

Molecular weight-functional group relations in softwood residual kraft lignins

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Abstract

An integrated picture of the distribution of functional groups should be provided as a function of molecular size within residual kraft lignins. With this goal we developed a reliable and reproducible method for determination of the molecular weight and molecular weight distribution of residual kraft lignins (RKLs) over the whole delignification range. In general, our data indicate that for reliable measurement of the molecular weight and its distribution of residual lignin in pulps, the lignin-carbohydrate bonds have to be cleaved prior to size exclusion chromatography. The recently developed method for isolating residual lignins, which involves cellulolytic treatment followed by a mild acid hydrolysis step, was found to be the most suitable approach to achieve this. The molecular weight and polydispersity of all RKLs decreased as a function of delignification. As anticipated, the observed decrease in molecular weight was clearly reflected in the concomitantly decreasing amount of β -O-4 structural linkages present. Similarly, the total phenolic hydroxyl content increased as the molecular weight of the RKLs decreased during kraft pulping. Despite the smaller size of the lignin remaining on the kraft fiber at the end of delignification, the preponderance of condensed phenolic structures within these lignins offers an explanation for delignification problems during bleaching.

Keywords: delignification; kappa number; molecular weight; ³¹P NMR; residual kraft lignin.

Introduction

In earlier work we made extensive attempts to clarify the structural characteristics of residual lignin in kraft pulp (RKL, residual kraft lignin) to better understand the structural alterations that occur during pulping and bleaching processes (Jiang and Argyropoulos 1994; Granata and Argyropoulos 1995; Sun and Argyropoulos 1996; Asgari and Argyropoulos 1998; Argyropoulos and Liu 2000; Tohmura and Argyropoulos 2001; Argyropoulos et al. 2002a,b; Jääskeläinen et al. 2003; Wu and Argyropoulos 2003). The coexistence of lignins and carbohydrates is an opportunity to study the nature of lignin carbohydrate bonds on the one hand, and a challenge when lignin analysis is the focus, on the other hand. For example, the presence of carbohydrates produces a variety of NMR signals that may interfere with lignin ¹³C, ¹H and/or ³¹P NMR signals. Furthermore, size exclusion chromatography of lignin-carbohydrate complexes (LCCs) is problematic due to the fact that the hydrodynamic radii of the components are different. Last, but not least, the presence of carbohydrates seriously affects sample solubility in a variety of otherwise good solvents for lignins.

There is abundant literature describing lignin isolation methods for wood or pulps aimed at achieving high yields with minimum chemical modification (Björkman 1956, 1957; Björkman and Person 1957; Pew 1957; Pew and Weyna 1962; Wayman and Obiaga 1974; Chang et al. 1975; Lundquist et al. 1977; Polčín and Bezúch 1978; Glasser and Barnett 1979; Fišerová and Šutý 1980; Yamasaki et al. 1981; Jiang and Chang 1987; Hortling et al. 1990; Gellerstedt et al. 1994; Bujanovic et al. 1999; Duarte et al. 2000, 2001; Hortling and Tamminen 2001; Argyropoulos et al. 2002b; Jääskeläinen et al. 2003; Wu and Argyropoulos 2003). Most of these methods used sample milling, solvent extraction, enzymatic treatments with cellulases and combinations thereof to prepare lignins with low carbohydrate content. The use of acid hydrolysis conditions was proposed for the isolation of lignin from kraft pulps (Gellerstedt et al. 1994). Most of the procedures evaluated offer advantages and limitations, as discussed previously (Argyropoulos et al. 2002b; Wu and Argyropoulos 2003). Recently, a good compromise between lignin yield and purity was achieved with a two-stage isolation method (Argyropoulos et al. 2002b) that uses a combination of cellulolytic enzymatic treatment and a mild acid hydrolysis step.

Here we examine the nature of representative, high-yield residual kraft lignin preparations (RKLs) isolated from kraft pulps (KPs) obtained at different extents of delignification, by measuring the changes in their molecular weight and functional groups during pulping. We tested a wide variety of techniques and solvents to completely dissolve RKLs prior to molecular weight analyses and quantitative ³¹P NMR. These experiments included a

novel solvent system (*N*-methylimidazole/DMSO) that was reported to completely dissolve finely milled cell-wall material (Lu and Ralph 2003). The molecular weight and molecular weight distribution (\bar{M}_w and \bar{M}_n) thus obtained are then plotted as a function of absolute lignin content to reveal their relation to the extent of delignification. Furthermore, plots of the content of functional groups such as β -ether bonds, phenolic OH groups (condensed, uncondensed and total) and aliphatic OH groups, expressed as mmol g⁻¹, are also presented as a function of molecular weight.

