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Isolation and characterization of lignins from *Eucalyptus grandis* Hill ex Maiden and *Eucalyptus globulus* Labill. by enzymatic mild acidolysis (EMAL)

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Abstract

Despite the growing importance of *Eucalyptus* wood as raw material for pulp and paper, there is a lack of knowledge on the chemistry of their macromolecular components. The present paper addresses this issue by applying the recently developed protocol for isolating enzymatic mild acidolysis lignins (EMAL) from *Eucalyptus grandis*, *Eucalyptus globulus* and the softwood species Douglas fir and white fir, which were used for comparative purposes. The structures of EMALs were investigated by quantitative ^{31}P NMR, DFRC/^{31}P NMR (derivatization followed by reductive cleavage followed by quantitative ^{31}P NMR) and size exclusion chromatography (SEC). Overall, the yields of EMALs isolated from *Eucalyptus* were higher than those from the softwoods examined. Lignin from *E. globulus* was found to contain higher contents of arylglycerol-β-aryl ether structures, free phenolic hydroxyl groups and syringyl-type units than lignin from *E. grandis*. New insights provided by the DFRC/^{31}P NMR revealed that up to 62.2% of arylglycerol-β-aryl ether structures in *E. globulus* are uncondensed, while in *E. grandis* the amount of such uncondensed structures was found to be lower than 48%. SEC analyses showed that lignins from *E. grandis* and softwoods associate in greater extension than lignin from *E. globulus*.

Keywords: derivatization followed by reductive cleavage (DFRC); enzymatic mild acidolysis lignin (EMAL); *Eucalyptus*; lignin; milled wood lignin (MWL); ^{31}P nuclear magnetic resonance spectroscopy.

Introduction

Eucalyptus wood has long been recognized in the Iberian Peninsula and South America as a fast-growing tree with potential for pulp and paper production (Evtuguin et al. 2001). Meanwhile, *Eucalyptus* wood has become the most important source of bleached market pulp in the world. The demand for eucalyptus pulp reached 8 million tons in 2003, and in 2015, it will be probably approximately 14 million tons (Colodette et al. 2005). Fibers derived from single species as *Eucalyptus* are currently the worlds preferred market pulps. Nowadays, *Eucalyptus globulus* and *Eucalyptus grandis* represent two of the most interesting species, among the more than 600 species comprising the genus *Eucalyptus*.

There is a lack of knowledge on the specific chemistry of the macromolecular components of *Eucalyptus* wood (Capanema et al. 2005; Evtuguin et al. 2001; Pinto et al. 2005). For example, few efforts have been made to better understand the lignin isolation from *Eucalyptus* species (Bland 1985; Evtuguin et al. 2001). Milled wood lignin (MWL) from *Eucalyptus* is always obtained in poor yields and with high proportion of attached hemicelluloses and tannins, which hinder its quantitative analysis (Evtuguin et al. 2001). There are few comprehensive models on the structure of *Eucalyptus* lignins (Evtuguin et al. 2001; Capanema et al. 2005). Isolating lignin samples in high yield from *Eucalyptus* wood species would contribute greatly to the elucidation of their lignin structure.

In a recent series of papers, we have shown that a novel procedure using the combination of enzymatic and mild acidolysis (EMAL) gives rise to lignin that may be more representative of the total lignin than milled wood lignin (MWL) (Guerra et al. 2006a,b). Because a mild acid hydrolysis can liberate lignin from lignin-carbohydrate complexes (known to preclude lignin isolation in high yields), it can be combined with low severity of milling, facilitating the isolation of less modified lignin in high yields and purities from milled wood (Wu and Argyropoulos 2003; Guerra et al. 2006a,b). As a consequence of such treatment, the yields of EMAL are from 2 to 5 times greater than the corresponding MWL and cellulolytic enzyme lignin (CEL) isolated from the same batch of milled wood (Guerra et al. 2006b). Comparison of the chemical structure of EMAL, MWL and CEL has revealed only subtle differences, evidencing that EMAL is released by cleaving lignin-carbohydrate bonds rather than other linkages within lignin macromolecule (Wu and Argyropoulos 2003; Guerra et al. 2006a,b). Consequently, the aforementioned protocol presents a real opportunity to improve yield and purity of lignin from *Eucalyptus*.

