COMPARISON BETWEEN ANALYTICAL PYROLYSIS AND NITROBENZENE OXIDATION FOR DETERMINATION OF SYRINGYL/GUAIACYL RATIO IN *Eucalyptus* spp. LIGNIN

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Pyrolysis-gas chromatography/mass spectrometry (Py-GC-MS) was applied to measure the lignin syringyl/guaiacyl (S/G) ratio in *E. dunni*, *E. grandis*, *E. nitens*, *E. urograndis*, and *E. urophylla* woods. A total of 41 compounds were identified, of which 11 were derived from carbohydrates and 30 from lignins. The S/G ratio was calculated on the basis of the areas of peaks recorded in the pyrograms and compared with the results obtained by alkaline nitrobenzene oxidation. The values of S/G found by pyrolysis were similar for all the species using the compounds guaiacol, 4-methylguaiacol, 4-vinylguaiacol, vanillin, 4-ethylsyringol, 4-vinylsyringol, homosyringaldehyde, acetosyringone, and syringylacetone, as lignin markers. The selected markers were efficient for the determination of S/G ratio in eucalyptus wood by Py-GC-MS. The Py-GC-MS technique produced results that are comparable to the nitrobenzene oxidation method, with the advantage of requiring small wood samples and a short analysis time.

Keywords: Analytical pyrolysis; S/G ratio; Lignin; Eucalyptus ssp.

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INTRODUCTION

Lignin is an amorphous, aromatic, and highly complex substance present in the cell wall and in the middle lamella of plants (Rowell 2005). It is an essential component of wood that supports and strengthens the cell wall, facilitates water transport, and inhibits degradation of cell wall polysaccharides (Hatfield and Vermerris 1991). It also protects wood from attack by pathogens, insects or other herbivores (Saliba et al. 2001; Hatfield and Vermerris 2001).

Lignin is composed of monomeric phenylpropanoid units known as *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S), originated from three main precursors: *trans*-*p*-coumarilic alcohol, *trans*-synapilic alcohol, and *trans*-coniferilic alcohol, respectively (Salo et al. 1989; Galletti and Bocchini 1995).

This natural polymer is an undesirable component in the chemical conversion of wood into pulp, and its content is an important parameter from the industrial point of view, since removal of lignin is a major step in pulp production (Campbell and Sederoff 1996; Rodrigues et al. 1999). Wood with increased S/G ratio is more easily delignified and provides higher pulp yield (Collins et al. 1990; Wallis et al. 1996; González-villa et

al. 1999; Del Río et al. 2005). This behavior is because the guaiacyl units have the C5 position in the aromatic ring (Fig. 1) available to make strong carbon-carbon bonds, making them more resistant to depolymerization during wood pulping (Gutiérrez et al. 2006). Besides, cleavage of *β*-aryl-ether bond in syringyl lignin occurs more easily than in the guaiacyl lignin under alkaline cooking conditions, contributing to increase the rate of delignification (Tsutsumi et al. 1995).

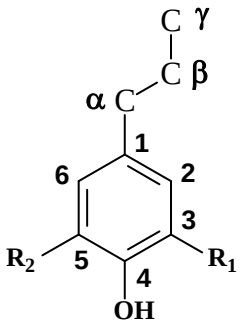

Fig. 1 - Basic lignin structural units: *p*- Hydroxyphenyl (R_1 and R_2 = H); Guaiacyl (R_2 = H and R_1 = OCH_3) and Syringyl (R_1 and R_2 = OCH_3).

The structural composition of lignin is therefore an important characteristic for eucalyptus clone selection, seeking to obtain better quality wood for pulp production in terms of delignification rate, reagent consumption, and pulp yield (Campbell and Sederoff 1996; Wallis et al. 1996).

Although several methodologies have been used to study lignin structure (Takayama et al. 1997; Ibarra et al. 2005; Morais et al. 2005; Zier et al. 1997; Kuroda et al. 2002a,b; González-Vila et al. 1999; Del Río et al. 2001a; Morais et al. 2005), the standard method most used to determine syringyl and guaiacyl proportions is nitrobenzene oxidation in alkaline medium. This procedure requires long analysis time and consumption of great amount of reagents, and thus there is a need for the development of faster, safer, and more economical analytical procedures.