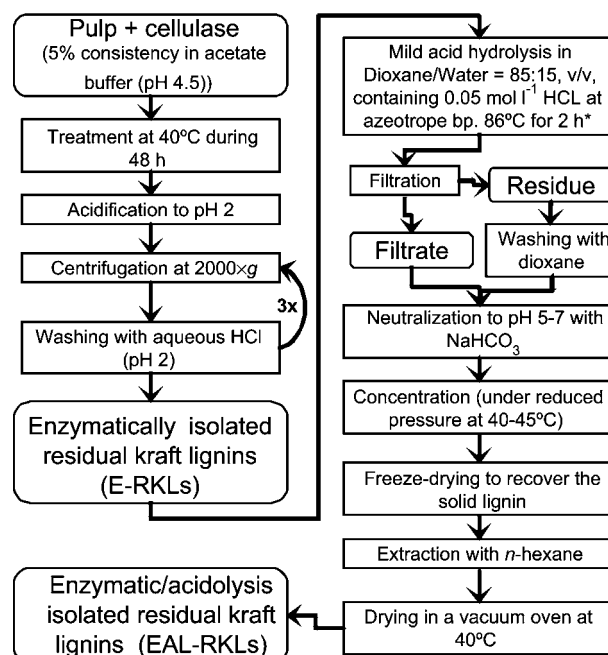
Experimental

Extraction and pulverization of kraft pulp

A series of loblolly pine (*Pinus taeda* L.) kraft pulps were prepared at different kappa numbers using conventional pilot-plant equipment at a wood/liquor ratio of 1:4, active alkali of 20% and sulfidity of 25%. By changing the H factor of the pulping procedure to values of 800, 1000, 1200, 1900 and 2100, the following kappa numbers were obtained: 104, 89.3, 70.7, 38.4 and 27.4. Pulp with kappa number 15.1 was obtained from the pulp with kappa 27.4 after a conventional O₂-delignification stage (consistency 10%, NaOH 2.5%, o.d. pulp, p(O₂) 100 psi, 105°C for 60 min). All pulps were first washed well with deionized water and then extracted with acetone for several hours until the color of the extract became clear. The extractives-free pulps were then air-dried prior to being pulverized by Planetary milling (Fritsch, Pulverisette 7). For pulverization, approximately 0.8 g of dry pulp was placed in a 50-ml sample chamber together with 16 milling balls made of zirconium dioxide (9.8 mm in diameter). The pulp was first roughly milled at a rotation speed of 600 rpm for 10 min to reduce its volume and then an additional 0.8 g of dry pulp was added and milled again in the same manner. This process was repeated 10 times until the total weight of the sample was 8 g. The resulting powdered pulp was subjected to further milling using a rotation speed of 600 rpm for 30 min, followed by a cooling period of 10 min. For each sample the whole cycle was repeated 10 times. Loblolly pine wood meal was prepared in an identical manner from wood flakes.

Enzymatic and mild acid hydrolyses

The enzymatic and mild acid hydrolysis procedure for lignin isolation is shown in Scheme 1. Pulverized and non-pulverized pulp samples were subjected to a cellulase (logen, Canada; filter paper activity 1000 IU ml⁻¹) treatment at 40°C for 48 h using acetate buffer (pH 4.5) at 5% consistency according to previously published conditions (Argyropoulos et al. 2002b). The material that remained was collected by centrifugation (2000 × g) after the pH was adjusted to pH 2 using a small amount of 2.0 mol l⁻¹ aqueous HCl. It was then repeatedly washed with dilute hydrochloric acid solution (pH 2; deionized water containing dilute HCl) and collected by centrifugation to yield enzymatically isolated residual kraft lignin-carbohydrate mixtures (E-RKLS). A sample of each of the E-RKLS was further subjected to mild acid hydrolysis using an azeotrope (b.p. 86°C) of aqueous dioxane (dioxane/water 85:15, v/v, containing 0.05 mol l⁻¹ HCl) under an argon atmosphere, as previously reported (Argyropoulos et al. 2002b). The resulting suspension was filtered, and the filtrate was neutralized to pH 5–7 with sodium bicarbonate. The solution was concentrated under reduced pressure at 40–45°C to reduce its volume. The solid in the concentrate was recovered by freeze-drying, and then extracted with *n*-hexane to remove remaining extractives. The extractives-



Scheme 1 Two-step enzymatic/acidolysis procedure for lignin isolation. For wood meal 0.01 M l⁻¹ HCl was used.

free solid was dried in a vacuum oven at 40°C overnight, and residual kraft lignins were finally obtained via the two-step enzymatic-acidolysis method (EAL-RKLS). The pulverized wood meal was also subjected to enzymatic hydrolysis and acidolysis (using dioxane/water 85:15, v/v, containing 0.01 mol l⁻¹ HCl) to yield enzymatic isolated wood lignin (E-WL) and enzymatic-acidolysis isolated wood lignin (EAL-WL), respectively.

