13,300	11,300
M_n	4300	3900	3500	3000
M_w/M_n	3.5	3.3	3.8	3.8

and therefore will have less pitch problems, while the woods of IP and DG × U2 have the highest content of them, and therefore it is foreseen that they will have more pitch problems than the wood from the hybrid U1 × U2.

3.3. Composition and structure of the lignins from the different eucalypt hybrids

The lignin content of the different eucalypt hybrids selected for this study, estimated as Klason lignin, is similar in all cases (ca. 24–25%) and slightly higher in comparison with other eucalypt woods, such as *E. globulus* (Rencoret et al., 2007, 2008). However, the delignification reactions and, therefore, the pulping efficiency are not only affected by the lignin content but are also greatly influenced by the lignin composition and structure. Therefore, we have thoroughly studied the lignin composition and structure of the Brazilian woods from the selected eucalypt hybrids. For this, the MWL, which is considered to be representative of the whole native lignin in the plant, was isolated by aqueous dioxane extraction from finely ball-milled wood according to the classical lignin isolation procedure (Björkman, 1956).

The values of the weight-average (M_w) and number-average (M_n) molecular weights, estimated from the GPC curves (relative values related to polystyrene), and the polydispersity (M_w/M_n) of the MWL from the selected eucalypt hybrids, are indicated in Table 3. The MWLs exhibited similar molecular weight distributions, in the range 11,300–15,000 g mol^{-1}, being slightly higher in the case of the MWL from IP and lower for the MWL from DG × U2. In addition, all the MWL exhibited relatively narrow molecular weight distributions, with $M_w/M_n < 4$. Those values are comparable to literature values for various isolated lignins (Baumberger et al., 2007).

The composition of the MWLs were analyzed by Py-GC/MS. All the eucalypt hybrid lignins yielded similar Py-GC/MS products, and a representative pyrogram is shown in Fig. 3. The identities

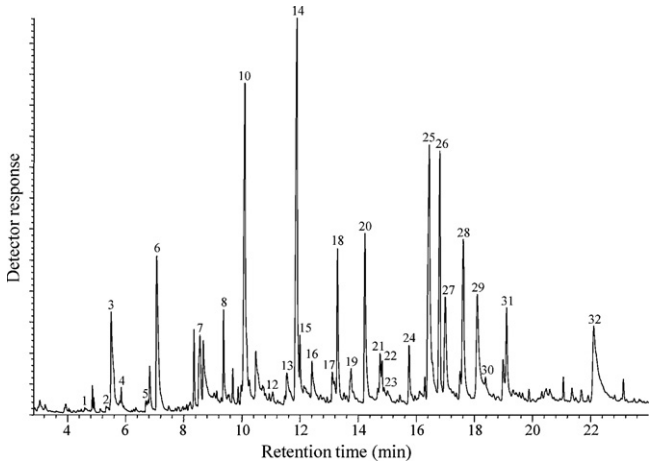

Fig. 3. Py-GC/MS chromatogram of a representative MWL isolated from a selected eucalypt hybrid (DG × U2) wood. The numbers refer to the compounds listed in Table 4.

Table 4
Identification and relative molar abundance of the compounds identified in the Py-GC/MS of MWL from wood of the different eucalypt hybrids selected in this study.

