

CHARACTERIZATION OF STRUCTURAL CHANGES IN LIGNIN DURING CONTINUOUS BATCH KRAFT COOKING OF *EUCALYPTUS GLOBULUS*

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Eucalyptus globulus wood originating from plantations in Uruguay was subjected to continuous batch kraft cooking (CBC), applying mill-like conditions. Pulp samples were taken at different stages of CBC cooking being representative for all three cooking phases. The residual lignin was successfully isolated in a reasonable yield by a new method, the dissolved wood lignin (DWL) protocol, which is based on the total dissolution of ball milled wood and pulp samples in dimethylsulfoxide and N-methylimidazole (DMSO/NMI) followed by precipitation in dioxane/water to separate lignin and carbohydrate fractions. For comparative reasons, the lignin was also isolated by a conventional mild acidolysis (AL) method. Extensive structural lignin characterization using 1D and 2D NMR revealed that the DWL protocol allows the isolation of less altered lignin than the AL method. During bulk and residual delignification, the S/G ratio of lignin remaining in the fibers continuously decreased, while the content of β -O-4 units and phenolic OH groups remained almost unaffected, suggesting that the CBC process permits enhanced delignification efficiency and good bleachability.

Keywords: *Eucalyptus globulus*; Continuous batch cooking; Dissolved wood lignin; Mild acidolysis lignin; Residual lignin.

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INTRODUCTION

Kraft pulping has developed as the principal cooking process, accounting for about 90% of the chemical pulps worldwide (Sixta 2006). The dominance of kraft pulping over sulphite pulping, which became apparent with the development of the Tomlinson recovery boiler and the chlorine dioxide bleaching technology, has further extended since the introduction of modified cooking technology in the early 1980's (Teder and Olm 1981). In the meantime, three generations of modified kraft pulping processes have emerged through continuous research and development (Axegard and Backlund 2003). The concept of continuous batch cooking (CBC) represents one of the latest developments of modified cooking procedures, where the four basic principles of modified kraft cooking are accomplished by combining batch displacement technology with continuous flow of cooking liquor through the digester of constant temperature and preset cooking liquor composition (Wizani et al. 2000). With this concept any desired concentration profile of the active cooking chemicals, hydroxide, and hydrogen sulfide

anions, necessary to achieve high cooking selectivity and efficiency, can be adjusted throughout the entire cook. This also includes the temperature profile and, at least in part, the dry solids content during the late stage of the cook (Sixta et al. 2007). The potential of the kraft cooking technology is, however, not exhausted by far.

The elucidation of structural details of residual lignins constitutes the most important challenge and prerequisite for further improvements in the kraft pulping technology. The changes in the residual lignin structure reflect the reactions taking place during the selected pulping operations. With this knowledge, reliable structure-property relationships can be established, which in turn creates the basis for further improving the selectivity and efficiency of kraft pulping technology. However, this implies that the chemical structure of lignin distributed in all morphological parts of the cell wall can be characterized in a chemically unaltered form. This may only be accomplished by analytical methods applicable directly on wood or pulp, such as scanning UV microspectrophotometry and confocal Raman spectroscopy (Koch and Kleist 2001; Roeder et al. 2004). Both of these analytical tools provide general information on the topochemistry of delignification during cooking processes with a spatial resolution of about $0.25 \mu\text{m}^2$, without affecting the structure of cell wall components (Koch et al. 2003). The obtained UV and Raman spectra, however, cannot provide the information needed to follow small changes in the chemical structure of the lignin embedded in a polysaccharide matrix as a result of the applied reaction conditions during e.g. kraft pulping.

Currently, the only way to comprehensively characterize residual lignin consists of its isolation from the pulp matrix. Many research groups have been attempting to completely isolate lignin from wood or pulp in an unaltered form. However, this seems to be nearly impossible, because the lignin polymer is associated with the polysaccharide matrix by physical and possibly covalent bonds. This raises the question as to which of the available isolation methods yields a pure, representative, and least altered lignin. Currently, two categories of methods are in use for the isolation of residual lignin from kraft pulp: acidolysis methods (Gellerstedt et al. 1994) and procedures that remove lignin after ball milling (Björkman 1956; Chang et al. 1975). During acidolysis, lignin is extracted with acidic dioxane-water solutions under reflux, usually adjusting the acid concentration to 0.2 M. The resulting material is of a rather high purity (low amount of carbohydrate contamination) and can be recovered in a yield of about 40% based on the sum of Klason and acid soluble lignin (Jääskeläinen et al. 2003). However, acidolysis leads to a cleavage of alkyl-aryl ether linkages and therewith to the increase of the lignin phenolic content (Wu and Argyropoulos 2003).

Alternatively, methods using ball-milling as a pretreatment offer less modified lignin. The classical approach was developed by Björkman, who extracted lignin from ball milled wood with aqueous dioxane (Björkman 1956). The yield is, however, rather low, which qualifies this method for substrates with a high amount of residual lignin only. Ball milling followed by an enzymatic hydrolysis applying cellulolytic enzymes has been shown to be a reliable method for lignin isolation also from unbleached pulps (Chang et al. 1975; Ikeda et al. 2002). In contrast to acidolysis, which yields rather pure lignin, the enzymatically released lignins are contaminated with cellulases, and purification is required. The first step of reducing contaminations of protein, is to select the most

appropriate enzyme mixture to minimize its charge. This is particularly important for the isolation of hardwood-based lignins (Capanema et al. 2004). The final purification of crude lignin can be achieved by a combination of protease hydrolysis and solvent extractions (Ibarra et al. 2004; Ibarra et al. 2005). Argyropoulos et al. finally proposed an enzymatic hydrolysis to expose the fibre structure as a first step followed by mild acidolysis to cleave the remaining lignin-carbohydrate bonds (Argyropoulos et al. 2000; Argyropoulos et al. 2002). This method, enzymatic and mild acidolysis (EMAL), was further improved (Wu and Argyropoulos 2003) and extensively tested, mainly using *E. globulus* as a substrate (Guerra et al. 2007, 2008a,b) thereby showing that mild ball milling is sufficient to obtain high yields. It is claimed that the EMAL protocol offers access to lignin samples that are more representative of the whole lignin as compared to the classical MWL (Guerra et al. 2006b).

Fairly recently we presented a new method for the isolation of lignin in high yield based on the total dissolution of ball milled wood in dimethylsulfoxide and N-methylimidazole (DMSO/NMI) adopted from Lu and Ralph (Lu and Ralph 2003), followed by precipitation of the carbohydrate fraction in dioxane/water (Fasching et al. 2008). A direct comparison of this dissolved wood lignin (DWL) with MWL obtained by the classical Björkman method on beech wood (*Fagus sylvatica*) revealed advantages of the former in terms of higher yield and a higher amount of β -O-4 linkages, indicating a less altered lignin structure. In the next step we have investigated the application of the DWL method to unbleached pulp, since using the same lignin isolation method for both wood and pulp would provide some advantages. To investigate the applicability of the DWL method also for pulps, we used it to study the alterations of the chemical lignin structure during the CBC cooking process of *E. globulus*.

The wood species was selected because eucalypt pulps increasingly dominate the world's hardwood pulp production (Patt et al. 2006), and among the eucalypt species, *E. globulus* has gained a high acceptance as a raw material for the production of kraft pulps due to their superior properties. Although the chemical composition among *E. globulus* woods of different origins and growth conditions may vary significantly, most of them are known to ensure high pulp yields upon kraft pulping ($\geq 50\%$) while applying a low H-factor (≤ 600) and a low alkali charge ($< 20\%$ NaOH/od wood) (del Rio et al. 2005). The high syringyl-to-guaiacyl ratio (S/G), 4-6, as determined by pyrolysis GC/MS (del Rio et al. 2005), by quantitative ^{13}C -NMR (Pinto et al. 2005), and by DFRC/ ^{13}P NMR (Guerra et al. 2006a), as well as the high abundance of arylglycerol- β -aryl ethers of which molar ratios of 0.56 per one aromatic ring (Ar) and 0.59/Ar were reported by Guerra et al. (2008a) and by Evtuguin et al. (2001), respectively, allow kraft pulping with low chemical charges and low H-factors.