Determination of lignin content

Klason lignin (acid-insoluble lignin) and acid-soluble lignin contents of wood meal and pulverized kraft pulps were measured according to TAPPI methods, T222 om-88 and UM250, respectively, with some modifications as reported in the literature (Effland 1977; Whiting et al. 1981; Yoshihara et al. 1984). Approximately 100 mg of wood meal and 170–1200 mg of pulverized kraft pulp was weighed, so that each sample contained 20–30 mg of lignin that was roughly estimated by its kappa number. A 25–100-ml beaker was charged with a sample, and then 72% sulfuric acid solution was added; 1.5 ml of 72% sulfuric acid was added for 100 mg of wood meal, and 2.0 ml for each 100 mg of pulverized pulps. The mixture was placed in a water bath at 20 ± 1°C and stirred occasionally to effect complete solution. After exactly 2 h, the mixture was diluted with deionized water to a final sulfuric acid concentration of 3%. The resulting mixture was transferred to a pressure-proof glass bottle and then placed in a pressurized autoclave kept at 121°C for 1 h. After cooling, the lignin residue was filtered through a crucible and washed well with boiling deionized water. Both the residue and filtrate were recovered. The residue was completely dried in an oven at 105°C overnight and then weighed to determine the Klason lignin content. The acid-soluble lignin in the filtrate was determined by UV spectrophotometry at a wavelength of 205–210 nm. The filtrate was diluted, when necessary, so that absorbance was within the range 0.2–0.7.

Molecular weight distribution of acetylated RKLS

Approximately 10 mg of each of the E-RKLS obtained from pulverized and non-pulverized pulps was suspended in two different solvent systems: namely, pyridine (1.0 ml) and *N*-

methylimidazole/DMSO (1:2, v/v; 1.0 ml). Then 1.0 ml of acetic anhydride was added to the suspension. Acetylation was carried out in a water bath set at room temperature overnight to dissipate any excess heat generated when the *N*-methylimidazole/DMSO solvent system was used. The resulting solution was then poured into an excess (200 ml) of aqueous methanol (1:1, v/v) and kept in a refrigerator overnight. Acetylated RKLs were then collected by centrifugation. The EAL-RKLs from pulverized pulps were acetylated and recovered in the same manner. E-WM and EAL-WM were also acetylated and served as a reference.

Acetylated samples (10 mg) were dissolved in 10 ml of THF (approx. 0.1% w/v) and characterized on a size exclusion chromatograph consisting of a Waters U6K injector, a Waters 510 pump, a UV Waters 484 detector and a Waters refractive index 410 detector. These analyses were carried out at 40°C using THF as eluent at a flow rate of 0.22 ml min⁻¹. HR 5E and HR 1 (Waters) columns connected in series were used. Polystyrene standards in the molecular weight range 890–186 × 10⁴ g mol⁻¹ were used to calibrate the columns. Millenium³² GPC software (Waters) was used for data processing.

Prior to analyses, the solubility of acetylated samples in THF, DMF, DMF containing 0.5% LiCl, and 0.05 M and 0.5 M aqueous NaOH was qualitatively examined using 5 mg of acetylated sample in 5 ml of each selected solvent.

Quantitative ³¹P NMR analysis of hydroxyl groups in RKLs

Quantitative ³¹P NMR spectra of EAL-RKLs were obtained on a Bruker 300 MHz spectrometer equipped with a Quad probe dedicated to ³¹P, ¹³C, ¹⁹F and ¹H acquisition. The sample preparation procedure was similar to that reported earlier (Granata and Argyropoulos 1995; Tohmura and Argyropoulos 2001; Jääskeläinen et al. 2003). Minor modifications were used due to the difficulty in dissolving some of the samples, especially those isolated from pulp with lower kappa number and from oxygen-delignified kraft pulp. The modified procedure was mainly developed for highly oxidized lignin samples, which have lower solubility in the solvent mixture system (pyridine/deuterated chloroform) typically used. Residual lignins isolated from oxygen-delignified and peroxide-bleached pulps also showed a similar tendency during sample preparation for ³¹P NMR (Liu et al., in preparation). These developments will be reported and clarified in subsequent publications (Gaspar et al. in preparation).