Label	Compound	IP	U1 × U2	G1 × UGL	DG × U2
1	Phenol	0.5	0.4	0.4	0.4
2	Methylphenol	0.2	0.2	0.2	0.4
3	Guaiacol	5.7	5.4	4.0	5.9
4	Methylphenol	0.4	0.4	0.1	0.5
5	Ethylphenol	0.0	0.0	0.1	0.1
6	4-Methylguaiacol	6.5	7.9	6.0	7.1
7	4-Ethylguaiacol	1.7	1.9	1.1	2.7
8	4-Vinylguaiacol	3.7	2.4	2.7	2.0
9	Eugenol	0.5	0.3	0.1	0.1
10	Syringol	10.5	9.5	8.1	12.5
11	4-Propylguaiacol	0.0	0.0	0.0	0.1
12	*cis*-Isoeugenol	0.5	0.3	0.3	0.3
13	Vanillin	1.8	1.8	1.6	1.6
14	4-Methylsyringol	11.4	15.5	13.6	13.1
15	*trans*-Isoeugenol	1.9	0.7	1.0	1.0
16	Homovanilline	0.7	0.9	1.3	1.3
17	Acetoguaiacone	1.0	1.6	1.7	1.4
18	4-Ethylsyringol	2.9	2.9	3.4	3.4
19	Guaiacylacetone	0.8	1.3	0.9	1.2
20	4-Vinylsyringol	7.1	5.0	6.5	4.9
21	4-Allylsyringol	1.9	1.4	0.9	1.0
22	4-Propylsyringol	0.4	0.5	0.3	0.7
23	Propiovanillone	0.1	0.4	0.4	0.2
24	*cis*-4-Propenylsyringol	1.6	1.1	0.9	1.5
25	Syringaldehyde	10.5	11.3	10.9	8.9
26	*trans*-4-Propenylsyringol	7.7	3.2	5.1	5.2
27	Homosyringaldehyde	2.5	2.6	5.1	3.5
28	Acetosyringone	4.1	6.1	5.2	5.1
29	Syringylacetone	3.8	2.8	2.9	2.9
30	*trans*-Coniferaldehyde	1.8	2.9	2.9	2.7
31	Propiosyringone	2.0	2.2	2.5	2.0
32	*trans*-Sinapaldehyde	5.6	7.1	10.0	6.2
	H	1	1	1	1
	G	27	28	24	28
	S	72	71	75	71
	S/G=	2.7	2.6	3.1	2.6

and relative molar abundances of the released lignin compounds are listed in Table 4. Among them, guaiacyl (**G**) and syringyl-type (**S**) phenols, were identified. Only minor amounts (ca. 1%) of phenol-type compounds from *p*-hydroxycinnamyl (**H**) units could be detected. The most important compounds identified were guaiacol (**3**), 4-methylguaiacol (**6**), 4-vinylguaiacol (**8**), syringol (**10**), 4-methylsyringol (**14**), 4-vinylsyringol (**20**), syringaldehyde (**25**), *trans*-4-propenylsyringol (**26**), homosyringaldehyde (**27**), acetosyringone (**28**), syringylacetone (**29**), propiosyringone (**31**) and *trans*-sinapaldehyde (**32**). The molar S/G ratios obtained from the molar areas of all the lignin-derived compounds are shown in Table 4 and ranged from 2.6 to 3.1. The lignin from the eucalypt hybrids IP, U1 × U2 and DG × U2 present similar lignin composition and S/G ratio, while the lignin from the hybrid G1 × UGL presents the highest content on S lignin and the lowest content on G-lignin (S/G ratio of 3.1). This composition will make the wood from the hybrid G1 × UGL easier to be delignified under kraft cooking than the other eucalypt hybrids due to the higher reactivity of the S-lignin in alkaline systems (Chang and Sarkanen, 1973; Tsutsumi et al., 1995). It has already been shown for eucalypt woods that higher S/G ratios imply higher delignification rates, less alkali consumption and therefore higher pulp yield (González-Vila et al., 1999; del Río et al., 2005).

The structure of the isolated lignins was also analyzed by 2D-NMR, that provides information of the structure of the whole macromolecule and is a powerful tool for lignin structural characterization. The side-chain and aromatic regions of the HSQC spectrum of a representative eucalypt MWL (from DG × U2) are shown in Fig. 4, together with the main lignin substructures present. Cross-signals were assigned by comparing with the