Only recently, this interesting wood species has attracted some researchers to initiate systematic studies on the behavior of *E. globulus* lignin during kraft pulping. Pinto et al. (2002a,b) revealed a gradual decrease of phenylcoumaran and pino-/syringaresinol lignin units while they detected a significant decrease of the β -O-4 structures in residual lignin in the final cooking phase only after the impregnation phase, where guaiacyl-rich lignin was preferably removed. Lignin enriched in syringyl units (S) was continuously removed, thus leading to decrease in the S/G ratio of the residual lignin (Pinto et al. 2002a). Additional information on the behavior of the wood polymers during

kraft pulping can be obtained by comprehensive kinetic studies. Unfortunately, the results on the kinetics of *E. globulus* are still rather conflicting, depending on the origin (del Rio et al. 2005), the experimental set-up, and the models applied. Santos et al. (1997) reported rather low kinetic orders for $[\text{OH}^-]$ and $[\text{HS}^-]$ of about 0.15 during bulk delignification, whereas we found orders of 0.6 and 0.3, respectively (Sixta and Rutkowska 2007). However, we also investigated the influence of $[\text{Na}^+]$ on the kinetics, which resulted in quite high negative orders of -0.63 for the bulk and -0.68 for residual delignifications. Thus, the presence of a high sodium cation concentration (≥ 1.5 M) significantly impedes delignification. Since $[\text{Na}^+]$ promotes cellulose degradation with an order of 0.7 (Sixta and Rutkowska 2007) due to attracting hydroxide ions inside the fibre wall following the Donnan equilibrium (Motomura et al. 1998), an increase in $[\text{Na}^+]$ should have a severe impact on the selectivity of delignification. Defining the selectivity as the ratio of the rates of delignification and cellulose degradation, $S = \frac{dK/d(1/DP_{n,t} - 1/DP_{n,0})}{dK/d(1/DP_{n,t} - 1/DP_{n,0})}$, kinetic orders of -0.5 for $[\text{OH}^-]$, 0.3 for $[\text{HS}^-]$ and -1.3 for $[\text{Na}^+]$ for the bulk as well as 0.4 for $[\text{OH}^-]$ and -1.4 for $[\text{Na}^+]$ for the residual delignification may be calculated (Sixta and Rutkowska 2007), indicating that pulping selectivity could be influenced by adjusting the concentration of the cooking chemicals appropriately. Very recently, Santiago et al. (2008) have investigated the impact of effective alkali and sulfide ion profiling on the selectivity of *E. globulus* kraft pulping. Surprisingly, they could not identify any advantage in evening out $[\text{OH}^-]$ and/or $[\text{HS}^-]$ during cooking on the yield-kappa number and viscosity-kappa number relationships, provided that the total chemical charges remained unaffected (Santiago and Pascoal Neto 2007). They concluded that the particular chemical structure of the components of *E. globulus* wood allows kraft pulping to rather low kappa numbers (about 13) under very mild conditions, which reduces the opportunities for improvements through the application of modern pulping technology.

The aim of the present work was to investigate the changes of the chemical structure of lignin during CBC cooking of *E. globulus* wood. In addition, this study applies the recently developed protocol to isolate DWL in comparison to a modified acidolysis method followed after a mild alkaline pre-extraction as proposed by Evtuguin et al. (2001). The isolated lignin samples were analyzed by quantitative 1D NMR, 2D NMR spectroscopy, and Fourier transform infrared (FTIR) spectroscopy to identify those structural modifications as occurred in the course of the CBC cooking process.

EXPERIMENTAL

Materials

Wood chips

Eucalyptus globulus wood chips from plantations in Uruguay, supplied by ENCE, were used for kraft pulping experiments after a laboratory screening according to standard method SCAN CM 40:94. The average characteristics of the screened wood chips were as follows: 22.7% Klason lignin (KL) (TAPPI test method T 222 om-98), 4.7% acid soluble lignin (TAPPI test method T om-250), 42.4% cellulose (C), 2.2% galactoglucomannan (GGM), 17% xylan assuming that 60% of the linkages between 4-*O*-methylglucuronic acid (MGA) and the xylan backbone survive the primary and

secondary hydrolysis step (Genco and Busayasakul 1990), 0.4% arabinan, 0.1% rhamnan, 4.8% total uronic acids (2.4% MGA, 1.9% galacturonic acid and 0.5% glucuronic acid), 0.6% acetone extractives, and 0.4% ash (methods T 211 om-93; ISO 14453:1997). The moisture content was 31.6%.

Chemicals

Fine chemicals were purchased from Sigma-Aldrich or Merck (dioxane was ordered without stabilizer) and used without further purification. Peroxide content of dioxane was tested prior to use. Deuterated solvents were purchased from Core-Tecnet. Fully deionized water was used for all experiments.

Cooking Experiments

The pilot plant trials were carried out in a 10-L digester with forced liquor circulation according to the CBC concept (Sixta et al. 2006). After about thirty minutes of impregnation, the cooking liquor is pumped through the digester, displacing the impregnation liquor back to the impregnation tank. As soon as the entire amount of impregnation liquor is discharged, the liquor outlet is transferred in the cooking liquor system. The circulation of the cooking liquor through the digester and the cooking liquor tank continues until the preset H-factor is reached. According to the CBC process principle, all the process-related liquors, such as the impregnation and cooking liquors are prepared already in the tank farm using different tank-to-tank circulation loops. Subsequent to cooking, the wash filtrate displaces hot black liquor. The cooking conditions are summarized in Table 1.

Table 1. Cooking Conditions

| Sample | Label | Impregnation conditions | | | | | Cooking conditions | | | | |
|--------|---------|-------------------------|--------------------------|--------------------------|------------|-------------|--------------------------|--------------------------|--------------------------|------------|---------------|
| | | L/S L/kg | OH ⁻ mol/L | HS ⁻ mol/L | Temp °C | time min | Na ⁺ mol/L | OH ⁻ mol/L | HS ⁻ mol/L | Temp °C | H-factor h |
| K109 | Precook | 10 | 0.34 | 0.13 | 100 | 60 | | | | | |
| K48 | CBC266 | 10 | 0.34 | 0.14 | 101 | 60 | 1.5 | 0.49 | 0.22 | 160 | 142 |
| K34 | CBC208 | 4 | 0.35 | 0.38 | 106 | 58 | 1.5 | 0.53 | 0.62 | 161 | 169 |
| K18 | CBC256 | 10 | 0.36 | 0.15 | 101 | 60 | 1.5 | 0.49 | 0.22 | 153 | 182 |
| K10 | CBC267 | 10 | 0.34 | 0.13 | 100 | 60 | 1.5 | 0.49 | 0.24 | 160 | 325 |

The pulping experiments were selected as representatives of the initial (kappa no. 109 (K109)), bulk (K48, K34) and residual delignification phases (K18, K10). With the exception of K34, the different delignification phases were obtained by adjusting the H-factor, while all other reaction conditions were largely kept constant. K34 was characterized by a lower liquor-to-wood ratio and higher sulfidity, as depicted in Table 1.