Samples were completely dried in a vacuum oven set at 40°C overnight. Approximately 40 mg of each sample (accurately weighed) was transferred into a sample vial and pyridine-d₅ (184 μl) was added to it. The mixture was then left at room temperature overnight with continuous stirring. DMF (300 μl) was then added to the mixture prior to phosphorylation. Cholesterol and chromium(III) acetylacetonate were dissolved in a solvent mixture composed of pyridine-d₅/chloroform-d (CDCl₃) (1.6:1, v/v). A cholesterol solution (50 μl, 80.0 mg ml⁻¹) or *N*-hydroxynaphthalimide solution (100 μl, 22.0 mg ml⁻¹) was used as an internal standard, and chromium(III) acetylacetonate (50 μl, 11.4 mg ml⁻¹), as a relaxation reagent. Chloroform-d (115 μl) was also added to the sample mixture. Finally, 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (100 μl) was added. The mixture was capped, left at room temperature for 20–30 min to complete the reaction and then transferred to a 5-mm-O.D. NMR tube. The NMR spectrum of EAL-WL was also obtained as a reference. To determine the amount of remaining β-ether bonds, another series of EAL-RKL and EAL-WL samples was prepared in the same manner, and phosphorylated as the final step with a different reagent, namely, 2-chloro-1,3,2-dioxaphospholane (100 μl) (Argyropoulos 1995).

Results and discussion

Molecular weight distribution

Almost all the E-RKL samples showed limited solubility in any of the solvents typically used for size exclusion measurements (Table 1), regardless of whether samples were finely milled and/or acetylated in pyridine or in *N*-methylimidazole/DMSO. The latter solvent mixture was reported to be a good solvent system for pulverized wood cell-wall materials (Lu and Ralph 2003) and *N*-methylimidazole is a known effective catalyst for acetylation (Connors and Pandit 1978; Wachowiak and Connors 1979; Bittner et al. 1980). After acetylation, these samples were soluble in the solvent mixture recommended by Lu and Ralph (2003), and were then further examined. However, the resulting molecular weight data produced unreliable results, since the lignin carbohydrate bonds were still intact and E-RKL samples after acetylation showed inadequate solubility in the organic phase used for size exclusion measurements.

Alternatively, pulps treated enzymatically followed by mild acidolysis resulted in EAL-RKL samples that, when carefully prepared (see Experimental), showed excellent solubility (Table 1, last two columns). Covalent linkages between lignin and carbohydrates are known to exist in wood (Minor 1986) but they can also be formed during the kraft pulping process. Such linkages are stable in alkali media (Gierer and Wännström 1984, 1986; Iversen and Wännström 1986; Taneda et al. 1987; Lawoko et al. 2003, 2004; Létumier et al. 2003; Laine et al. 2004).

Cellulolytic enzyme treatment does not split covalent bonds between carbohydrates and lignins. Thus, the solubility of LCCs is low, even after acetylation. A variety of earlier papers offered fractionation protocols for enzymatically isolated RKLs as a means to alleviate solubility problems (Hortling et al. 1990; Fukagawa et al. 1992; Bujanovic et al. 1999; Duarte et al. 2000). It is conceivable that mild acid hydrolysis cleaves lignin-carbohydrate linkages, so that molecular weight determination will be representative for the RKLs, because the whole system will be dissolved, rather than fractions of it. EAL-RKLs still have small amounts of carbohydrates (ca. 1.5%) (Jääskeläinen et al. 2003), but this will not greatly affect the molecular weight determination.

The kappa numbers and lignin contents of the kraft pulps investigated are listed in Table 2. Weight and number average molecular weights (\bar{M}_w and \bar{M}_n , respectively) of acetylated EAL-RKLs are plotted versus the degree of delignification for the overall kraft delignification range (based on actual Klason and UV-determined pulp lignin contents) in Figure 1A. This plot clearly shows for the first time that both molecular weight averages of EAL-RKLs monotonously decrease with progressing delignification. As anticipated, \bar{M}_w is more sensitive to the relative changes in the degree of delignification than \bar{M}_n .