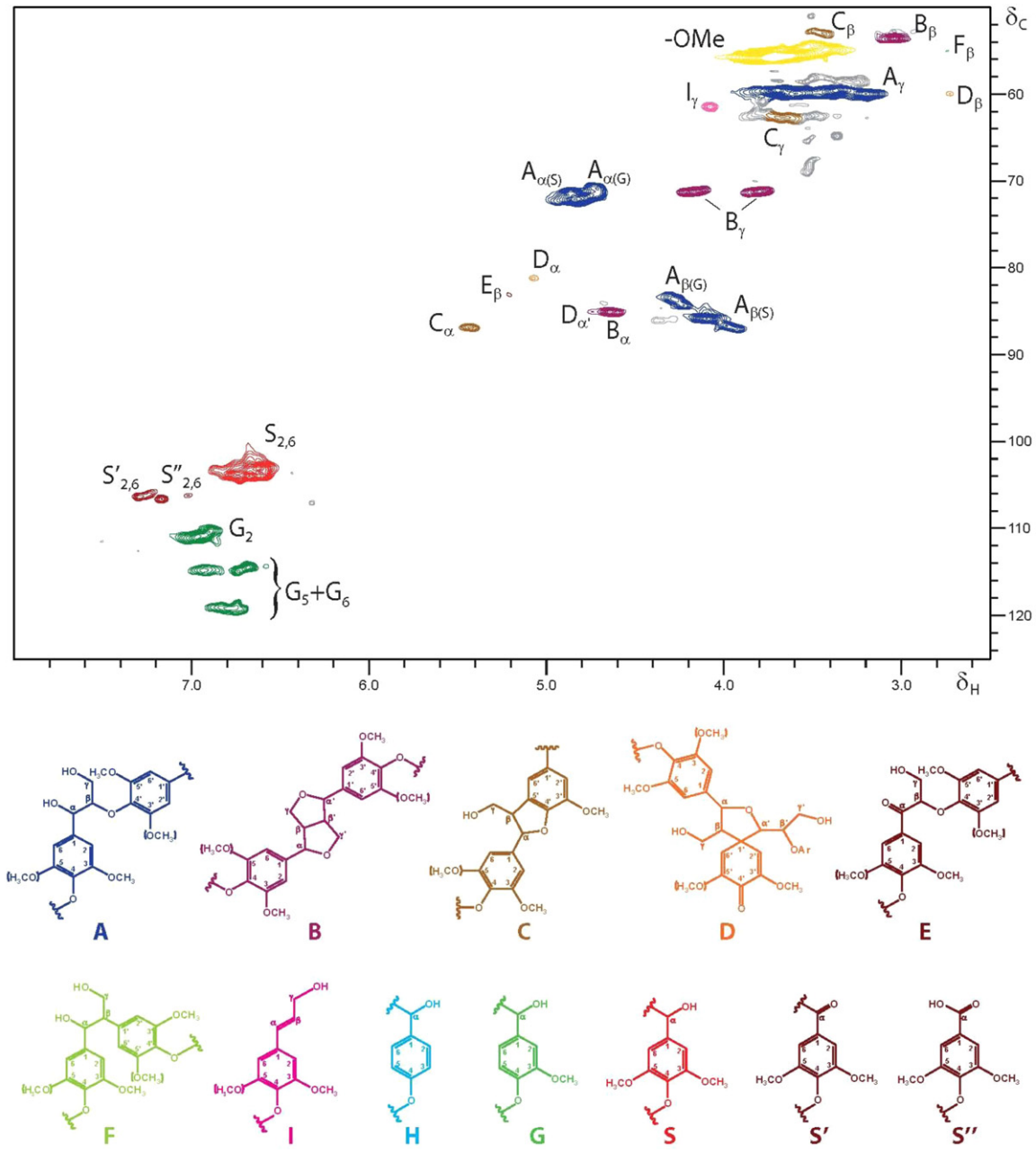

Fig. 4. HSQC NMR spectra (δ_C/δ_H 50–125/2.5–8.0) of a representative MWL (from DG × U2). See Table 5 for signal assignment. Main structures present in the lignin from the Brazilian eucalypt hybrid woods: (**A**) β-O-4′ substructures; (**B**) resinol substructures formed by β–β′/α-O-γ′/γ-O-α′ linkages; (**C**) phenylcoumarane substructures formed by β-5′/α-O-4′ linkages; (**D**) spirodienone substructures formed by β-1′/α-O-4′ linkages; (**E**) C_α-oxidized β-O-4′ substructures; (**F**) conventional open β-1′ structures; (**I**) *p*-hydroxycinnamyl alcohol end-groups; (**G**) guaiacyl units; (**S**) syringyl units; (**S′**) oxidized syringyl units bearing a carbonyl group at C_α (phenolic); (**S″**) oxidized syringyl units bearing a carboxyl group at C_α.