Pyrolysis coupled with gas chromatography/mass spectrometry (Py-GC-MS) comes as an alternative analysis commonly used in the study of polymeric samples such as lignins (Silvério et al. 2008). The methodology is based on thermal degradation of lignin, producing components that can be analyzed by gas chromatography/mass spectrometry. This technique also stands out for allowing the analysis of very small samples (approximately 100 *μ*g), without any preparation, over short periods of time (Ishida et al. 2007).

Considering the need for faster methods for determination of lignin S/G composition, the Py-GC-MS technique was investigated to analyze wood samples from different eucalyptus species and to determine the S/G ratio from pyrogram areas of peaks of wood degradation products. The results were compared with results obtained using the reference method that uses nitrobenzene in alkaline medium.

This study compared the Py-GC-MS and nitrobenzene oxidation methods for measuring *Eucalyptus spp*. wood lignin S/G ratio. The main goal was establishing a fast

and reliable method to analyze this important wood trait, which is largely used for massive eucalyptus clone selection for pulp manufacture.

EXPERIMENTAL

Sample Preparation

Five species of seven year old eucalyptus trees were used, namely: *Eucalyptus dunni, E. grandis, E. urograndis* from Brazil and *E. urophylla* and *E. nitens* from Chile, were selected for wood samples. Wood chips (3 × 2 x 0.2 cm) from samples of each species were prepared using a laboratory chipper, homogenized in mixer, air-dried, and made into sawdust using a Wiley mill. The sawdust samples (2 g s.a.) sieved to pass 40-60 mesh were subjected to Soxhlet extraction with acetone for 5 h, and the extractive-free fraction was used for lignin analysis by alkaline nitrobenzene oxidation and Py-GC-MS.

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 nitrobenzene oxidation according to Lin and Dence (1992). Approximately 0.2 g (oven dried) 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 ºC for 2.5 hours, with analyses repeated twice. The oxidized material was then extracted with chloroform (6 x 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 regenerate cellulose membrane, pore size 0.45 μm, and analyzed by high efficiency 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 x 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^{-1} was used. Vanillin and syringaldehyde standards (Aldrich, Milwalkee, 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 acetonitrile/water mixture (1:1 v/v), in pH 2.6.

Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC-MS)

The pyrolysis of the samples was performed in duplicate with a microfurnace pyrolyser Pyr A-4 model (Shimadzu) coupled to a GC-MS apparatus (Shimadzu, model

PQ5050A), using a fused silica capillary column (DB-5, 30 m x 0.25 mm ID, 0.25 μm film thickness). A finely divided sawdust sample (100 μg) was deposited in a small platinum cup that was then inserted into a quartz tube (2 mm x 40 mm) placed in the pyrolysis chamber. The pyrolysis was carried out at 550 ºC for 10s, as previously described (Del Río et al. 2005; Silva 2006; Barbosa et al. 2008). The pyrolysis chamber was kept at 250 ºC and purged with helium to transfer the pyrolysis products as quickly as possible to the GC column. The chromatograph oven was programmed from 45 ºC (4 min) to 240 ºC at a rate of 4 ºC min^{-1}. The final temperature was held for 20 min. The injector and GC-MS interface were kept at 200 ºC. The mass spectrometer was operated by the electron impact ionization mode at 70 eV and the mass scan range was 40 to 600 Da. The temperatures of the detector and the GC-MS interface were 250 and 290 °C, respectively. Compounds were identified by comparing their mass spectra with the GC-MS spectral library (Willey 333,000), with data from the literature (Van der Hagge et al. 1993; Del Río et al. 2005; Oudia et al. 2007) and when necessary by mass fragmentography.