Lignin Isolation

Wood preparation

Wood samples were dried under vacuum at 50°C for 48 h and milled with a cutting mill to pass a 40-mesh sieve. The milled wood was extracted with acetone/water (9:1) in a Soxhlet apparatus for 24 h. The extractive free wood was dried under vacuum for several days until it could be utilized for further lignin preparation methods.

Dissolved wood lignin (DWL)

The lignin from wood and pulps was isolated according to a novel DWL method presented recently. A detailed protocol on the DWL isolation can be found elsewhere (Fasching et al. 2008).

1 g of wood prepared as described above was subjected to planetary ball milling (Retsch PM2) at 500 rpm for 48 h under nitrogen. 20 mL of DMSO was placed in a round bottomed flask where 10 mL of NMI was added. The mixture was stirred at room temperature for 3 h. The dark but clear solution was transferred to a separating funnel using a minimum amount of additional DMSO/NMI (2:1). The solution was transferred to a flask containing dioxane/water (9:1) in a twenty-fold excess of its total amount by means of a peristaltic pump to precipitate the carbohydrates. The dispersion formed in the flask was centrifuged for 10 min at 5000 rpm. After phase separation and washing with dioxane/water (9:1), the lignin-containing solutions were combined and gradually concentrated under vacuum at 65 °C. The solid residue was re-dissolved in approximately 10 mL of acetic acid (75%), centrifuged, and the supernatant was filtered over a G4 glass sinter plate. The clear solution was slowly introduced into a large excess (twelve times) of deionized water. The formed dispersion was cooled at 4 °C overnight, frozen, thawed, and centrifuged at 5000 rpm. The separated supernatant was re-dispersed in water, centrifuged two times, and finally filtered over a 0.45- μ m polyamide filter. The solid precipitates and the residue on the filter were combined and dried under vacuum. In the next step the lignin sample was again extracted by means of accelerated solvent extraction (ASE) with dichloromethane (DCM) and subsequently with acetone. Paramagnetic impurities introduced by ball milling have to be removed because they significantly deteriorate the subsequent NMR analysis. The removal was achieved by the addition of Amberlite™ IRC748, an iminodiacetate acid chelating cation exchange resin. The detailed protocol can be found elsewhere (Fasching et al. 2008).

Modified acidolysis lignin (AL)

For comparative reasons, the lignins were also isolated by a modified acidolysis comprising a mild alkaline extraction prior to wood extraction with dioxane/water solution (Evtuguin et al. 2001). The mild alkaline extraction was suggested as a pre-treatment particularly for *E. globulus* to remove the condensed tannins of catechin type which could not be removed even after successive extraction with toluene/ethanol. The dioxane lignin which can be recovered in a high yield and purity was still considered to be representative even though it was reported that about 10% of the initially present and guaiacyl-enriched lignin was eliminated in the alkaline extraction step. The isolation protocol followed exactly the protocol published by Evtuguin et al. (2001).

Methods

Carbohydrate analysis

The neutral sugar monomers were determined by anion exchange chromatography (AEC) with pulsed amperometric (PAD) detection after a total hydrolysis with H₂SO₄ according to (Sixta et al. 2001), while the uronic acids were quantified by methanolysis (Vuorinen and Alen 1999).

The methoxyl group content was quantified by the method of Vieböck-Schwappach (1930). The elemental analysis was carried out by Fraunhofer Institut Angewandte Polymerforschung, Golm, Germany. Klason lignin and acid soluble lignin of the wood were determined according to TAPPI methods (TAPPI 1991, 1998).

NMR analysis

Prior to NMR measurements all lignin samples were acetylated following the protocol described by Lundquist (1992). All NMR spectra were recorded in CDCl₃ on a Bruker Avance DPX 300MHz spectrometer using a 5mm BBO inverse probe equipped with z-gradients, at 300K. Chemical shifts were referenced to solvent signals at $\delta(^{13}\text{C})=77\text{ppm}$ and $\delta(^1\text{H})=7.26\text{ppm}$. All quantitative 1D ¹³C NMR experiments were performed at sample concentrations of 200mg.ml⁻¹. Chromium(III)acetylacetonate (0.01M) was added as a relaxation reagent. ¹³C T₁ relaxation times were checked with the inversion recovery technique for each sample. To ensure quantitative conditions, inverse-gated ¹H decoupled ¹³C NMR spectra were recorded with 5s relaxation delay, 0.4s acquisition time, and 245ppm spectral width. For each spectrum 40000 to 60000 transients were accumulated, resulting in an experiment time of 2.5 to 4 days. Quantitative ¹H NMR experiments were acquired with 7s relaxation delay, 4.2s acquisition time, and 26ppm spectral width at sample concentrations of 100mg.ml⁻¹. Processing quantitative 1D spectra involved exponential window multiplication (lb=0.3Hz for ¹H and lb=5Hz for ¹³C) of the free induction decay (FID) prior to Fourier transform. Qualitative 2D ¹H/¹³C HSQC NMR spectra were recorded at sample concentrations of 100mg.ml⁻¹. Each spectral dataset comprised 2048 data points in the ¹H-dimension and 500 time domain increments in the ¹³C dimension. 290 scans were accumulated per transient with 1s relaxation delay and 0.13s acquisition time at 26ppm (¹H) and 165ppm (¹³C) spectral width, resulting in 2.75 days total experiment time. 2D time domain data were multiplied with shifted square sine bell (SSB=2; ¹³C dimension) functions in both dimensions and zero-filled to 2048 times 2048 real data points prior to Fourier transform. All 1D and 2D NMR data were processed and analyzed using the Bruker software TopSpin 2.1.

FT-IR analysis

The FT-IR spectra were recorded on a Bruker Tensor 27 spectrometer using the Golden Gate ATR. The lignins spectra were recorded between 700 and 4000 cm⁻¹ with the spectral resolution of 2 cm⁻¹ and were baseline corrected.

RESULTS AND DISCUSSION

Cooking Results

Five cooks were selected as representatives of the three pulping stages: initial, bulk, and residual delignification. The former coincides very well with the impregnation stage prior to CBC cooking as demonstrated by the lignin removal rate of 23%, which is in excellent agreement with the values 23% and 24% obtained by Wilder and Daleski (1965) and Kondo and Sarkanen (1984), respectively. At the same time about 8% of the initial xylan was removed, presumably mostly through dissolution (Table 2). Bulk delignification was represented by two cooks, denoted as K48 and K34, of which the latter was produced with a significantly higher $[HS^-]/[OH^-]$ -ratio in both impregnation and cooking and a lower liquor-to-wood ratio during impregnation. Obviously, the better delignification selectivity of the K34-cook may be explained by the higher sulfidity of the impregnation and cooking liquors (Table 2). Two further cooks, denoted as K18 and K10, were made by simply prolonging the H-factor to provide pulps comprising lignin structures that were characteristic of the transition from bulk to residual delignification. Table 1 demonstrates that the range of cooking intensity, expressed as H-factor, necessary to obtain low kappa number is extremely low. In comparison to other hardwood species, such as birch or poplar, only about half of the H-factor is required for *E. globulus* kraft cooking to achieve the same extent of delignification (Pinto et al. 2005; Sixta 2007). This has been assigned to the particular lignin structures comprising the high S/G ratio and the low proportion of noncondensed aromatic units (Pinto et al. 2004; Pinto et al. 2005).