literature (Balakshin et al., 2003; Capanema et al., 2001, 2004, 2005; del Río et al., 2008, 2009; Ibarra et al., 2007; Liitiä et al., 2003; Martínez et al., 2008; Ralph et al., 1999; Ralph and Landucci, 2010; Rencoret et al., 2008, 2011) and the main assignments are listed in Table 5.

The side-chain region of the spectra gave useful information about the different inter-unit linkages present in the eucalypts lignins. All the spectra showed prominent signals corresponding to β-O-4′ alkyl aryl ether linkages (substructure **A**). In addition to β-O-4′ substructures, other linkages were also observed. Thus, strong signals for resinol (β–β′/α-O-γ′/γ-O-α′) (**B**) and phenylcoumaran (β-5′/α-O-4′) substructures (**C**) and small signals corresponding to spirodienone (β-1′/α-O-α′) substructures (**D**) were observed in all spectra. Other small signals in the side-chain region of the HSQC spectra corresponded to C_β–H_β correlations of β-O-4′ substructures bearing a C_α carbonyl group (**E**), the C_β–H_β correlations of β-1′ open substructures (**F**) and C_γ–H_γ correlations of *p*-hydroxycinnamyl (**I**) end-groups. The main cross-signals observed in the aromatic region of the HSQC spectra corresponded to the benzenic rings of lignin units. Signals from syringyl (**S**) and guaiacyl (**G**) units could be observed in all the spectra. However, signals of H units were not detected in any of the HSQC spectra as corresponds to their very low abundances observed by Py-GC/MS.

The percentage of lignin side-chains involved in the main substructures and terminal structures found in the different eucalypt lignins (referred to total side-chains) are indicated in Table 6. In all

Table 5
Assignments of the lignin ^{13}C–^{1}H correlation signals observed in the HSQC spectra of the MWL from the eucalypt hybrids.

Labels	δ_C/δ_H (ppm)	Assignment
B_β	53.4/3.06	C_β–H_β in β–β′ (resinol) substructures (**B**)
C_β	53.4/3.45	C_β–H_β in β-5′ (phenylcoumaran) substructures (**C**)
F_β	54.8/2.75	C_β–H_β in β-1′ substructures (erythro forms) (**F**)
OMe	55.6/3.73	C–H in methoxyls
A_γ	59.5/3.38-3.71	C_γ–H_γ in β-O-4′ substructures (**A**) and others
D_β	59.8/2.75	C_β–H_β in β-1′ (spirodienone) substructures (**D**)
J_γ	61.4/4.10	C_γ–H_γ in cinnamyl alcohol end-groups (**J**)
C_γ	62.0/3.75	C_γ–H_γ in β-5′ (phenylcoumaran) substructures (**C**)
B_γ	71.1/3.82 and 4.18	C_γ–H_γ in β–β′ (resinol) substructures (**B**)
$A_{\alpha(S)}$	71.8/4.87	C_α–H_α in β-O-4′ substructures linked to a S unit (**A**)
$A_{\alpha(G)}$	71.3/4.77	C_α–H_α in β-O-4′ substructures linked to a G unit (**A**)
$D_{\beta'}$	79.3/4.11	$C_{\beta'}$–$H_{\beta'}$ in β-1′ (spirodienone) substructures (**D**)
D_α	81.1/5.10	C_α–H_α in β-1′ (spirodienone) substructures (**D**)
E_β	82.9/5.22	C_β–H_β in C_α-oxidized β-O-4′ substructures (**E**)
$A_{\beta(G)}$	83.5/4.29	C_β–H_β in β-O-4′ substructures linked to a G unit (**A**)
B_α	84.6/4.66	C_α–H_α in β–β′ (resinol) substructures (**B**)
$A_{\beta(S)}$	86.0/4.11	C_β–H_β in β-O-4′ substructures linked to a S unit (**A**)
$D_{\alpha'}$	84.7/4.76	$C_{\alpha'}$–$H_{\alpha'}$ in β-1′ (spirodienone) substructures (**D**)
C_α	86.8/5.42	C_α–H_α in β-5′ (phenylcoumaran) substructures (**C**)
$S_{2,6}$	103.9/6.68	C_2–H_2 and C_6–H_6 in syringyl units (**S**)
$S'_{2,6}$ $S''_{2,6}$	106.3/7.32 and 7.20	C_2–H_2 and C_6–H_6 in C_α-oxidized syringyl units (**S′** and **S″**)
G_2	110.8/6.96	C_2–H_2 in guaiacyl units (**G**)
$D_{2'}$	113.5/6.26	$C_{2'}$–$H_{2'}$ in β-1′ (spirodienone) substructures (**D**)
G_5	114.9/6.70 and 6.94	C_5–H_5 in guaiacyl units (**G**)
G_6	118.9/6.76	C_6–H_6 in guaiacyl units (**G**)
$D_{6'}$	118.9/6.08	$C_{6'}$–$H_{6'}$ in β-1′ (spirodienone) substructures (**D**)