In another front, Tohmura and Argyropoulos (2001) have recently described a novel approach for the quantification of different lignin structures by combination of the degradation technique, derivatization followed by reductive cleavage (DFRC) (Lu and Ralph 1998) with quantitative ^{31}P nuclear magnetic resonance (^{31}P NMR).

Quantitative ^{31}P NMR is well suited to determine the various hydroxyl groups after phosphitylating lignin with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (Granata and Argyropoulos 1995). Thus, the spectra "before DFRC" provide quantitative information on the aliphatic hydroxyls, carboxylic groups, and condensed and uncondensed units bearing phenolic hydroxyl groups within lignin. Unfortunately, quantitative ^{31}P NMR cannot offer detailed information on the etherified or carbon-carbon linked bonding pattern of lignin. However, when the aryl ether linkages are selectively cleaved by DFRC, the phenolic hydroxyls released can be quantified by ^{31}P NMR. In this way, the ^{31}P NMR spectra obtained "after DFRC" offer detailed information on condensed and uncondensed units connected through β-aryl ether linkages (Tohmura and Argyropoulos 2001; Guerra et al. 2006a,b).

Overall, this study applies the recently described procedure to isolate EMAL and then uses the combination of DFRC with quantitative ^{31}P NMR spectroscopy (DFRC/^{31}P NMR) to provide new insights into the structure of lignins from *E. grandis* and *E. globulus* wood. For comparative purposes, Douglas fir and white fir as softwoods were also included in this study. The yields of EMAL obtained were compared to MWL and CEL isolated and purified from identical batches of milled wood.

Materials and methods

Isolation of EMALs, MWLs and CELs

EMALs and MWL were isolated from Douglas fir (*Pseudotsuga menziesii*), white fir (*Abies concolor*), *Eucalyptus globulus* and *Eucalyptus grandis* according to procedures described previously (Björkman 1956, 1957; Guerra et al. 2006a,b). CEL was isolated from the insoluble material obtained after isolating MWL, according to the method of Chang et al. (1975) and modified by Ikeda et al. (2002). Both preparations were purified as described elsewhere (Björkman 1956).

Acetobromination derivatization procedure

Approximately 2.5 ml of a mixture composed of 8 parts of acetyl bromide and 92 parts (v/v) of glacial acetic acid were added to approximately 10 mg of a lignin sample in a 15 ml round-bottom flask. The flask was sealed and placed in a water bath set at 50°C for 2 h with continuous magnetic stirring. The solvent was rapidly evaporated at 25–28°C in a rotary evaporator connected to a high vacuum pump and a cold trap. The residue was immediately dissolved in tetrahydrofuran (THF, 5 ml) and subjected to size exclusion analyses (SEC).

DFRC/^{31}P NMR

DFRC was performed as described by Lu and Ralph (1998). The precise amounts of the lignin and precautions due to the resulting ^{31}P NMR steps were nearly identical to those reported elsewhere (Tohmura and Argyropoulos 2001; Guerra et al. 2006a,b).

Quantitative ^{31}P NMR

Quantitative ^{31}P NMR spectra of all lignin preparations were obtained according to the literature (Argyropoulos 1994; Granata and Argyropoulos 1995). To improve resolution, a delay time of 5 s was used and a total of 256 scans per sample were acquired.

Size exclusion chromatography

Size exclusion chromatography (SEC) of EMAL samples were performed on a Waters system (Waters Corporation, Milford, Massachusetts, USA) equipped with UV set at 280 nm, as described previously (Guerra et al. 2006a). The analyses were carried out at 40°C with THF as eluent at a flow rate of 0.44 ml min^{-1}. A total of 120 ml of the sample dissolved in THF (2 mg ml^{-1}) was injected into a HR5E and HR1 system of columns connected in series (Waters). The HR5E column specification allow for molecular weights (Mw) up to 4×10^6 g mol^{-1} to be reliably detected. The SEC system was calibrated with polystyrene standards in the Mw range of 890–1.86×10^6 g mol^{-1}. A Millennium 32 SEC software (Waters) was used for data processing.

Incubation of EMALs in THF

Following acetobromination, one aliquot (4.0 ml) of each lignin sample was withdrawn from the starting solution in THF and maintained at room temperature (25±3°C) under vigorous stirring (5000 rpm) for periods of up to 30 days (Guerra et al. 2007). These samples were used to evaluate the effects of incubation at room temperature on the molecular weight distribution (MWD) of the evaluated lignins.