Polydispersity, defined by the ratio \bar{M}_w/\bar{M}_n , also progressively decreased with increasing delignification (Figure 1B). It is obvious that the molecular weight distribution narrows as delignification proceeds. This can also be visualized from the gel permeation chromatograms in Figure 2. As delignification proceeds, the chromatograms progressively shift to higher elution volumes

Table 1 Qualitative solubility tests of enzymatically isolated wood meal lignin (E-WM), enzymatically isolated residual kraft lignins (E-RKLs) and enzymatic/acidolysis-isolated residual kraft lignins (EAL-RKLs).

Treatment	E-WM	E-RKL					EAL-RKL	
		κ 104	κ 89.3	κ 70.7	κ 38.4	κ 27.4	κ 89.3	κ 89.3
Pulverization	Yes	No	Yes	No	No	No	No	No
Acetylation ^a	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
THF							S	S
0.05 M NaOH							S	S
0.5 M NaOH							S	S
DMF							S	S
DMF + 0.5% LiCl							S	S

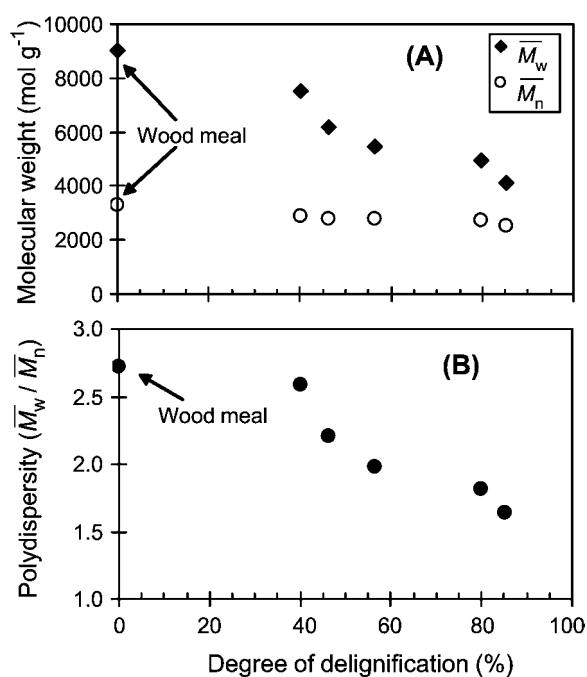
I, insoluble or only partly soluble; S, completely soluble.

^aAcetylation in pyridine/acetic anhydride; *N,N*-methylimidazole/DMSO system instead of pyridine did not offer improved solubility (data not shown).

Table 2 Kappa number and lignin content of kraft pulps and wood meal used in this study.

	Wood meal	Kraft pulps				
		κ 104	κ 89.3	κ 70.7	κ 38.4	κ 27.4
Klason lignin (%)	25.0	14.5	13.0	10.5	3.9	2.2
Acid-soluble lignin (%)	0.9	1.0	0.9	0.9	1.4	1.7
Total lignin content (%)	25.9	15.5	13.9	11.3	5.2	3.9
Degree of delignification (%)	0	39	46.4	56.4	80.0	85.0

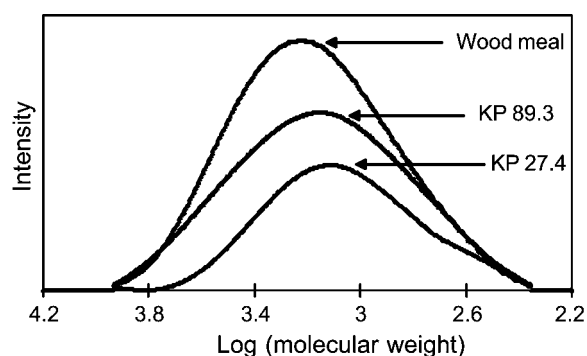
(i.e., to lower molecular weights) with concomitant changes in shape to narrower peaks. Figure 1A,B reflects to a certain degree the initial, bulk and residual delignification phases (Kleinert 1965, 1966; Kleppe 1970; Axegård et al. 1978; Ljunggren 1980; Teder and Olm 1981; Axegård and Wiken 1983). In this context, the segments 0–40%, 40–80% and >80% delignification should be noted. During the initial phase, \bar{M}_w and polydispersity of the residual lignins gradually decrease, while in the latter phases, these parameters decrease more rapidly.

**Figure 1** (A) Weight and number average molecular weights of residual kraft lignins and (B) polydispersity as a function of degree of delignification.