Semi-quantification was based on the peak areas, considering the total peak area as 100%. The syringyl/guaiacyl (S/G) ratio was calculated by dividing the sum of peak areas from syringyl units (4-ethylsyringol, 4-vinylsyringol, homosyringaldehyde, acetosyringone, syringilacetone) by the sum from the peak areas of guaiacyl derivatives (guaiacol, 4-methylguaiacol, 4-vinylguaiacol, vanillin), obtained by integration of the peak areas. All the analyses were carried out in triplicates.

RESULTS AND DISCUSSION

Identification of Fragments of Wood Pyrolysis

The pyrolysis method is based on the fragmentation of lignin into monomeric compounds induced by thermal energy under inert atmosphere, producing a mixture of relatively simple aromatic compounds. These compounds can be directly separated by gas chromatography and identified by mass spectrometry (Ralph et al. 1991; Zier et al. 1997). In this way, the lignin can be characterized on the basis of the products from pyrolysis.

In a previous investigation, we have studied the influence of the pyrolysis temperature on the composition of lignin derived products formed (Barbosa et al. 2008). It was found that better results were obtained when the wood was pyrolized at 550 ºC for 10s. So, five wood samples from different eucalyptus species (*E. dunni*, *E. grandis*, *E. nitens*, *E. urograndis* and *E. urophylla*) were pyrolized and the pyrolysis products were separated and characterized by GC-MS. The sample pyrograms showed similar profiles, with the same fragmentation patterns of carbohydrates and lignins. A typical pyrogram of *E. urograndis* wood is shown in Fig. 2. The identified compounds are listed in Table 1.

The pyrogram of the *E. urograndis* wood showed the prevalence of guaiacyl and syringyl units, characterizing lignin derived from angiosperm wood (Rowell 2005; Del Río et al. 2001a). Forty-eight compounds were identified, 11 corresponding to carbohydrate degradation products, whereas 30 were lignin derivatives and one modified lignin derivative (Table 1). The identification was confirmed by comparing mass spectra

of compounds detected with data from the literature (Van der Hagge et al. 1993; Del Río et al. 2005; Nonier et al. 2006; Oudia et al. 2007).

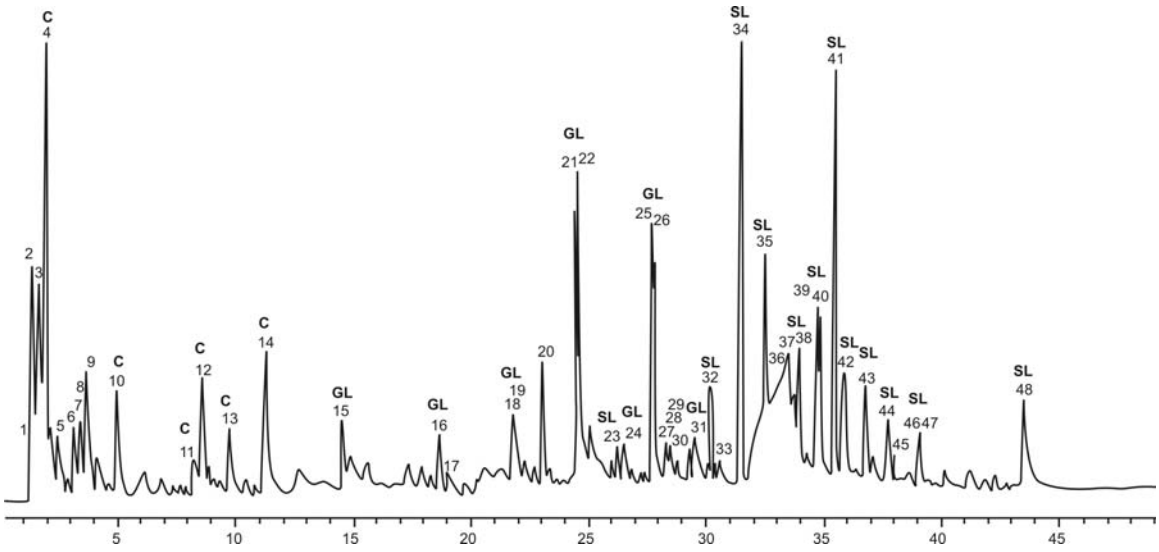

Fig. 2 - Pyrogram of a sample of *E. urograndis* wood. Peak identities for the lignin-derived compounds are shown in Table 1. C: carbohydrates; GL: guaiacyl lignin; SL: syringyl lignin