Table 2. Characterization of Substrates Utilized for Lignin Isolation

| Sample | Label | pre-treatment | Yield | | Klason Lignin % odw | AS Lignin % odw | Kappa K % odw | Xylan X % odw | S* dK/dX |
|--------|---------|---------------|----------------|--------------|------------------------|--------------------|---------------------|---------------------|-------------|
| | | | unscr % odw | scr % odw | | | | | |
| K141 | Wood | | 100.0 | | 22.7 | 4.7 | 141 | 15.8 | 0.0 |
| K109 | Precook | unscreened | 90.2 | | 19.5 | 4.3 | 109 | 14.6 | 2.9 |
| K48 | CBC266 | unscreened | 65.0 | | 5.9 | 3.4 | 48 | 10.4 | 1.9 |
| K34 | CBC208 | unscreened | 60.6 | 52.9 | 3.0 | 1.2 | 34 | 11.0 | 2.5 |
| K18 | CBC256 | screened | 54.3 | 51.3 | 1.4 | 0.8 | 18 | 9.1 | 2.1 |
| K10 | CBC267 | screened | 50.1 | 49.4 | 0.6 | 0.6 | 10 | 8.7 | 2.1 |

*S = selectivity of delignification = $dK/dX = (1-K/K_0)/(1-X/X_0)$

The extent of xylan re-precipitation onto the cellulose surface can be controlled by the hydroxide ion concentration in the cooking liquor, in particular during the final cooking stage. The relatively high and constant $[OH^-]$ throughout the entire cook of about 0.5 M can be seen as the reason for the continuous decrease in xylan content with the increasing H-factor.

Lignin Yield and Chemical Composition

The yields of DWL and AL isolated from wood and pulps are shown in Table 3. In agreement with the literature (Evtuguin et al. 2001), the yields of AL were found to be consistently at about 60% based on the total lignin content, regardless of the kappa number of the pulp, while the level of sugar and ash impurities slightly increased from very low levels. The yields of DWL were lower, but at least between 40 and 50%, with the exception of the sample isolated from wood. It is not likely that this can be attributed to the type of substrate, since DWL could be isolated from beech wood in yields between 41 and 52% based on Klason lignin (Fasching et al. 2008). It is interesting to note that the DWL samples isolated from pulps showed very low amounts of carbohydrate impurities. The elemental composition of AL and DWL differ mainly in two aspects:

- First, it can be noticed that the methoxy group content of DWL passed a maximum after the initial delignification (K109), while no maximum, but rather a continuous decrease with progressive delignification was observed for the AL samples. This was confirmed by quantitative ^{13}C NMR spectroscopy, as will be shown later. The maximum in S/G ratio after the initial delignification was also found by Pinto et al. (2002a), however, on AL samples. The results were explained by a preferential dissolution of lignin enriched in guaiacylpropane (G) type units.
- Secondly, the AL samples generally contained less hydrogen and oxygen as compared to the DWL samples on the basis of a C₉ formula, indicating a loss of hydroxyl groups in the aliphatic side chain (Table 3). This is in agreement with model compound studies of Li and Lundquist (2000), who reported that moderately acidic conditions, as prevailing during acidolysis, initiated the loss of aliphatic OH groups through a homolytic cleavage of β -ether linkages.

Table 3. Yield, Amount of Impurities, Elemental Composition and Methoxy Group Content of the Isolated DWL and AL Fractions

| Fraction | Sample | Yield * | Impurities | | Elemental analysis | | | | | Methoxy group content % od | Empirical C ₉₀₀ formula** | | | | |
|----------|--------|------------|--------------------------|------------------------|--------------------|-----------|-----------|-----------|-----------|-------------------------------------|--------------------------------------|-------------|-----|---------------------|--|
| | | | Sugar content % od | Ash content % od | C % od | H % od | N % od | O % od | S % od | | [H] | [O] | [S] | [OCH ₃] | |
| | | | | | | | | | | | | molar ratio | | | |
| DWL | K141 | 28 | 7.1 | 3.8 | 57.7 | 6.1 | 0.5 | 35.7 | 0.0 | 20.8 | 877 | 339 | 0 | 145 | |
| | K109 | 51 | 0.9 | 2.3 | 59.2 | 5.9 | 0.5 | 35.0 | 0.4 | 22.5 | 804 | 313 | 2 | 155 | |
| | K48 | 47 | 0.7 | 4.4 | 59.3 | 6.0 | 0.9 | 32.9 | 0.9 | 18.2 | 878 | 304 | 6 | 121 | |
| | K18 | 40 | 1.7 | 5.6 | 58.8 | 6.0 | 1.2 | 32.9 | 1.1 | 21.2 | 838 | 293 | 8 | 146 | |
| AL | K141 | 64 | 0.5 | 3.4 | 60.7 | 6.0 | 0.1 | 33.2 | 0.0 | 23.6 | 782 | 275 | 0 | 159 | |
| | K109 | 69 | 0.7 | 2.6 | 60.9 | 6.0 | 0.1 | 32.5 | 0.4 | 21.7 | 806 | 274 | 2 | 144 | |
| | K48 | 54 | 0.7 | 3.4 | 61.5 | 5.7 | 0.0 | 32.0 | 0.7 | 21.1 | 747 | 268 | 4 | 138 | |
| | K34 | 67 | 3.4 | 4.1 | 61.9 | 5.9 | 0.1 | 30.9 | 1.2 | 20.2 | 782 | 256 | 8 | 130 | |
| | K10 | 65 | 4.3 | 7.2 | 63.0 | 5.9 | 0.0 | 30.0 | 1.0 | 19.7 | 778 | 241 | 6 | 124 | |

*based on sum of Klason and acid soluble lignin

**values are corrected for the sugar and ash impurities

FTIR Characterization

The FTIR spectra of samples from both preparation methods were quite similar. The major discernible differences were the clearly higher intensities of the carbonyl band at 1720 cm^{-1} and the band at 1029 cm^{-1} for the DWL sample. The former indicates a partial acetylation of DWL during the purification step involving 75% acetic acid (Fasching et al. 2008), and the latter can be attributed to both a higher amount of residual carbohydrate structures, at least for the K141 sample, and a higher amount of primary alcohols in the lignin side chain, respectively. This will be confirmed by the NMR studies, as we will see later.

Quantitative evaluations of selected bands from baseline-corrected normalized spectra are shown in Fig 1. They reveal that the relative intensities of the bands at 1120 and 835 to that of the band at 1503 cm^{-1} decreased with progressive delignification, with the intensity of the DWL samples remaining on a slightly higher level as compared to the AL samples. At the same time the ratio of the $1720/1503$ bands signaled a rising tendency with decreasing kappa number, suggesting an increase in carbonyl group content, which may originate from esters (acetate in case of DWL), aldehydes, or carbonyl groups conjugated or unconjugated with the aromatic ring (Lin and Dence 1992). The substantial increase of the 1720 band for both lignin samples, particularly below kappa number 50, is a strong indication that the increase in carbonyl group content in the residual lignin samples is not an artifact but caused by oxidation reactions during alkaline pulping.