Results and discussion

Effects of milling on lignin yields

The effects of different ball milling times on the yield of the resulting EMALs are shown in Figure 1. The longer

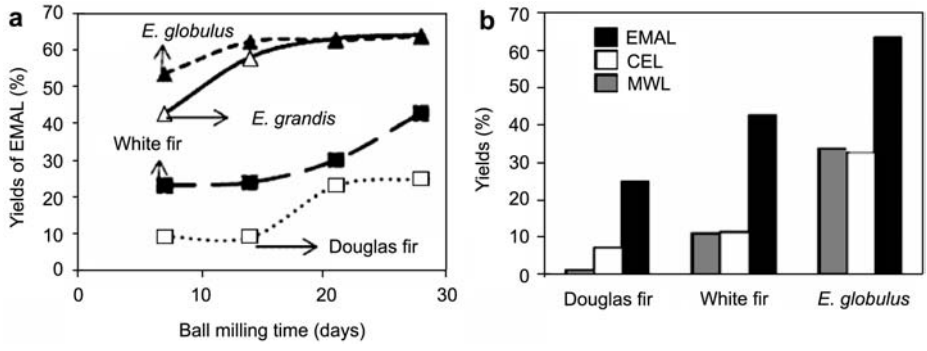

Figure 1 Yields of EMALs isolated from *E. globulus* (black filled triangles), *E. grandis* (open triangles), Douglas fir (open squares) and white fir (black filled squares) as a function of the ball milling time (a); yields of EMAL, CEL and MWL isolated from the same batch of milled-wood ball milled for 28 days (b).

the milling time the higher the EMAL yield, regardless of the wood species evaluated (Figure 1a). The yields of EMAL from *E. globulus* reached 54% (w/w, b.o. Klason lignin of the starting wood) after 7 days of ball milling, whilst the yields of *E. grandis*, white fir and Douglas fir were lower than 45%, 25% and 10%, respectively. Extending the milling time to 14 days increased only the yields of *E. grandis* and *E. globulus* lignin, which reached 58% and 62.2%, respectively. These values already represent nearly the maximal yields obtainable of these species. On the other hand, 21 days of milling time was necessary for Douglas fir to reach 24.8%, and 28 days milling time for white fir to obtain ca. 42% lignin.

The yields of EMAL isolated from the same batch of Douglas fir, white fir and *E. globulus* ball-milled for 28 days were always greater than MWL and CEL (Figure 1b). The yields of MWL and CEL from *E. grandis* were not determined in this study. However, Capanema et al. (2005) has pointed out that such yield of MWL is not higher than 25%. These data confirm our previous findings (Guerra et al. 2006a,b) and demonstrate that the concerted effect of cellulolytic action and mild acid hydrolysis offer significant yield improvements over the traditional procedures for isolating lignin from both hardwoods and softwoods. However, the present and previous studies (Guerra et al. 2006b) underline that the yields and the milling requirements are wood specific.

Determination of units bearing free phenolic hydroxyl groups

Various functional groups (total phenolic OH, S/G ratios with free phenolic OH, carboxylic acids) and β-aryl ether contents of EMALs obtained under different ball milling conditions (Figure 2) were determined by quantitative ^{31}P NMR spectroscopy. The total free phenolic hydroxyl contents and carboxylic groups were quantified by ^{31}P NMR after phosphitylating lignin with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (Granata and Argyropoulos 1995) and the β-aryl ether contents were determined indirectly from the α-hydroxyl groups after derivatization with 2-chloro-1,3,2-dioxaphospholane (Guerra et al. 2006a,b).

As the integrity of the polymeric chains of lignin is largely due to the presence of β-aryl ether structures, it is conceivable that any chain length degradation should manifest itself by a decrease of the corresponding signal (α-hydroxyl in β-aryl ether structures) in the ^{31}P NMR spectra (Wu and Argyropoulos 2003; Koda et al. 2005; Guerra et al. 2006a,b). Furthermore, cleaved β-aryl ether linkages give rise to detectable increments of phenolic hydroxyl groups, while oxidation reactions may result in oxidative fragmentation of the lignin macromolecule with concomitant creation of carboxylic acid groups, which are also detectable by ^{31}P NMR spectroscopy (Argyropoulos 1994). However, the data in Figure 2 demonstrate that prolonged ball milling time do not have any effect with this regard. The phenolic hydroxyl contents (Figure 2a), S/G ratios (Figure 2b), carboxylic groups (Figure 2c) and the contents of β-aryl ethers (Figure 2d) were remarkably constant up to 28 days of ball milling. This finding concurs with those of Fujimoto et al. (2005), Ikeda et al. (2002) and Guerra et al. (2006a) and supports the contention that low intensity milling does not affect the integrity of the lignin macromolecule. Furthermore, the data in Figure 2 together with the data in Figure 1 demonstrate the advantages of the EMAL method over MWL and CEL protocols.