From the lignin pyrolysis products, 14 compounds belong to guaiacyl unit (GL) and 16 derive from the syringyl unit (SL). The main pyrolysis products derived from guaiacyl units showing relative areas above 1.0% were: guaiacol (15), 4-methylguaiacol (16), 4- vinylguaiacol (20) and *trans*-isoeugenol (25), except those compounds 15 and 16. Among the compounds with syringyl units, those showing relative areas above 1.0% stand out: 4-methylsyringol (26), 4-vinylsyringol (34), syringaldehyde (39), *trans*-4-propenylsyringol (41), homosyringaldehyde (42), acetosyringone (43), and sinapaldehyde (48). Other less abundant compounds were: *cis*-isoeugenol (23), vanillin (24), homovanillin (27) and guaiacylacetone (33) as guaiacyl derivatives, and 4-ethylsyringol (32), 4-allylsyringol (36), *cis*-4-propenylsyringol (38), syringylacetone (44), and sinapaldehyde (48) as syringyl derivatives. These substances were identified in previous works involving lignin degradation by analytical pyrolysis (Yokoi et al. 2001; Del Río et al. 2002; Ibarra et al. 2005; Del Río et al. 2007).

Peaks related to products derived from carbohydrate pyrolysis were more intense and less resolved compared with those from lignin products. The main product of cellulose degradation was 1,6-anhydro-β-D-glucopyranose (levoglucosan) (35), in agreement with the results reported by Alén et al. (1996). Other compounds also detected included acetic acid (4), 3-hydroxypropanal (7), pyranic (13 and 14), and furanic (6, 8, 10, 11, 12 and 17) derivatives.