Table 6
Percentage of lignin side-chains forming different inter-unit linkages (**A–E**) and terminal structures (**F**) from integration of ^{13}C–^{1}H correlation signals in the HSQC spectra of MWL from wood of the different eucalypt hybrids analyzed (referred to total side-chains).

	IP	U1 × U2	G1 × UGL	DG × U2
β-O-4′ Alkyl aryl ethers (**A**)	75	74	77	74
Resinols (**B**)	11	11	9	11
Phenylcoumarans (**C**)	5	5	5	5
Spirodienones (**D**)	4	4	3	4
β-O-4′ (C_α=O) (**E**)	1	1	2	1
β-1′ Substructures (**F**)	1	2	1	2
p-Hydroxycinnamyl alcohol end-groups (**I**)	3	3	3	3
S/G ratio	2.2	2.2	2.8	2.2

cases, the main lignin substructure present was the β-O-4′ alkyl-aryl ether (**A**) that amounted to 75–79% of all side-chains (including the oxidized β-O-4′ ones, **E**). The second most abundant linkage in the eucalypt lignin corresponded to the resinol substructure (**B**) that involved around 9–11% of all side-chains. The other linkages, such as phenylcoumaran (**C**), spirodienone (**D**) or β-1′ open substructures (**F**), were present in lower proportions (1–5% of all side-chains). A NMR estimation of the molar S/G ratios in the lignins from the different Brazilian eucalypt hybrids is included in Table 6, and ranges from 2.2 to 2.8. The highest S/G value corresponded to the lignin from the hybrid G1 × UGL, as similarly observed by Py-GC/MS. The higher S/G ratio observed in this lignin is related to the highest proportion of β-O-4′ linkages present in this lignin. Ether linkages are cleaved during alkaline cooking, while condensed linkages (such as β–β′, β-5′ and β-1′) resist cooking conditions (Gierer, 1985; Gierer and Norén, 1980; Ibarra et al., 2007). Therefore, it is foreseen that the wood from the hybrid G1 × UGL will be more easily delignifiable than the other selected Brazilian eucalypt hybrids.

3.4. Morphological characteristics of the wood components in the different eucalypt hybrids

According to the MorFi analysis (Fig. 5), the fiber length and width distributions for the different eucalypt hybrids were quite equivalent, except for the IP hybrid. For all the eucalypts, fiber length varied between 350 and 3000 μm and the fiber width between 12 and 23 μm. The mean area-weighted length versus vessels content for the hybrid eucalypt woods is depicted in Fig. 6. Eucalypt hybrids U1 × U2 and G1 × UGL presented the