For these experiments THF was used as the GPC mobile phase, despite the fact that Sjöholm et al. (1999a,b) reported that size exclusion chromatography is solvent-dependent. The limitation of polystyrene standards for molecular mass determination of lignins is also well known. Of course, the application of fractionated lignin preparations as a calibration standard would be ideal (Evtuguin et al. 1999; Pinto et al. 2002).

β -O-4 ether structures

The β -aryl ether structures were determined by quantifying the C α hydroxyl groups using quantitative ³¹P NMR spectroscopy (Jiang and Argyropoulos 1994; Argyropoulos 1995; Akim et al. 2001). As anticipated, the molecular weights decreased as the degree of delignification increased (Figure 3A). Since the integrity of the polymeric chains of lignins is largely due to the presence of β -O-4 structures, it is evident that once the chain length of a given RKL decreases, the content of such structures will also be reduced (Figure 3B). The content of β -O-4 link-

**Figure 2** Typical size exclusion chromatograms of residual kraft lignins and lignins isolated from wood meal.

ages of samples investigated earlier (Gellerstedt and Gustafsson 1988) showed a similar trend as in our study.

^{31}P NMR spectroscopy also allows quantitation of the diastereomer forms of $\beta\text{-O-4}$ structures possessing $\text{C}\alpha$ hydroxyl groups. In Figure 4 the amounts of *erythro* and *threo* isomers are plotted as a function of \bar{M}_w . Both diastereomeric forms decreased in parallel to the molecular weight. Closer examination of the data reveals that the degradation of *erythro* structures is greater than that of the *threo* counterparts. This phenomenon is well known from the literature (Obst 1983; Taneda et al. 1989; Jiang and Argyropoulos 1994). Evidently, RKLs with lower molecular weight seem to be greatly enriched in $\beta\text{-O-4}$ structures composed almost entirely of the stable *threo* diastereomer (Figure 4).

Phenolic functional groups

The fact that cleavage of $\beta\text{-O-4}$ ether bonds leads to phenolic OH groups is corroborated in Figure 5A. Total phenolic hydroxyl groups present in RKLs increased concomitantly with decreasing $\beta\text{-aryl}$ ether bonds (Figure 5A). Figure 5B shows that phenolic groups formed and $\beta\text{-O-4}$ structures decreased as a function of \bar{M}_w .

An increase in total phenolic content of approximately 1.0 mmol g^{-1} (from 1.25 to 2.25 mmol g^{-1}), as observed for RKLs between \bar{M}_w 9040 and 5470, was accompanied by a decrease in $\beta\text{-O-4}$ structures of 0.5 mmol g^{-1} (from 1.2 to 0.7 mmol g^{-1}). The overproportional increase in phenolic OHs hints at the high degradation power of kraft pulping, which is able to liberate phenolic hydroxyl groups from a variety of structures other than $\beta\text{-O-4}$ bonds. This observation is further supported, to a small extent, by the fact that structures carrying benzylic carbonyl or sulfur atoms are not detectable during measurement of $\beta\text{-O-4}$ bonds by quantitative ^{31}P NMR.

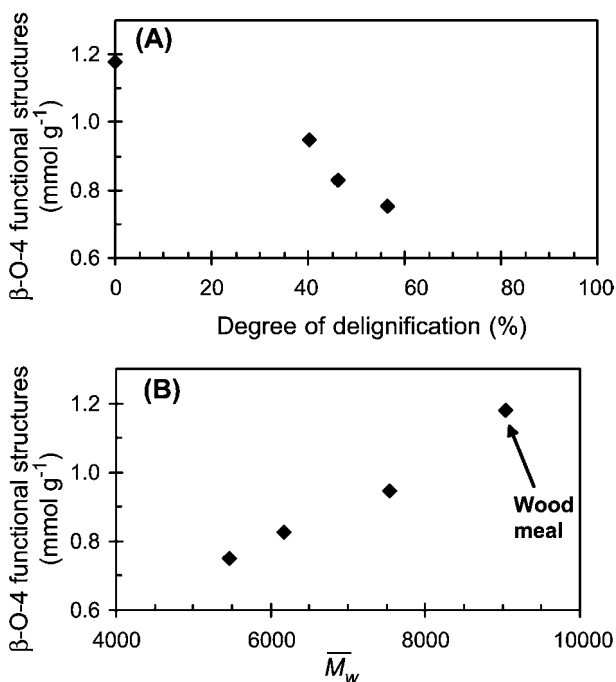


Figure 3 $\beta\text{-O-4}$ functional structures of residual kraft lignins as a function of (A) degree of delignification and (B) \bar{M}_w .