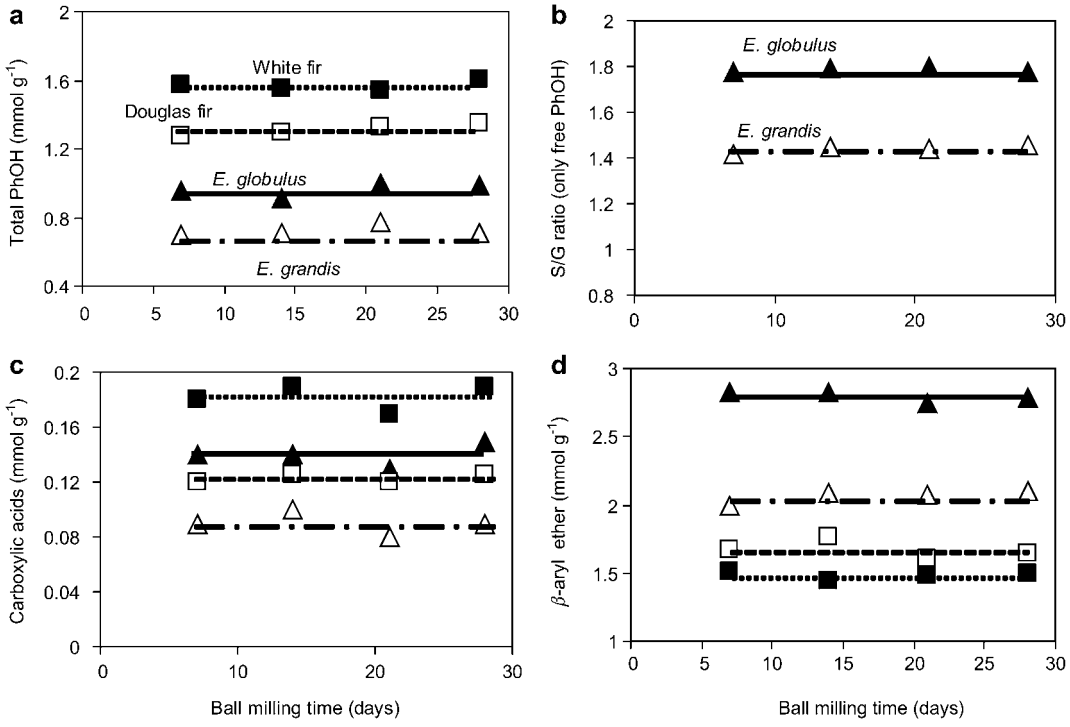

Figure 2 Total phenolic hydroxyl contents (a), S/G ratio (b), carboxylic groups (c) and β-aryl ether structures (d) of EMALs isolated from *E. globulus* (black filled triangles), *E. grandis* (open triangles), Douglas fir (open squares) and white fir (black filled squares) as a function of the ball milling time, determined by ^{31}P NMR before DFRC.

The data in Figure 2a also reveal that *E. grandis* and *E. globulus* have lower total phenolic hydroxyl contents (less than 1.0 mmol g^{-1} EMAL) than Douglas fir and white fir (1.35 and 1.6 mmol g^{-1}, respectively). This finding is not surprising, as the total contents of phenolic hydroxyl groups in softwood have been reported to be somewhat higher than hardwoods (Lai and Guo 1991; Guerra et al. 2006b). The higher OH_{phen} content of *E. globulus* in comparison to *E. grandis* may explain, at least in part, the superior pulping and bleaching performance of the former (Pinto et al. 2005). Furthermore, the S/G ratios (Figure 2b) calculated from units bearing free phenolic hydroxyl group were also higher in *E. globulus* (1.8) than *E. grandis* (1.5), and higher content of syringyl units may also contribute to an easy delignification. It is worth emphasizing that the aforementioned S/G ratios are valid for units bearing free phenolic hydroxyl group rather than the whole S/G ratio in both *E. globulus* and *E. grandis*. No syringyl units were detected in the softwood species examined in this study.