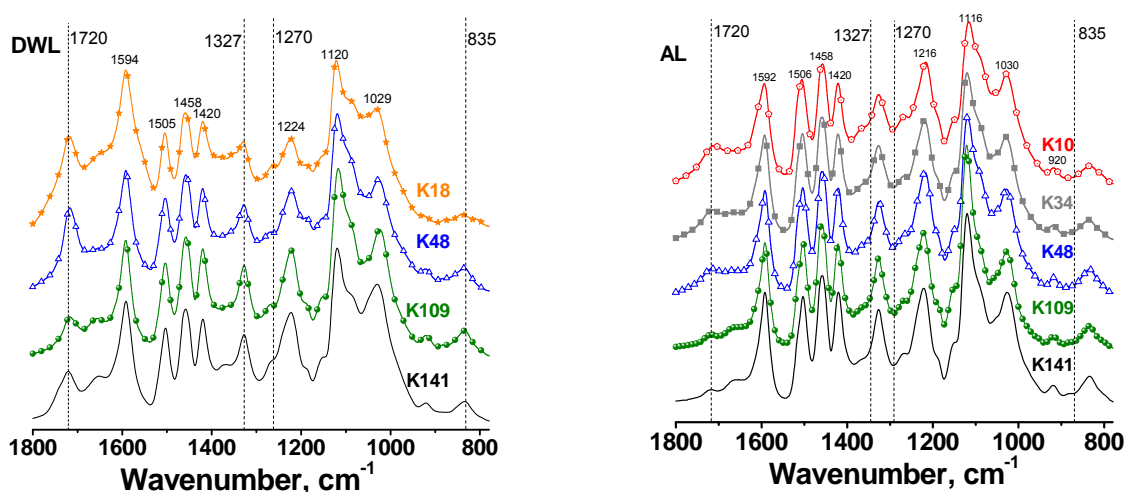


Fig. 1. FTIR spectra, $1800\text{-}800\text{ cm}^{-1}$ region, of DWL and AL samples isolated from *E. globulus* wood and corresponding pulps during all stages of CBC cooking. Assignment of the labeled peaks: 1720 cm^{-1} : stretching C=O in esters, carboxylic acids and carbonyls conjugated or unconjugated with the aromatic ring; 1327 cm^{-1} : aromatic ring breathing (S and condensed G); 1270 cm^{-1} : aromatic ring breathing (G), and C=O stretch; 835 cm^{-1} : C-H bending out-of-plane in positions 2 and 6 of S (Lin and Dence 1992).

The FTIR spectra of residual lignins from both sample preparations are characterized by higher intensity of signals assigned to S-type units (1327 cm^{-1} band) than to G-type units (1270 cm^{-1} shoulder). Part 1 of Fig. 2 shows that the ratio of the

1327/1270 bands gradually decreases over cooking intensity, indicating a decrease in the S/G ratio. In agreement with the elemental composition of the DWL samples displayed in Table 3, the ratio of the 1327/1270 bands passes a maximum after impregnation, while this cannot be observed for the AL sample. Since the spectra were normalized to the band at 1505 cm^{-1} , representing the aromatic units, this behavior cannot be explained by the higher sugar content of the DWL sample isolated from the initial wood as compared to that derived from the wood after the impregnation step. Again, the maximum of the ratio of the 1327/1270 bands may be explained by the preferential dissolution of lignin enriched in G type units (Pinto et al. 2002a).

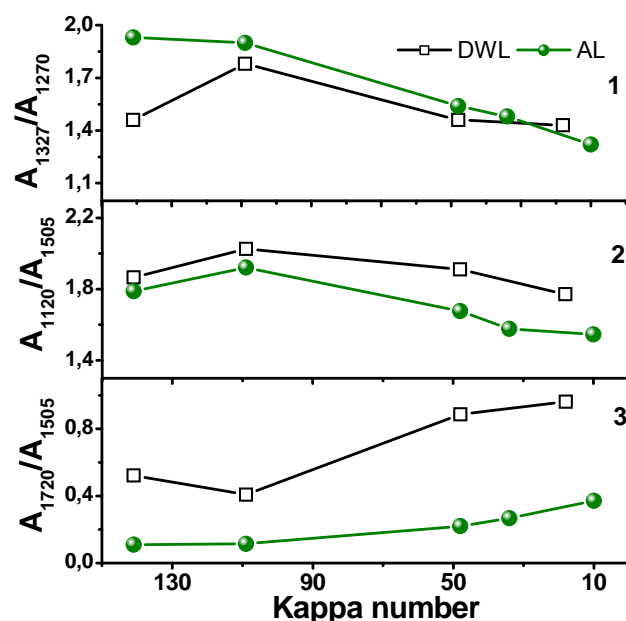


Fig. 2. Relationship between selected band ratios of the normalized spectra measured on both AL and DWL samples and the Kappa number of the pulps of which the lignin was isolated. 1: ratio of the 1327/1270 bands related to the S/G ratio; 2: ratio of the 1120/1505 bands typical for the aromatic C-H in-plane deformation in S units including the amount of secondary alcohols; 3: ratio of the 1720/1505 bands characterizing the specific amount of carbonyl groups in esters, carbonic acids, and carbonyls conjugated or not conjugated with aromatic units

NMR Studies

The changes of the most important lignin structures along the CBC cooking process were monitored by both ^{13}C and ^1H -NMR spectroscopies. The assignment of the resonances was based on 2D NMR spectroscopy (HSQC), using the vast lignin NMR databases (Ämmälähti et al. 1998; Fasching et al. 2008; Kilpeläinen et al. 1994). The lignin structures identified by HSQC spectra in samples originating from *E. globulus* and the corresponding kraft pulps are shown in Fig. 4, and their assignments in Table 4. An overlay of the HSQC spectra of both lignin preparations, DWL and AL, isolated from the native wood is shown in Fig. 3. Both lignin preparations show a few additional resonances: Stilbene structures, characterized by the resonance at 6.93/128.5 ppm (**D**),

were identified only in AL preparations. They occurred in all AL samples along the cooking process, while no signals were detected for the DWL samples. The formation of stilbene may indicate the homolytic cleavage of β -O-4 bonds as initiated by weak acid treatment (Li and Lundquist 2000). Weak signals at 5.1+4.8/93 ppm were detectable only in AL preparations as well. They indicate the presence of a $-\text{O}-\text{CH}_2-\text{O}-$ group in a 1,3,5-trioxane structure, since the methylene group is not associated with other groups, as predicted by HMBC and COSY spectra. By contrast, the HSQC spectrum of DWL is characterized by rather intensive carbohydrate peaks as evidenced by **E1**, **E2**, **E3** and **E5** resonances (Fig. 3). As predicted from carbohydrate analysis (Table 3), these resonances disappear in the DWL samples with progressive delignification. The resonance at 7.46/105.9 ppm was only visible in DWL preparation. It originates from an oxidized syringyl unit bearing a carbonyl group at C_α (Rencoret et al. 2008).

The relative ease of kraft pulping was shown to be primarily determined by the ratio of syringyl-to-guaiacyl (S/G) units and the proportion of noncondensed aromatic units (del Rio et al. 2005). In addition, the content of β -O-4 structures characterizes the reactivity of residual lignin towards delignification though no clear relationship could be established between pulping efficiency and the amount of β -O-4 units in a recent study (Pinto et al. 2005).

