

# Modification of the Lignin Structure during Alkaline Delignification of Eucalyptus Wood by Kraft, Soda-AQ, and Soda-O<sub>2</sub> Cooking

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## Supporting Information

**ABSTRACT:** The modification of the lignin structure of an eucalyptus feedstock during alkaline delignification by kraft, soda-AQ, and soda-O<sub>2</sub> cooking processes has been investigated by different analytical techniques (size exclusion chromatography (SEC), pyrolysis gas chromatography–mass spectroscopy (Py-GC/MS), <sup>1</sup>H–<sup>13</sup>C two-dimensional nuclear magnetic resonance (2D-NMR), and <sup>31</sup>P NMR). The characteristics of the lignins were compared at different pulp kappa levels, and with the native lignin isolated from the wood. The structural differences between the kraft, soda-AQ, and soda-O<sub>2</sub> residual lignins were more significant at earlier pulping stages. At the final stages, all the lignin characteristics were similar, with the exception of their phenolic content. Strong differences between lignins from pulps and cooking liquors were observed, including enrichment in guaiacyl units in pulp residual lignin and enrichment in syringyl units in black liquor lignin. A comparison of the alkaline cookings indicate that soda-O<sub>2</sub> process produced higher lignin degradation and provided promising results as pretreatment for the deconstruction of eucalyptus feedstocks for subsequent use in lignocellulose biorefineries.

## 1. INTRODUCTION

Alkaline deconstructions appear as attractive pretreatments for lignocellulose biorefinery purposes. Pretreatment and delignification processes are aimed at disrupting the cellulose–hemicelluloses–lignin complex, and they are important technological steps for fractionating the lignocellulosic materials into their main components.<sup>1–4</sup> Kraft cooking is the alkaline process most widely used for delignification of hardwoods and softwoods for the production of paper pulp. The key to the kraft process is the recovery furnace, which is quite efficient at recovering the pulping chemicals NaOH and Na<sub>2</sub>S.<sup>5</sup> When the cooking liquor is used for further fractionation into valuable components, the soda-antraquinone (soda-AQ) process, in which AQ is used as a pulping additive to decrease the carbohydrate degradation,<sup>6</sup> is favorable because the absence of sulfur compounds in the liquor enables recovery of sulfur-free raw lignin (and lignin products) for the production of bio-based materials. On the other hand, the soda-O<sub>2</sub> process greatly improves the delignification of biomass and is especially promising as a pretreatment for biorefinery processes, as the oxidative conditions affect the fiber ultrastructure in a deconstructive manner.<sup>7–11</sup> In contrast to paper-grade pulps, the fiber strength properties are not important in some biorefinery applications, as biofuel production, as the deconstruction of the lignocellulosic matrix is aimed at the removal and/or modification of the lignin in order to improve substrate digestibility.

The chemistry and efficiency of alkaline delignification is influenced by several process parameters, including cooking time and temperature, active alkali charge, and the possible presence of other cooking chemicals such as sulfide or anthraquinone

(AQ).<sup>4–15</sup> In addition, the structural characteristics of the native lignin in feedstocks also play an important role in the chemistry of the pulping process. Hence, in hardwoods, the content of syringyl units, which are predominantly associated to β-aryl ether linkages, is one of the most determinant characteristics.<sup>16–18</sup> Numerous studies have attempted to correlate the chemical structure of residual lignin in pulp and dissolved lignin in cooking liquors to the pulping efficiency, contributing to a more comprehensive study of the delignification mechanisms involved.<sup>8–27</sup>