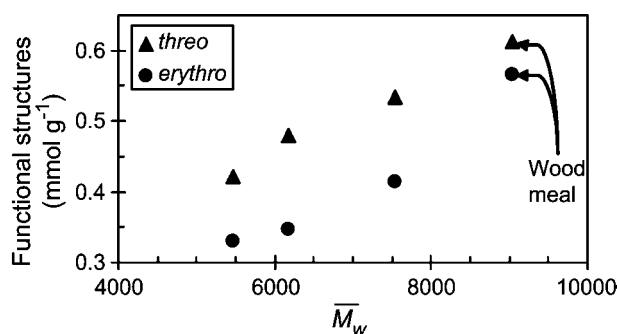


Figure 4 *Threo* and *erythro* diastereomeric forms of $\beta\text{-O-4}$ groups within residual kraft lignins as a function of \bar{M}_w .

Total and condensed phenolic hydroxyl groups are depicted in Figure 6A. Previous findings of our group (Jiang and Argyropoulos 1994; Hosoya 1992) are strengthened by taking into consideration \bar{M}_w of the residual lignins. As expected, the total phenolic hydroxyl content monotonically increased with decreasing RKL molecular weight, since OH groups are the predominant end groups in lignin. Simultaneous consideration of Figure 6B and 3B is informative and allows the following conclusion. As delignification proceeds, progressively smaller fragments of lignin remain on the fiber, containing lower amounts of aryl ether structures and higher amounts of condensed phenols.

In the initial stage of delignification, the formation and/or accumulation of condensed phenolic hydroxyl groups within a residual lignin is more pronounced than for non-

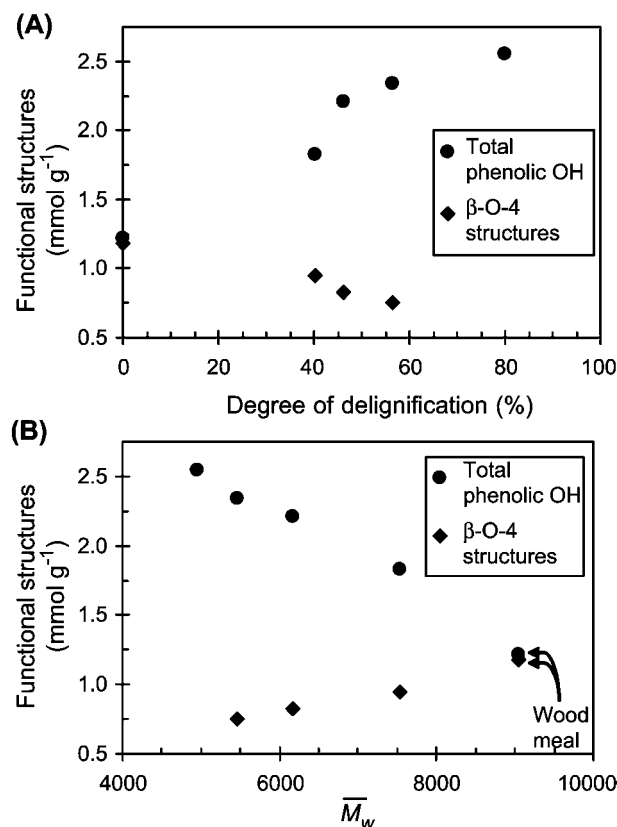


Figure 5 Total phenolic hydroxyl groups and $\beta\text{-O-4}$ functional structures of residual kraft lignins as a function of (A) degree of delignification and (B) \bar{M}_w .