Table 1 - Main Compounds Identified by Py-GC-MS in *Eucalyptus* spp. Wood

Peak*	RT	MM	*m/z*	Compounds	Area (%)					Origin
					E. dunni	*E. grandis*	*E. nitens*	*E. urograndis*	*E. urophylla*	
1	1.25	58	58, 56	N.I.	0.59	0.57	0.54	0.89	0.92	-
2	1.35	72	72, 68, 56, 55	N.I.	3.42	4.10	3.89	3.81	4.17	-
3	1.62	60	60, 57, 55	N.I.	4.16	4.89	4.28	5.00	4.96	-
4	1.96	60	60	Acetic acid	5.37	6.24	6.59	5.93	4.94	C
5	2.40	86	86	N.I.	0.27	0.30	0.34	0.30	0.29	-
6	3.12	84	84, 55	Furan-2(3*H*)-one	0.86	1.03	1.09	1.07	0.91	C
7	3.39	74	74, 73	3-Hidroxypropanal	0.80	0.99	0.99	1.12	0.88	C
8	3.40	84	84, 55	Furan-2(2*H*)-one						
9	3.66	?	58, 57	N.I.	0.96	1.67	1.57	1.57	1.34	-
10	4.93	96	96, 95, 67	2-Furfuraldehyde	1.73	1.71	1.88	1.67	1.53	C
11	8.19	84	84, 55	Furan-2(5*H*)-one	0.45	0.72	0.67	0.41	0.31	C
12	8.53	98	98, 69, 55	2,3-Dihydro-5-methylfuran-2-one	1.65	2.09	2.06	1.99	1.74	C
13	9.69	114	114, 86, 55	Methyl-2-tetrahydropyranone	0.82	0.78	0.89	0.69	0.62	C
14	11.28	114	114, 58, 57	5,6-Dihydro-4-hydroxypyran-2(2*H*)-one	2.22	2.63	3.38	2.36	2.14	C
15[#]	**14.54**	**124**	**124, 109, 81**	**Guaiacol**	**0.47**	**0.80**	**0.67**	**0.87**	**0.72**	**GL**
16	**18.58**	**138**	**138, 123, 95**	**4-Methylguaiacol**	**0.51**	**0.76**	**0.59**	**0.99**	**0.74**	**GL**
17	19.04	144	82, 70, 69, 57	5-Hidroxymethyl-2-tetrahydrofuraldehyd-3-one	1.40	0.66	0.66	0.70	0.60	C
18*	21.74	152	152, 137, 122	4-Ethylguaiacol	1.68	1.79	1.21	1.52	1.53	GL
19*	21.75	140	140, 125, 97	3-Methoxycatechol						ML
20	**23.03**	**150**	**150, 135, 107**	**4-Vinylguaiacol**	**0.96**	**1.60**	**1.19**	**1.98**	**1.61**	**GL**
21*	24.49	154	154, 139, 111	Syringol						SL
22*	24.50	164	164, 149, 77	Eugenol	2.27	4.32	3.60	4.06	3.85	GL
23	26.19	164	164, 149, 77	*cis*-Isoeugenol	0.22	0.24	0.21	0.28	0.24	GL
24	**26.46**	**152**	**152, 151, 109**	**Vanillin**	**0.46**	**0.52**	**0.59**	**0.63**	**0.50**	**GL**
25	27.62	164	164, 149, 131	*trans*-Isoeugenol	1.20	2.15	1.24	2.06	1.98	GL
26	27.66	168	168, 153, 152	4-Methylsyringol	2.17	2.56	2.60	2.79	1.99	SL
27	28.29	166	166, 137, 122	Homovanillin	0.49	0.56	0.51	0.64	0.56	GL
28	28.45	178	162, 147, 91	G-CH=C=CH_2	0.18	0.22	0.25	0.30	0.26	GL
29	28.70	178	162, 147, 91	G-CH=C=CH_2	0.09	0.06	0.08	0.11	0.09	GL
30	29.23	166	166, 151, 123	Acetoguaiacone	0.21	0.28	0.28	0.43	0.29	GL
31	29.49	166	166, 151, 123	N.I.	0.55	0.63	0.76	0.48	0.73	GL

32	**30.11**	**182**	**182, 167, 107**	**4-Ethylsyringol**	**0.58**	**0.75**	**0.62**	**0.64**	**0.63**	**SL**
33	30.55	180	180, 137, 122	Guaiacylacetone	0.51	0.48	0.49	0.45	0.48	GL
34	**31.44**	**180**	**180, 165, 137**	**4-Vinylsyringol**	**3.71**	**5.62**	**4.92**	**5.06**	**4.46**	**SL**
35	32.45	194	194, 119, 91	1,6- Anhydrous-*β*-D-glycopyranose	47.55	32.22	35.18	33.79	41.00	SL
36	32.60	196	196, 167, 123	4-Allylsyringol	1.02	1.31	1.22	1.21	0.96	SL
37	33.62	162	73, 60, 57	4-Propylsyringol	0.12	0.18	0.16	0.19	0.12	C
38	33.90	194	194, 179, 91	*cis*-4-Propenylsyringol	0.60	0.76	0.71	0.81	0.60	SL
39	34.67	182	182, 181, 167	Syringaldehyde	1.63	2.03	2.69	2.17	1.58	SL
40	34.81	192	192, 177, 131	S-CH=C=CH_2	0.20	0.23	0.25	0.46	0.22	SL
41	35.43	194	194,179, 91	*trans*-4-Propenylsyringol	2.57	4.56	4.04	4.46	3.58	SL
42	**35.83**	**196**	**196, 167, 123**	**Homosyringaldehyde**	**1.07**	**1.43**	**1.65**	**1.35**	**1.38**	**SL**
43	**36.70**	**196**	**196, 181, 153**	**Acetosyringone**	**1.28**	**1.40**	**1.57**	**1.25**	**1.25**	**SL**
44	**37.67**	**210**	**210, 167**	**Syringylacetone**	**0.56**	**0.80**	**0.77**	**0.64**	**0.62**	**SL**
45	37.96	208	208, 161, 133	N.I.	0.13	0.15	0.14	0.11	0.11	-
46	38.92	210	210, 182, 167	4-Propanalsyringol	0.16	0.39	0.28	0.29	0.32	SL
47	39.01	210	210, 182, 181	Propiosyringone	0.54	0.68	0.69	0.57	0.55	SL
48	43.45	208	208, 165, 137	Sinapaldehyde	1.60	2.13	2.01	1.90	1.69	SL
				% Area of carbohydrates derivatives	**62.85**	**49.07**	**53.39**	**49.73**	**54.67**	
				% Area of lignin derivatives	**27.61**	**39.24**	**35.85**	**38.59**	**33,53**	