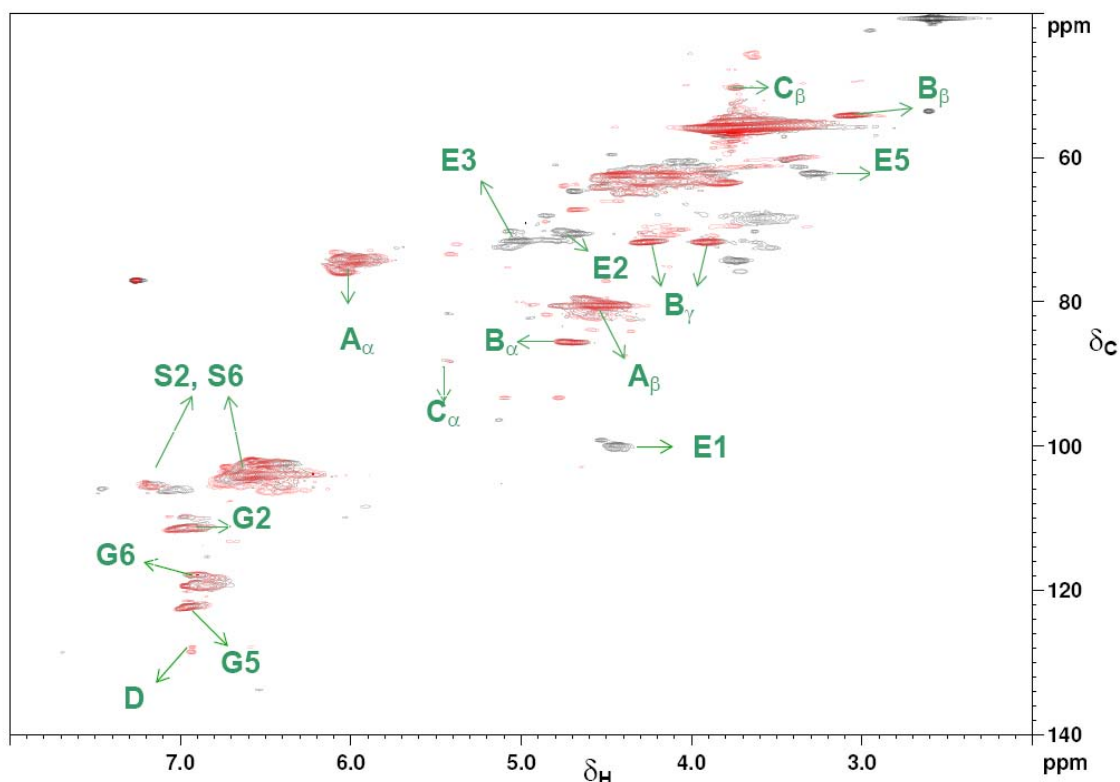


Fig. 3. Overlay of the HSQC spectra of DWL (black) and AL (red) samples isolated from *E. globulus*

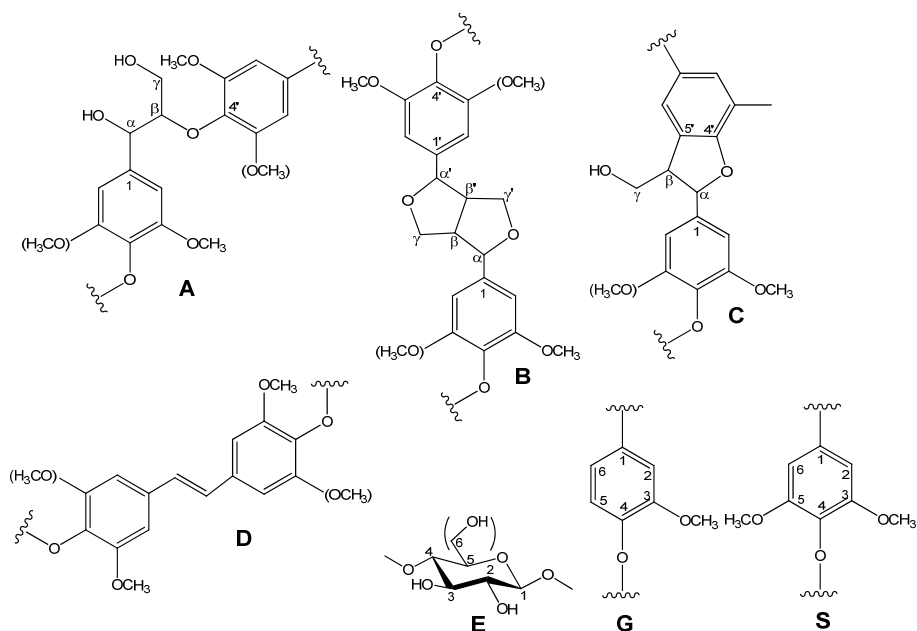


Fig. 4. Lignin structures identified by HSQC spectra in samples isolated from *E. globulus* and the corresponding kraft pulps

Table 4. Assignments of HSQC Data

| Assignment | | Chemical shift, ppm | |
|---------------|----------------------|---------------------|----------------|
| | | ¹³ C | ¹ H |
| β-O-4/α | A_α | 73.9 - 75.7 | 6.0 - 6.1 |
| β-O-4/β | A_β | 80.7 | 4.6 |
| β-β/α | B_α | 85.5 | 4.7 |
| β-β/β | B_β | 54.1 | 3.0 |
| β-β/γ | B_γ | 71.7 | 3.9, 4.3 |
| β-5/α | C_α | 87.6 | 5.4 |
| β-5/β | C_β | 50.2 | 3.8 |
| Stilbene | D | 128.5 | 6.9 |
| Syringyl | S2, S6 | 103.9 | 6.6 |
| Syringyl | S2, S6 | 105.8 | 7.1 |
| Guaiacyl | G2 | 110.9 | 6.9 |
| Guaiacyl | G6 | 119.3 | 6.9 |
| Guaiacyl | G5 | 122.3 | 6.9 |
| Carbohydrates | E1 | 99.6 | 4.4 |
| Carbohydrates | E2 | 70.5 | 4.7 |
| Carbohydrates | E3 | 71.5 | 5.0 |
| Carbohydrates | E5 | 62.2 | 3.3, 3.9 |

As demonstrated in Fig. 6 and Table 5, the S/G ratio calculated from the ^{13}C resonances, $(\delta_{\text{C}} \text{ S-2,6/2})/[(\delta_{\text{C}} \text{ G-2,5,6})/3]$, increased during the initial pulping stage, which is in agreement with the elemental analysis of the DWL lignin sample and also with the results reported by Pinto et al. (2004, 2005). They suggested that lignin that is rich in G units is predominantly dissolved during the impregnation. In accordance with the ^1H NMR spectroscopy (not shown), the increase in the S/G ratio was less pronounced for the AL sample. In the subsequent bulk and residual phase delignification, the S/G ratio decreased continuously. This was observed concurrently for ^{13}C and ^1H NMR spectroscopy and both types of lignin preparations, DWL and AL. The results clearly confirm that the residual lignin enriches in G-type lignin units due to the preferential degradation of the S-type lignin units.

The specific amount of bonds between an aromatic carbon and an aliphatic carbon, ArC-C, per aromatic unit (Ar) relates to both the S/G ratio and to the degree of condensation. Interestingly, no changes in the amount of ArC-C bonds, up to approximately 75% degree of delignification, were observed. The subsequent pronounced increase in the amount of ArC-C bonds may originate from the decrease in the S/G ratio or from condensation reactions. The principal pattern of the frequency of ArC-C bonds throughout pulping was similar for both lignin preparations. The calculated amount of the ArC-C bonds, however, was significantly higher for the AL than for the DWL, indicating a higher degree of condensation of the former. Following the results of the DWL preparation it can be concluded that the content of condensed units in the residual lignin of CBC pulps is quite low indicating that modified kraft cooking processes provide advantages in delignification efficiency as compared to their conventional counterparts (Jiang and Argyropoulos 1999).