As shown in Figures 2d and 3, *E. globulus* had more arylglycerol-β-aryl ethers (2.78 mmol g^{-1}) than *E. grandis* (2.10 mmol g^{-1}) and the corresponding data of the softwoods were altogether lower than that of the *Eucalyptus* species. Considering that the average molecular weight for one phenylpropane (C9 unit) in the lignins from *E. globulus* and *E. grandis* are 211 g mol^{-1} (Evtuguin et al. 2001) and 205 g mol^{-1} (Capanema et al. 2005, one may conclude that up to 58.7% of the units of *E. globulus* and 43% of *E. grandis* are linked by β-aryl-ether linkages. These values correlate very well with the 56% and 47% of arylglycerol-β-aryl structures reported for *E. globulus* and *E. grandis* by Evtuguin et al. (2001) and Adler (1977). Our findings are also in line with those of Akiyama et al. (2005), who analyzed different species of hardwoods by ozonation and demonstrated that lignins with a higher syringyl/guaiacyl ratio are richer in arylglycerol-β-aryl structures. Amongst softwoods, Douglas fir was found to contain 1.6 mmol g^{-1} of such structures and white fir only 1.5 mmol g^{-1}.

Both *erythro*- and *threo*-stereoisomeric forms of arylglycerol-β-aryl structures can also be determined by ^{31}P NMR after derivatization of lignins with 2-chloro-1,3,2-dioxaphospholane, by integrating the regions from 136 to 134.5 ppm and from 134.5 to 133.4 ppm, which have been attributed to Cα-OH in *erythro* and *threo* forms of β-*O*-4 structures, respectively (Argyropoulos 1994). As expected, Figure 3 shows that the *erythro/threo* ratios were similar in softwoods, while the predominance of the *erythro*-form is obvious in both *E. globulus* and *E. grandis* (with the *erythro/threo* ratio being higher in the former). These data corroborate previous findings reported by Akiyama et al. (2005), where the proportion and amount of *erythro*- and *threo*-forms were described to be similar in softwoods, whereas in contrast, the *erythro*-form predominates in hardwoods and the corresponding data are species dependent. The higher contents of the *erythro*-form in *E. globulus* are also in accordance with its superior performance in Kraft pulping when compared to *E. grandis* (Pinto et al. 2005). This is because the *erythro* diastereomers have been found to cleave somewhat faster than their *threo* counterparts under alkaline treatment (Ahvazi and Argyropoulos 1997).

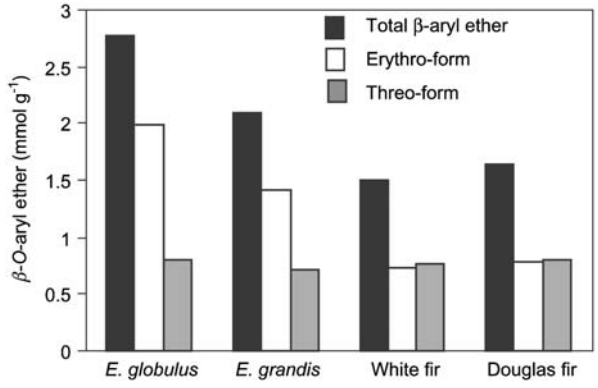

Figure 3 *Erythro* (open bars), *threo* (gray bars) and total (black filled bars) arylglycerol-β-aryl ether functional groups of EMALs isolated from white fir, Douglas fir, *E. globulus* and *E. grandis*, determined from the α-hydroxyl groups within β-aryl ether structures by ^{31}P NMR before DFRC.