N.I.: Unidentified; C: carbohydrates; GL: guaiacyl lignin; SL: syringyl lignin; RT: Retention Time; MM: Molecular Mass;*Partial peaks overlap; # In bold : Selected compounds used as chemical markers of lignin for the determination of S/G ratio.

Determination of Syringyl/Guaiacyl (S/G) Ratio by Nitrobenzene Method and Py-GC-MS

Nitrobenzene oxidation in alkaline medium is amongst the most used methods for determination of syringyl/guaiaciyl ratio in lignins (Lin and Dence 1992). This method is based on the amounts of products formed by lignin oxidation (Lin and Dence 1992). For hardwood the oxidation produces vanillin (G) and syringaldehyde (S) (Morais et al. 2005); thus, the quantification is given in terms of the S/G ratio obtained by the chromatogram peak areas of those substances.

S/G ratio can also br determined by Py-GC/MS by dividing the areas of the peaks corresponding to all syringyl and guaiacyl derivatives, as described in recent work (Nonier et al. 2006; Silva 2006). The values of the S/G ratio were obtained for five eucalyptus species by analytical pyrolysis and by the nitrobenzene oxidation standard method are compared in Table 2.

Table 2 - Values of the S/G Ratio Estimated in Samples of *Eucalyptus* spp. by Py-GC-MS and Alkaline Nitrobenzene Oxidation

Species	Py-GG-MS		Nitrobenzene Oxidation	
	S/G	**SD**	**S/G**	**SD**
E. dunni	3.0	0.12	3.0	0.04
E. grandis	3.0	0.12	2.7	0.02
E. nitens	3.5	0.06	3.2	0.01
E. urograndis	2.6	0.29	2.0	0.01
E. urophylla	2.4	0.25	2.4	0.01

S/G: Syringyl/Guaiacyl ratio. SD: Standard deviation

Data from Table 2 show that the values of the S/G ratio found by Py-GC-MS were similar to those obtained by alkaline nitrobenzene oxidation for samples of *E. dunni* (3±0.12 and 3±0.04, respectively) and *E. urophylla* (2.4±0.25 and 2.4±0.01, respectively). There was, nevertheless, a small variation in the S/G ratios estimated with the two methods for the samples of *E. grandis* (3±0.12 and 2.7±0.02) and *E. nitens* (3.5±0.06 and 3.2±0.01). However, the values of the S/G ratio obtained among two methods (Py-GC-MS and nitrobenzene oxidation) were significantly different for the *E. urograndis* (2.6±0.29 and 2±0.01, respectively).

To minimize these differences and simplify the methodology, some pyrogram peaks were selected regarding products of degradation that are free from interferences (co-elutions), called lignin chemical markers.

Del Rio and co-workers have used as markers guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-vinylguaiacol, syringol, 4-methylsyringol, 4-ethylsyringol, 4-vinyl-syringol, and *trans*-4-propenylsyringol for the determination of the S/G ratio in eucalyptus pulp (Del Río et al. 2001b). However, we could not use the same compounds as markers in our work, because of problems of co-elution of some of then. So, we selected as markers the following compounds: guaiacol (15), 4-methylguaiacol (16), 4-

vinylguaiacol (20) and vanillin (24) as guaiacyl derivatives, and homovanillin (27), 4-ethylsyringol (32), 4-vinylsyringol (34), syringaldehyde (39), and syringylacetone (44) as syringyl derivatives. The values found for S/G ratio using these nine markers is shown in Table 3.