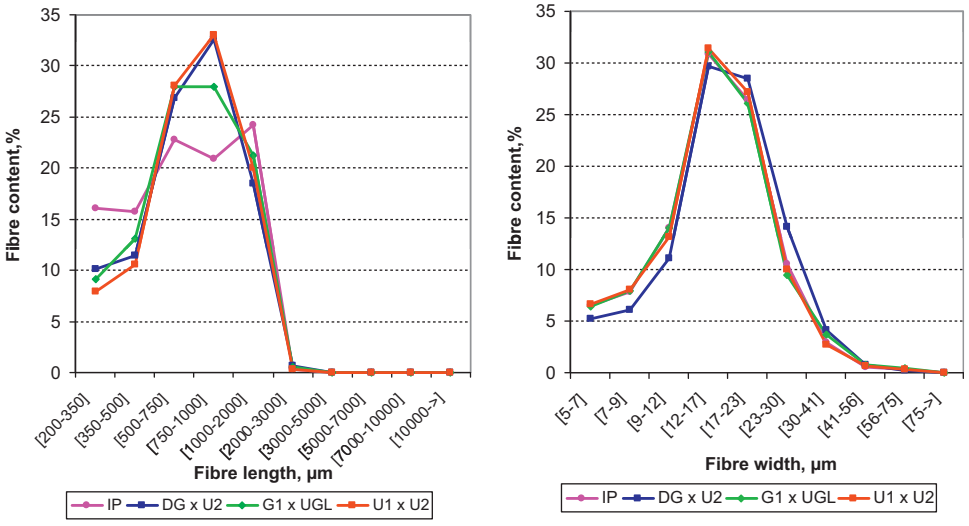

Fig. 5. Distribution of the fiber length and fiber width for the hybrid eucalypt woods.

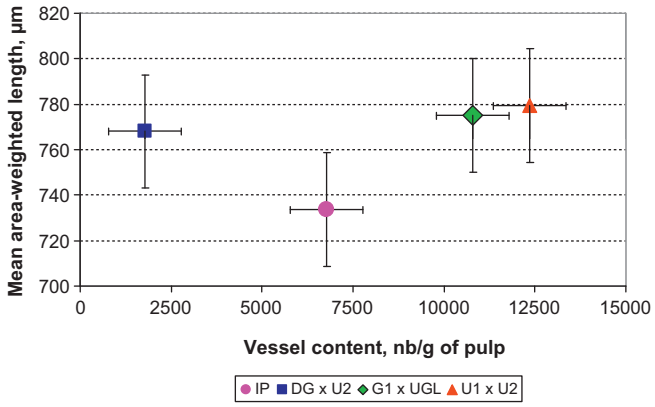

Fig. 6. Mean area-weighted length versus vessels content for the hybrid eucalypt woods.

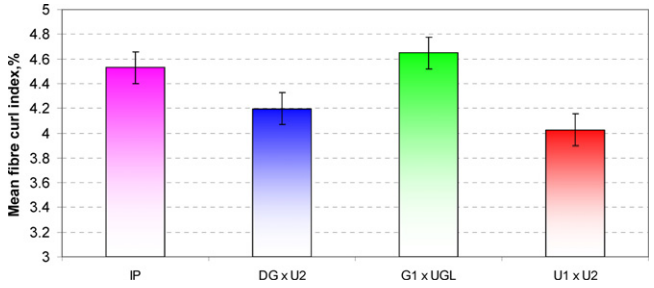

Fig. 9. Curl index of the fibers of the different eucalypt hybrid clones, allowing to give information about the fiber flexibility.

highest vessel content, while DG × U2 present the best compromise between fiber length and vessels content, with longer fibers and the lower vessel content. The difference in vessel content among the selected eucalypt hybrids was not correlated to the wood growth, but most probably to the growing conditions and genetic variability. Microscopic examination of cross sections of wood samples confirmed that hybrid DG × U2 contained fewer vessels with a less marked growing ring, while U1 × U2 contained more vessels (Fig. 7). Eucalypt hybrid DG × U2 presented two families of vessels: earlywood vessels with a square form and latewood vessels with a high length/width ratio (Fig. 8). On the contrary, hybrid U1 × U2 contained more earlywood vessels. The earlywood vessels are more detrimental to papermaking than latewood vessels because they are not degraded during pulping, bleaching, refining and papermaking, whereas the latewood ones are reduced into fragments that can be easily retained in the sheet thickness. This confirms the interest for the DG × U2 eucalypt hybrid for paper pulp manufacture.