Determination of units bearing etherified phenolic hydroxyl groups in arylglycerol-β-aryl ether structures

Detailed information on the etherified or carbon-carbon linked bonding pattern of lignin can be determined by DFRC/^{31}P NMR (Tohmura and Argyropoulos 2001; Guerra et al. 2006b). The results of DFRC/^{31}P NMR are presented in Figure 4. As expected, the total amount of uncondensed β-aryl ether structures within *Eucalyptus* was significantly higher than that within lignins from softwoods. The higher condensation grade of softwood lignins is well known and recognized (Ralph et al. 2004). The value of 1.73 mmol of uncondensed β-aryl ether structures per g of lignin obtained for *E. globulus* corresponds to 36.5% uncondensed β-aryl ether structures within lignin, based on the previously presented average molar mass data. The data in Figures 3 and 4 demonstrate that 62.2% of the β-*O*-aryl ether structures in *E. globulus* is unconden-

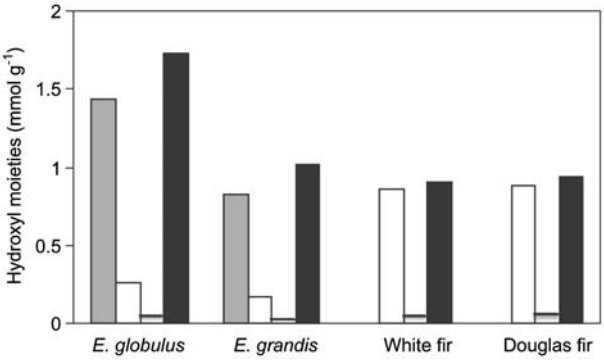

Figure 4 Values of hydroxyl groups released from arylglycerol-β-aryl ether structures by DFRC and quantified by ^{31}P NMR. The different colors in the bars refer to: syringyl units (gray bars), guaiacyl units (open bars) and *p*-hydroxyphenyl units (vertical striped) involved only in uncondensed β-*O*-aryl structures; and total uncondensed β-*O*-aryl structures (filled black bars) of EMALs isolated from white fir, Douglas fir, *E. globulus* and *E. grandis.*

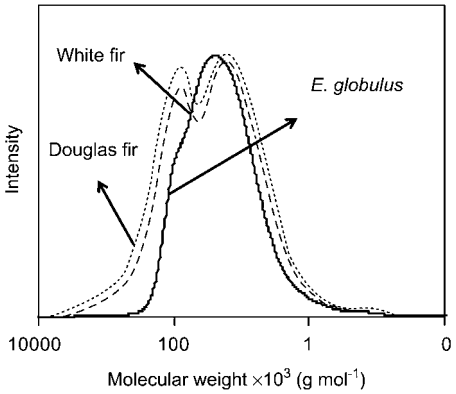

Figure 5 Size exclusion chromatograms (SEC) of lignin samples (EMALs) isolated from Douglas fir, white fir, *E. globulus* and *E. grandis* (curve overlapped by *E. globulus*).

sed (1.73 mmol/2.78 mmol), which is in good agreement with thioacidolysis data of the same wood reported by Evtuguin et al. (2001). The S/G ratio obtained after DFRC (5.5) (Figure 4) is somewhat different from that observed before DFRC (1.8) (Figure 2), indicating that the syringyl units present in *E. globulus* are primarily of the non-phenolic type (Adler 1977; Evtuguin et al. 2001).

However, the total amount of uncondensed β-aryl ether structures within *E. grandis* was somewhat lower than in *E. globulus* (Figure 4). For this wood, only 47.6% of the total amount of β-*O*-aryl ether structures was found to be uncondensed (calculated from 1000 μmol g^{-1} vs. 2100 μmol g^{-1}). This finding corroborates previous data obtained by permanganate oxidation, which indicated that *E. grandis* is more condensed than *E. globulus* (Evtuguin et al. 2001). Furthermore, S/G ratio before (1.4) and after DFRC (5.1) reveals that syringyl-type units in *E. grandis* are also preponderantly non-phenolic.

Associations of lignin macromolecules

The MWD of freshly acetobrominated EMALs was found to be strongly dependent upon the wood species from which they were isolated (Guerra et al. 2007). A highly polydisperse behavior is apparent in the SEC chromatograms of the EMAL samples (Figure 5). The elution profiles are different for the wood species. While the MWDs of the softwoods display a clear bimodal behavior, the MWDs of *Eucalyptus* (Figure 5) show a small shoulder extending over 100×10^3 g mol^{-1} instead of a maximum. The SEC chromatogram of *E. grandis*, which was omitted to prevent data over-crowding in Figure 5, would have overlapped with that of the *E. globulus* curve. Moreover, a high Mw fraction, extending into approximately 500×10^3 g mol^{-1}, is apparent in the chromatograms of EMALs from softwoods. Such a fraction is absent in the lignins from *Eucalyptus* species.