It is well accepted that the major delignification pathway during kraft pulping is characterized by the cleavage of the β -O-4 linkages. The question arises as to whether the decrease in β -O-4 linkages (per Ar) is reflected in both the dissolved and the residual lignins or only in the former. The quantification of β -O-4 bonds in ^{13}C NMR is critical because of the close solvent signal, but a relative comparison of the integrals should reveal the tendencies. The results of ^{13}C NMR on the AL indicate a decrease in the frequency of β -O-4 bonds, while only a moderate decrease was observed for the DWL preparation in the later stages of pulping. The ^1H NMR spectra revealed an even more pronounced difference between the two lignin preparations. The content of β -O-4 structures in the DWL samples seems less affected by the extent of delignification, as exemplified by Fig 5. The same trend, but mirror-inverted, was obtained for the content in free phenolic hydroxyl groups; a continuous increase for the AL sample and an almost unchanged level for the DWL sample indicate the most pronounced differences between the two lignin preparations. In addition, the frequency of β -O-4 linkages is significantly higher and, consequently, the content of free phenolic hydroxyl groups is lower in the DWL sample as compared to the AL sample. This may be an indication for a more severe impact of the acidolysis on lignin structure during the AL preparation than for the DWL preparation protocol. The higher frequency of β -O-4 linkages and hence the lower amount of phenolic hydroxyl groups in the DWL as compared to the AL was confirmed

by 2D NMR (HSQC). The high and almost unchanged content of β -O-4 units in the residual lignin of CBC pulps may be an explanation of the enhanced delignification efficiency and selectivity of this pulping technology over conventional kraft cooking provided that this result can be confirmed in future investigations (Sixta et al. 2006).

The specific amount of β - β structural elements showed no significant changes during CBC kraft pulping. A slight decrease can be anticipated on the basis of ^{13}C NMR (Fig. 6). However, the mode of lignin preparation does not reveal any major difference in this respect.

The content of primary and secondary hydroxyl groups continuously decreased in the course of pulping, as indicated by ^{13}C NMR spectroscopy of both DWL and AL samples. This can be explained by the cleavage of formaldehyde from the γ -C and the fragmentation and progressive dissolution of the aliphatic side chain (Gierer 1985). Pinto et al. (2002a) reported on an initial increase in aliphatic hydroxyl groups and a rather moderate decrease in the subsequent cooking phase using ^1H NMR spectroscopy. The lower content of aliphatic hydroxyl groups in AL compared to DWL in all preparations (Fig. 7) may again refer to a more severe impact during the preparation of the former. Li and Lundquist have conclusively shown that slightly acidic hydrolysis, as occurred during the AL preparation, initiated the loss of aliphatic hydroxyl groups through homolytic cleavage of the C β -O- bond (Li and Lundquist 2000).

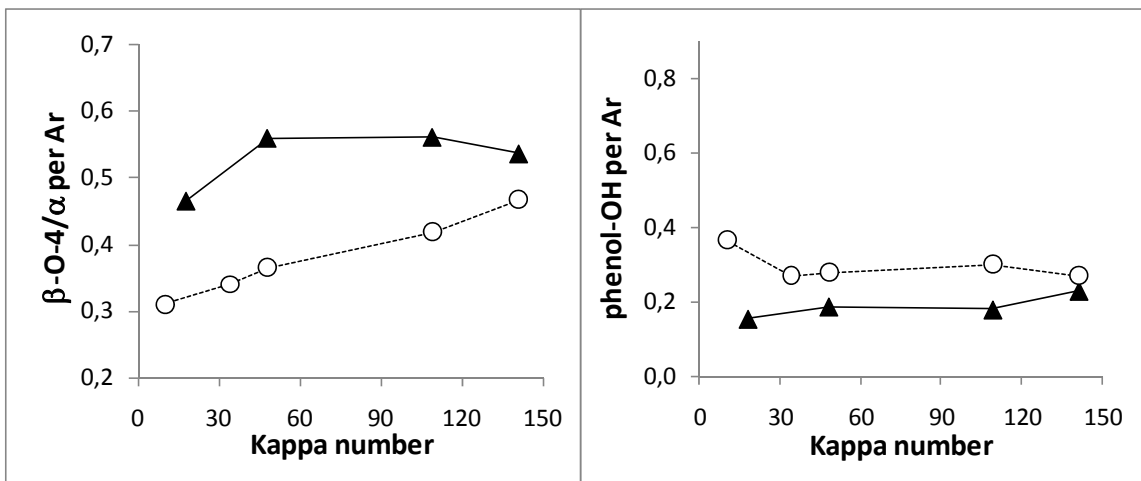


Fig. 5. Changes of β -O-4/ α structures (signals integrated between 6.18 and 5.76 ppm) and phenolic hydroxyl groups (signals integrated between 2.40 and 2.20 ppm) along with CBC kraft cooking of *E. globulus*, monitored by quantitative ^1H NMR of DWL (\blacktriangle) and AL (\circ) samples. The integrals of the respective structures were referenced to the integral area corresponding to the acetyl $-\text{CH}_3$, 2.40 – 1.60 ppm) The amount of Acetyl groups per aromatic unit (171.0 – 167.5 ppm) was calculated from the ^{13}C spectra normalized to the aromatic carbons.

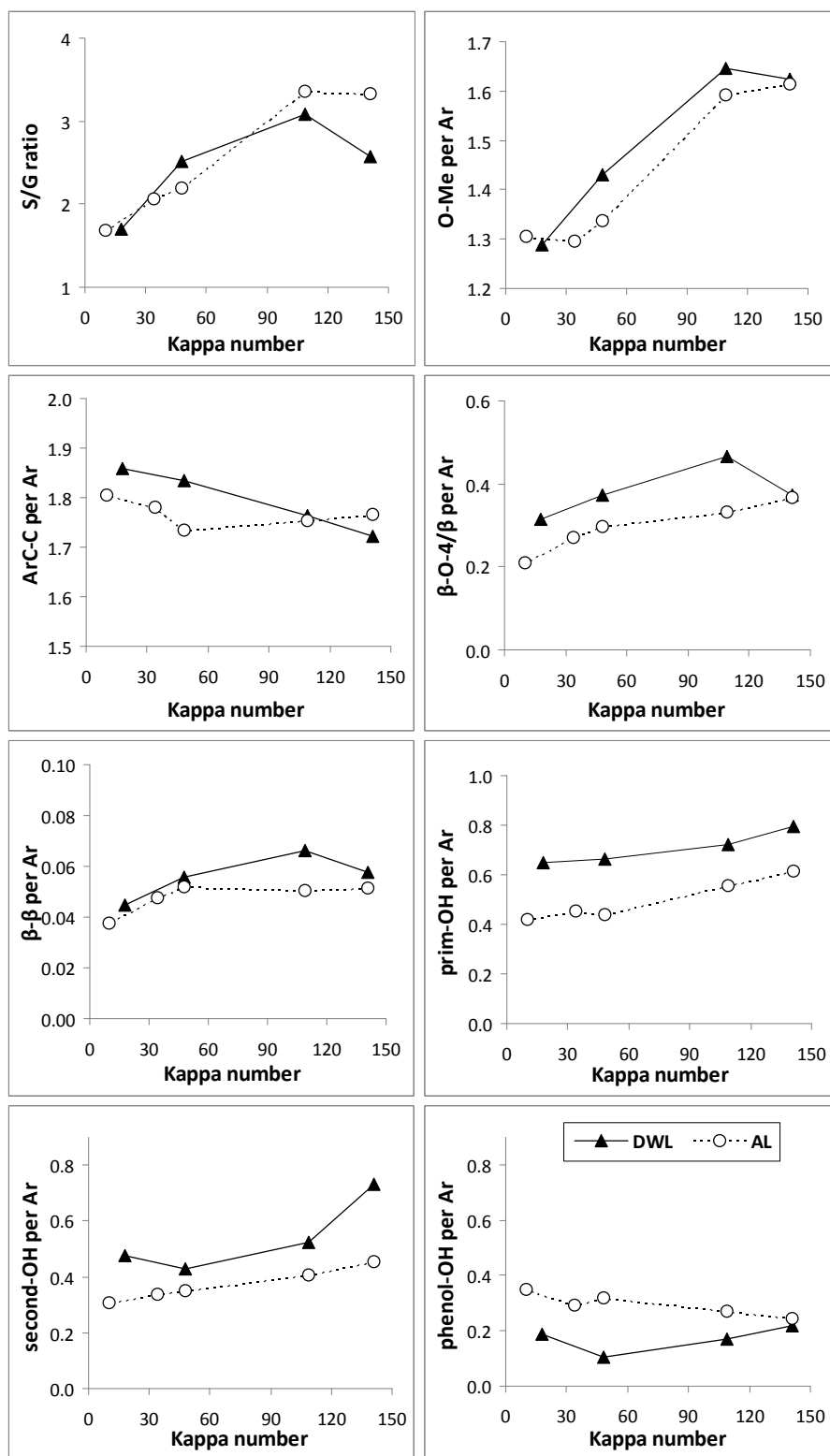


Fig. 6. Changes of important lignin structures along with CBC kraft cooking of *E. globulus*, monitored by quantitative ^{13}C NMR of DWL (▲) and AL (○) samples. The integral referring to six aromatic carbons at 162.0-101.5 ppm was set as the reference.