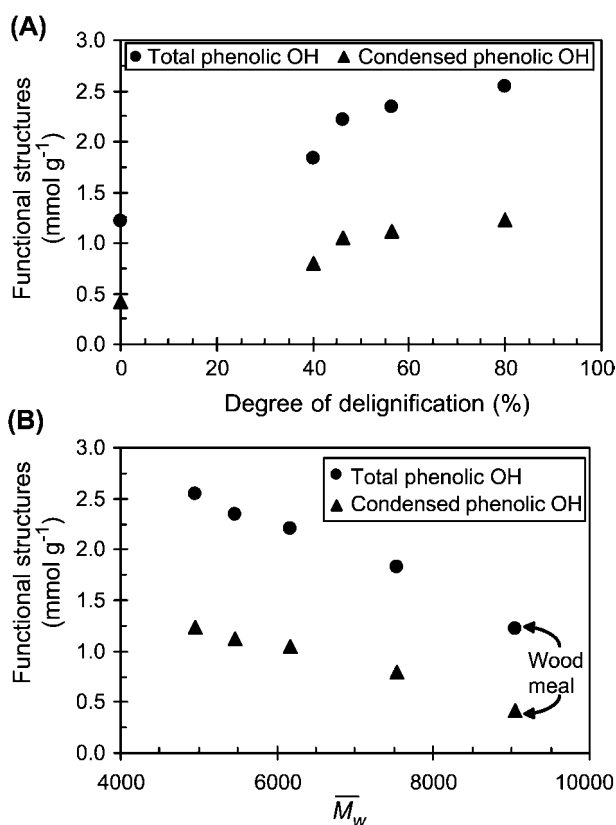


Figure 6 Total and condensed phenolic hydroxyl groups of residual kraft lignins as a function of (A) degree of delignification and (B) \bar{M}_w .

condensed phenolic groups (Figure 6). This observation corroborates the data of Liu et al. (2000) on the kraft delignification of black spruce wood (*Picea mariana*). Gellerstedt and Lindfors (1984a,b) proposed that lignin-carbohydrate linkages inhibit delignification in its final stages, after most of the β -aryl ether linkages have been eliminated. Subsequent study of model compounds confirmed that lignin fragments containing non-phenolic and non-condensed β -aryl ether structures linked to polysaccharides, via α -ether linkages, were relatively stable in alkali (Taneda et al. 1987). These can be cleaved only in the advanced phases of delignification. Consequently, such structures, once formed, would contribute to the accumulation of non-condensed phenolic OH groups only in the last two phases of delignification. Our findings (Figure 6A) are in line with this interpretation, since the accumulation of phenolic hydroxyl groups dramatically slowed near the end of the bulk phase and throughout the final delignification phase.

Aliphatic hydroxyl groups

The decrease in aliphatic hydroxyl content (Figure 7) is certainly related to the progress of delignification, with two factors contributing to this: the progressive elimination of C α hydroxyl groups and the well-known γ -elimination of formaldehyde with the accompanying formation of β -guaiacoxy styrene structures (Ekman 1965).

A notable feature of the data in Figure 7 is the initial rapid reduction in the aliphatic group content, i.e., from ~ 5 mmol g⁻¹ for wood meal lignin to ~ 2.9 mmol g⁻¹

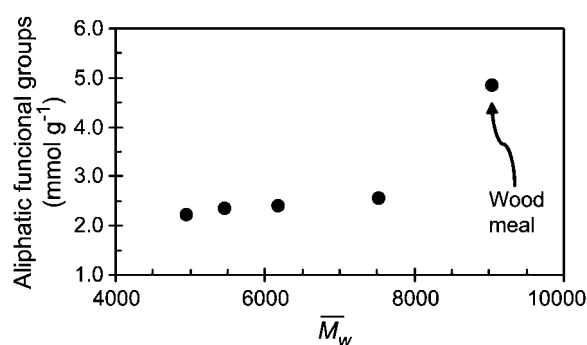


Figure 7 Aliphatic hydroxyl groups of residual kraft lignins as a function of \bar{M}_w .

for the RKL obtained at 39% delignification (\bar{M}_w approx. 7500 g mol⁻¹). This represents a very large decrease in terms of aliphatic OH group content, which cannot be attributed solely to the elimination of β -O-4 structures, since the sample of wood meal lignin contained only approximately 1.2 mmol g⁻¹ β -O-4 structures (see Figure 3). Consequently, the significance of aliphatic OH-group elimination (other than α -hydroxyl groups belonging to β -O-4 structures) becomes apparent.

Conclusions

During the initial phase of delignification, \bar{M}_w and the polydispersity index of the residual lignins gradually decreased, while during the bulk and residual delignification phases, \bar{M}_w and polydispersity decreased more rapidly. RKLs with lower molecular weight seemed to be greatly enriched in β -O-4 structures composed almost entirely of the stable *threo* diastereomer. The molecular weight of residual kraft lignins progressively decreased as a function of delignification, despite the observed accumulation of condensed phenolic structures within them. Consequently, fragmentation reactions of β -aryl ethers and other moieties most likely take place at higher rates than condensation reactions. Despite the smaller size of the lignin remaining on the kraft fiber at the end of delignification, the preponderance of condensed phenolic structures within these lignins offers an explanation for the difficulty in carrying out delignification by bleaching.

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