Table 3 - Relative Area of the Markers found by Lignin Pyrolysis and S/G Ratios of Five *Eucalyptus* Species

Markers	*E. dunni*		*E. grandis*		*E. nitens*		*E.urograndis*		*E. urophylla*	
	RA%	SD	RA%	SD	RA%	SD	RA%	SD	RA%	SD
Guaiacyl										
Guaiacol	0.47	0.01	0.80	0.04	0.67	0.07	0.87	0.16	0.72	0.08
4-Methylguaiacol	0.51	0.03	0.76	0.04	0.59	0.12	0.99	0.12	0.74	0.08
4-Vinylguaiacol	0.96	0.09	1.60	0.16	1.19	0.11	1.98	0.14	1.61	0.18
Vanillin	0.46	0.09	0.52	0.07	0.59	0.08	0.63	0.01	0.50	0.07
Syringyl										
4-Ethylsyringol	0.58	0.03	0.75	0.05	0.62	0.11	0.64	0.11	0.63	0.08
4-Vinylsyringol	3.71	0.18	5.62	0.33	4.92	0.41	5.06	0.99	4.46	0.42
Homosyringaldehyde	1.07	0.17	1.43	0.16	1.65	0.17	1.35	0.12	1.38	0.14
Acetosyringone	1.28	0.19	1.40	0.03	1.57	0.17	1.25	0.01	1.25	0.02
Syringilacetone	0.56	0.13	0.80	0.04	0.77	0.12	0.64	0.10	0.62	0.06
S/G (Py-GC-MS)	**3.0**	**0.00**	**2.7**	**0.10**	**3.1**	**0.12**	**2.0**	**0.17**	**2.3**	**0.06**
S/G (Nitrobenzene)	**3.0**	**0.04**	**2.7**	**0.02**	**3.2**	**0.01**	**2.0**	**0.01**	**2.4**	**0.01**

RA%: Relative Area (in percentage); SD: Standard deviation; S/G: Syringyl/Guaiacyl ratio.

The woods from *E. dunni* and *E. nitens* presented the highest S/G ratios (3.0 and 3.2, respectively), which in principle makes them easier to delignify under Kraft cooking, owing to the higher reactivity of S-lignin in alkaline conditions. In addition, it is anticipated that these two wood species would require less effective alkali for cooking and, as a consequence, would produce higher yields. On the other hand, wood from *E.urograndis* and *E. urophylla* presented the lowest S/G ratios (2.0 and 2.4, respectively), which, together with their higher lignin content, makes them the most difficult to delignify amongst the five studied species

We have also found the same values of S/G ratio when the wood samples were pyrolyzed without the removal of the extractives, as found in a previous investigation (Barbosa et al. 2008). This makes the method even more attractive, requiring less time and basically no sample preparation.

Therefore, the Py-GC-MS technique was shown to be valuable in the characterization and determination of S/G ration for five eucalyptus species, with the advantage of requiring small wood samples and a short analysis time. Further research is required to evaluate if the substances selected as markers could be effectively used for the determination of S/G ratio in other wood species.

CONCLUSIONS

1. The lignin S/G ratio can be determined by Py-GC-MS, submitting the woods to pyrolysis at 550 ºC for 10 seconds and using the following substances as markers: guaiacol, 4-methylguaiacol, 4-vinylguaiacol, vanillin, 4-ethylsyringol, 4-vinyl-syringol, homovanillin, syringaldehyde, and syringylacetone.
2. The results of S/G ratio, obtained by Py-GC-MS, for five eucalyptus species (*E. dunni, E. grandis*, *E. nitens*, *E. urograndis* and *E. urophylla*) were comparable to the values obtained by the routine nitrobenzene oxidation.
3. An advantage of the methodology described, compared to the nitrobenzene method, is a short analysis time, and almost no sample preparation is required.
4. Further studies with other eucalyptus species are required to confirm the efficiency of the methodology described for plants with larger and smaller S/G values.

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