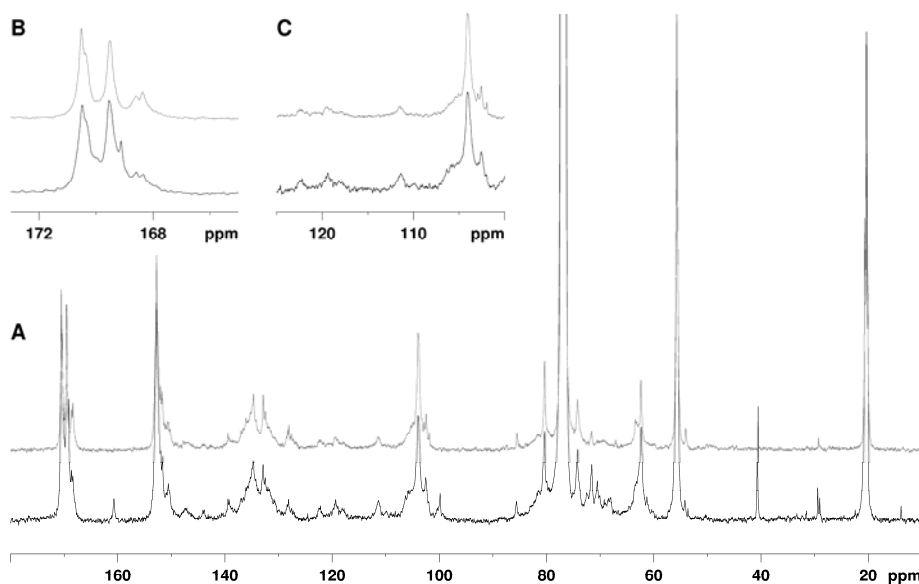


Fig.7. Comparison of quantitative ¹³C NMR spectra (A) of acetylated DWL and AL lignins isolated from native wood (*E. globulus*). Inserts B and C show expansions of the carbonyl bands of the acetylated hydroxyl groups and protonated aromatic regions, respectively. In all overlays A, B, and C black spectra at the bottom show DWL, while grey spectra on top correspond to AL lignin.

Table 5. Signal Assignment and Integration of ¹³C-NMR Resonances of the Acetylated DWL and AL Samples Isolated from *E. globulus* and the Corresponding CBC Pulps Made Thereof. (The Aromatic carbon of non-etherified phenolic C-OAc is shifted into the aromatic C-C region and can be calculated from the signal of the acetyl group of phenolic OAc.)

| Assignment | Integration limits, ppm | | Integral area** | | | | | | | | |
|-----------------------------------|-------------------------|-------|-----------------|------|------|------|------|------|------|------|------|
| | | | K141 | | K109 | | K48 | | K34 | K18 | K10 |
| | from | to | AL | DWL | AL | DWL | AL | DWL | AL | DWL | AL |
| Primary aliphatic OH* | 171.0 | 169.8 | 0.61 | 0.80 | 0.56 | 0.72 | 0.44 | 0.66 | 0.45 | 0.65 | 0.42 |
| Secondary aliphatic OH* | 169.8 | 168.8 | 0.45 | 0.73 | 0.41 | 0.53 | 0.35 | 0.43 | 0.34 | 0.47 | 0.31 |
| Phenolic OH* | 168.8 | 167.5 | 0.24 | 0.22 | 0.27 | 0.17 | 0.32 | 0.10 | 0.29 | 0.19 | 0.35 |
| O substituted aromatic C*** | 162.0 | 142.0 | 2.18 | 2.22 | 2.18 | 2.15 | 2.18 | 2.21 | 2.17 | 2.17 | 2.15 |
| C substituted aromatic C**** | 142.0 | 125.0 | 1.77 | 1.72 | 1.75 | 1.76 | 1.73 | 1.84 | 1.78 | 1.86 | 1.81 |
| H substituted aromatic C | 125.0 | 101.5 | 2.05 | 2.06 | 2.06 | 2.09 | 2.09 | 1.96 | 2.06 | 1.97 | 2.05 |
| Guaiacyl, G2,5,6 :3 | 125.0 | 109.5 | 0.21 | 0.27 | 0.21 | 0.23 | 0.28 | 0.24 | 0.29 | 0.31 | 0.32 |
| Syringyl, S2,6 :2 | 109.5 | 101.5 | 0.71 | 0.70 | 0.71 | 0.70 | 0.62 | 0.61 | 0.60 | 0.52 | 0.54 |
| Carbohydrates, E1 | 101.0 | 98.5 | 0.01 | 0.14 | 0.02 | 0.03 | 0.04 | 0.03 | 0.04 | 0.05 | 0.02 |
| Aliphatic-O / + CDCL ₃ | 90.0 | 58.0 | 2.51 | 3.72 | 2.54 | 3.01 | 2.20 | 3.41 | 2.18 | 3.96 | 2.12 |
| β-β/∅ | 86.0 | 85.2 | 0.05 | 0.06 | 0.05 | 0.07 | 0.05 | 0.06 | 0.05 | 0.04 | 0.04 |
| β-O-4/∅ | 81.0 | 79.8 | 0.37 | 0.37 | 0.33 | 0.46 | 0.30 | 0.37 | 0.27 | 0.32 | 0.21 |
| Methoxyl | 57.0 | 54.5 | 1.61 | 1.62 | 1.59 | 1.65 | 1.34 | 1.43 | 1.29 | 1.29 | 1.31 |
| S/G ratio | | | 3.32 | 2.58 | 3.36 | 3.09 | 2.20 | 2.51 | 2.07 | 1.70 | 1.68 |

* acetylated, ** Normalized to the integral area corresponding to aromatic carbon

*** non-etherified phenolic C-O-Ac added to integral area, **** non-etherified phenolic C-O-Ac deduced from integral area

CONCLUSIONS

1. The dissolved wood lignin (DWL) protocol has been successfully applied to unbleached kraft pulps. The results of the structural lignin characterization indicate that the DWL protocol offers a possibility to isolate lignin samples with less alterations, being therefore more representative of the residual lignin than the AL method.
2. The study confirmed the clear relationship between the ease of delignification and the particular chemical structure of the residual lignin, such as a high S/G ratio, a high amount of β -O-4 units, and a high proportion of uncondensed aromatic structures, as demonstrated for kraft pulping of *E. globulus* wood.
3. The results from the analysis of residual lignins isolated on different stages of kraft pulping suggest that the continuous batch cooking (CBC) process permits high delignification efficiency, as evidenced by an almost unchanged amount of β -O-4 structures and a low proportion of condensed aromatic units throughout the entire pulping process.
4. Comparative studies between CBC and conventional kraft cooking processes are required to evaluate the impact of alkali profiling on the final pulping results of *E. globulus*.

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