In this context, the question arises about the role of molecular associations during SEC analysis (Sarkanen et al. 1981). Figure 6 shows the effect of incubation on the MWD of acetobrominated EMALs. Incubation in the present study refers to the aging of the acetobrominated lignins conducted at 2.0 g l^{-1} in THF at 25±3°C under vigorous magnetic stirring (5000 rpm) (Guerra et al. 2007).

As demonstrated in Figure 6a, the bimodality of softwoods was clearly observed only in freshly prepared lignin solutions (analyzed immediately after derivatization). After 2 days of incubation under stirring, the fragments with high hydrodynamic volumes extending over 500×10^3 g mol^{-1} disappeared, while the high-Mw maximum became a shoulder, which was no longer observed after 4 days of incubation. Furthermore, the low-Mw peak was shifted towards lower hydrodynamic volumes and a well-resolved peak appeared after 1 day of incubation in this range (Figure 6a). This peak corresponds to the molar mass of dimeric species as demonstrated by the co-elution of 1-(3,5-dimethoxy-4-hydroxyphenyl)-2-(4-methoxy-phenyl)-propanodiol-1,3 with an EMAL sample (SEC not shown). However, after longer incubation times, the bimodal elution pattern of softwoods was replaced by a single broad elution peak and the signal due to the dimers was no longer resolved. Probably, lignin oligomers released from the association complex during the incubation superimposed the bimodal elution pattern in the low-Mw zone. Sarkanen et al. (1981) observed a pronounced reduction in the apparent weight average Mw of lignins of various types (organosolv, synthetic, Kraft and Brauns native lignin) during incubation in alkaline conditions. However, it is of significance that such notable incubation effects were not observed for the size exclusion chromatograms of EMAL from both *Eucalyptus* species. As demonstrated in Figure 6b, an almost unimodal elution profile was found for freshly derivatized lignin. After 10 days of incubation, the low-Mw peak shifted toward lower Mw and the peak due to the accumulation

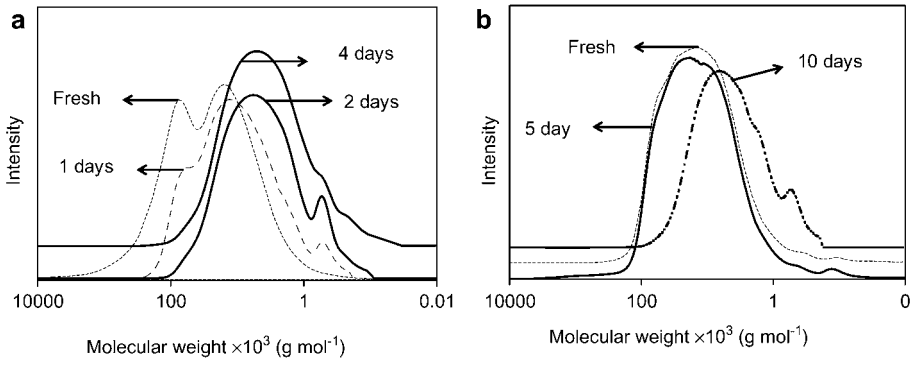

Figure 6 Effects of incubation at room temperature on the molecular weight distribution of Douglas fir (a) and *E. globulus* (b).

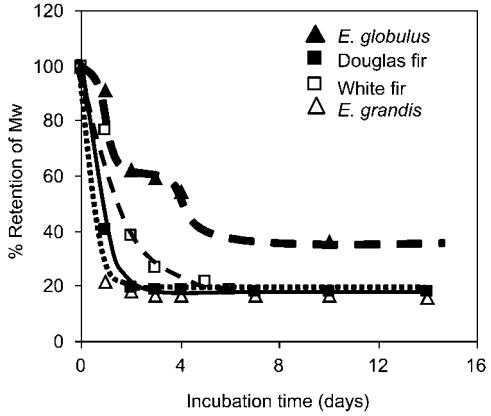

Figure 7 Effects of incubation at room temperature on the apparent weight average molecular weight (Mw) of lignins isolated from different wood species.

of oligomeric species also appeared. However, longer incubation times had negligible effects on the MWD of EMAL from *Eucalyptus*.