Fiber flexibility is another important parameter for papermaking. The curl index, as measured by the MorFi analyzer, is an indirect approach of the fiber flexibility, the lower the curl index, the higher the fiber flexibility. DG × U2 and U1 × U2 eucalypt hybrids produce more flexible fibers than the other hybrids (Fig. 9). As the lignin structure is more condensed for DG × U2 and U1 × U2 hybrids, as seen above, the delignification affected more severely the fiber wall structure, rendering the fibers more flexible when the same delignification occurred. The higher the fiber flexibility, the higher the pulp strengths.

The hybrid IP has lower content of lignin and acetone extractives compared to the other hybrids and thus, similar cooking conditions will produce higher delignification rate and higher carbohydrate degradation and hence will generates fibers more sensitive to mechanical action. This was illustrated by the higher broken fibers and fines content for this hybrid (Fig. 10). This indicates that the eucalypt hybrid IP would have a slightly different behavior during

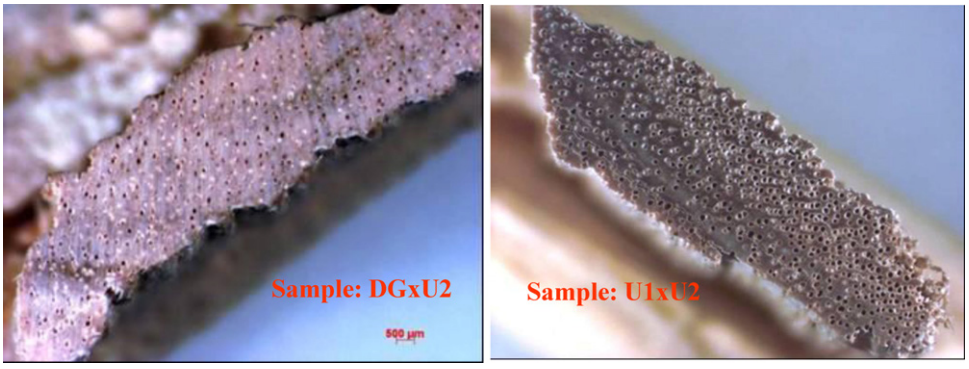

Fig. 7. Microscopy examination of the cross sections of DG × U2 and U1 × U2 eucalypt wood chips.

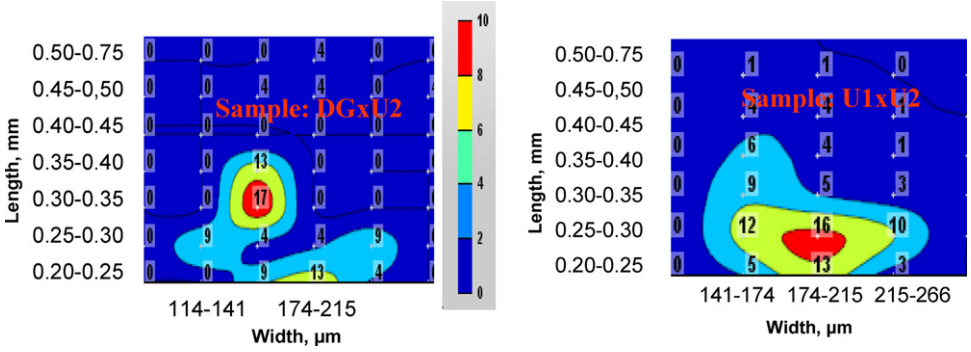

Fig. 8. Vessel distribution for DG × U2 and U1 × U2 eucalypt wood chips.