To ensure that the aforementioned effects are not due to degradation of covalent linkages within lignin, the incubation was also monitored by quantitative ^{31}P NMR. Samples were first acetobrominated and dissolved in THF. After examining their SEC profiles, the THF was immediately removed under nitrogen and the remaining lignin was dissolved in pyridine/$CDCl_3$ and phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane. As mentioned before, by dissolving a lignin sample in neat acetyl bromide diluted with glacial acetic acid (acetobromination), the primary alcoholic and the phenolic hydroxyl groups are acetylated, while the benzylic α-hydroxyls are displaced by bromide (Lu and Ralph 1998). Such acetylation of the hydroxyl groups precludes them from being detected by ^{31}P NMR, as they can no longer be phosphitylated. As a result, no signals due to phenolic or aliphatic hydroxyl groups were detected in the ^{31}P NMR spectra (spectra not shown) of the freshly prepared lignin solutions. It should be noted that an absence of such signals in the spectra of the starting acetobrominated lignins facilitates the monitoring of the incubation process. Accordingly, cleavage of aryl ether linkage or oxidative reactions taking place within these lignin preparations would be promptly recognized by the appearance of the corresponding signal in the ^{31}P NMR spectra after incubation. As pointed out above, when the aryl ether linkages are cleaved, the corresponding phenolic hydroxyls released can be quantified by ^{31}P NMR, and oxidation reactions leading to carboxylic acid groups can also be detected by ^{31}P NMR (Argyropoulos 1994). However, under the incubation conditions no such reactions were apparent. The OH_{phen} and COOH contents of the incubated EMALs derivatives were found to be remarkably constant throughout the incubation period of 30 days (spectra not shown). This finding supports the claim that the observed effects in the MWDs are due to the disruption of physical association forces rather than the cleavage of covalent bonds within the lignin macromolecules.

The apparent Mw of the lignins are presented as a function of the incubation time (Figure 7). Here, the dissociation behavior of *E. globulus*, which differs from the others, is noteworthy. To compare this dissociation behavior in numerical terms, the Mw reduction factor F, was calculated from the relation between the Mw before and after complete dissociation in THF (Table 1). It was ensured that the second measurement was performed in the final stage of dissociation (after incubation for 20–30 days in the presence of LiCl). LiCl eliminates potential residual association by the shielding of dipole effects (Cathala et al. 2003). No alteration in the MWs is apparent in the presence of LiCl, indicating a complete dissociation. The data listed in Table 1 show that the apparent weight average Mw of softwoods decreased as a whole by factors F ranging between 5 and 7.6, while the F factor of *E. globulus* was 3.0. However, as anticipated the Mw reduction factor for *E. grandis* (6.2) was found to be closer to those of softwoods than for *E. globulus.* Accordingly, the association/dissociation behavior in THF cannot be generalized; it is very wood and lignin specific.

The Mw data decreased greatly after dissociation in comparison to the initial values (Table 1). We suggest that lignin association phenomena also contribute to a large extent to the huge variety of MWs reported for lignins.

Conclusions

The combination of enzymatic and mild acid hydrolysis offered the possibility to isolate lignin samples that are more representative of the total lignin in *Eucalyptus* wood species than the traditional lignin preparations, such as MWL and CEL. A detailed comparison of the lignin structure revealed significant differences between *E. globulus*

Table 1 Effect of dissociation on apparent weight average molecular weight (Mw), number average molecular weight (Mn) and polydispersity (D) of EMALs isolated from *E. globulus*, *E. grandis*, Douglas fir and white fir.

EMAL from	State[a]	Mw (g mol^{-1})	Mn (g mol^{-1})	D	Factor ($Mw_{initial}/Mw_{final}$)
Douglas fir	Fresh	49 500	7700	6.4	4.9
	Dissociated	10 100	3740	2.7	
White fir	Fresh	57 000	7700	7.4	7.6
	Dissociated	7500	2800	2.7	
E. globules	Fresh	23 400	6500	3.6	2.9
	Dissociated	8100	2890	2.8	
E. grandis	Fresh	27 700	7500	3.7	6.2
	Dissociated	4500	1600	2.8	

[a]Fresh and dissociated refer to SEC analyses performed immediately after acetobromination and complete dissociation in THF, respectively.

and *E. grandis*, which can affect their pulping performances.

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