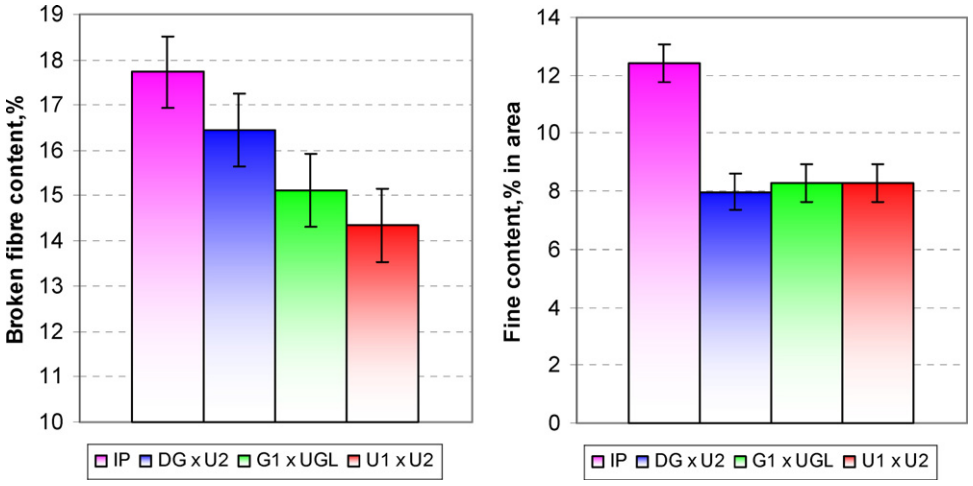

Fig. 10. Broken fibers and fines content of the different eucalypt hybrid woods.

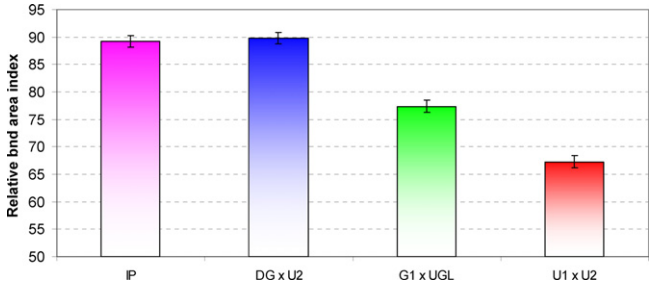

Fig. 11. Relative bonded area index of the fibers from the different eucalypt hybrid woods.

pulping and the fibers would be more sensitive to cutting into the pumps and the mixers.

Finally, the bonding potential of the fibers for papermaking was evaluated by the way of the relative bonded area index (Fig. 11). Eucalypt hybrids DG × U2 and IP fibers presented the highest index, confirming that the hybrid DG × U2 was the most promising for paper pulp manufacture based on morphological properties.

4. Conclusions

The morphological and chemical characteristics of the woods from several eucalypt hybrids grown in Brazil were studied. The lipid and lignin content of these Brazilian woods were very similar, but a thorough analytical study indicated some differences in their composition. The detailed analysis of the composition of the lipophilic extractives indicated the presence of comparatively high amounts of neutral compounds in these woods, and particularly, the high abundances of free and conjugated sterols, which have a high propensity to form pitch deposits, would point to a high pitch deposition tendency of the lipophilics from these woods. Among them, the wood from U1 × U2 had the lowest content of these detrimental compounds and therefore is less prone to pitch problems, while the woods IP and DG × U2 have the highest content of them, with greater pitch potential than the hybrid U1 × U2. The lignin content of the different eucalypt hybrids, estimated as Klason lignin, is similar in all cases (ca. 24–25%). However, some differences were found in the lignin composition, with the lignin from the hybrids IP, U1 × U2 and DG × U2 having similar and lower S/G ratios and higher abundance of condensed linkages, and the lignin from the hybrid G1 × UGL presenting the highest S/G ratio and the highest proportion of uncondensed β-O-4′ linkages. This composition makes the wood from the hybrid G1 × UGL more easily delignifiable under kraft cooking than the other eucalypt hybrids. However, based on dimensional and morphological characterization of the fibers and the vessels, eucalypt hybrid DG × U2 seemed to be the most interesting raw material for pulp manufacture. It presented the highest forest productivity, longer and flexible fibers with a high bonding potential, and the lowest vessels and fines contents.

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