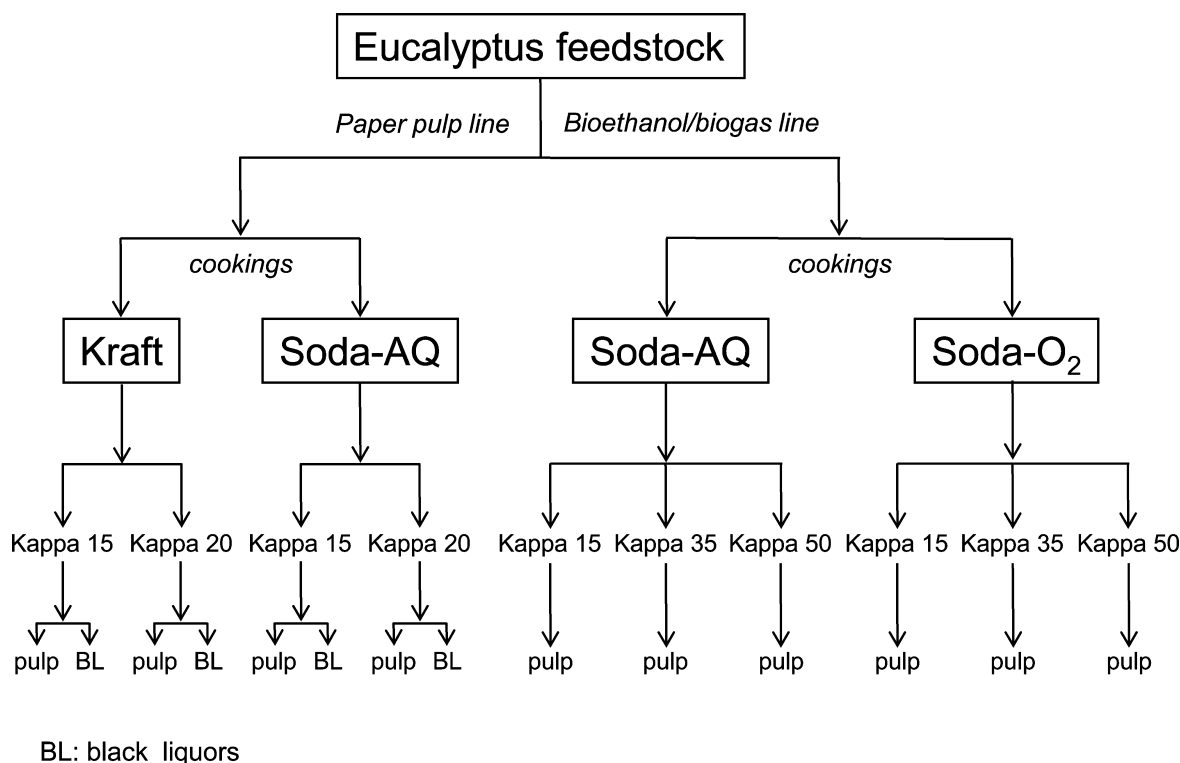
In this context, the objective of this study is to investigate and compare the modifications of the lignin structure of an eucalyptus feedstock during alkaline delignification by kraft, soda-AQ, and soda-O<sub>2</sub> cooking processes. The study intends to provide useful information about the chemistry of the delignification process necessary to optimize the processing of eucalyptus wood for traditional pulping purposes, as well as for other biorefinery processes. For this, two different sets of pulps were produced from an eucalyptus feedstock: (i) pulps intended for paper production by the kraft and soda-AQ processes, at kappa 20 and 15, and (ii) pulps intended for bioethanol and/or biogas production by the soda-AQ and soda-O<sub>2</sub> processes, at kappa 50, 35, and 15. The residual lignins (isolated from the pulps by acidolysis) and the lignins precipitated from the black liquors were analyzed by different analytical techniques, including size exclusion chromatography (SEC), pyrolysis gas chromatography–mass

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**Figure 1.** Flowchart indicating the production of the selected pulps and black liquors from kraft, soda-AQ, and soda-O<sub>2</sub> cookings at different kappa numbers.

spectrometry (Py-GC/MS), <sup>1</sup>H–<sup>13</sup>C two-dimensional nuclear magnetic resonance (2D-NMR) and <sup>31</sup>P NMR, and their structural characteristics were compared to those of the milled wood lignin (MWL) isolated from the initial raw material, a lignin preparation that is still considered to be the representative of the native lignin in the plant, despite its limitations.<sup>28,29</sup>

## 2. MATERIALS AND METHODS

**2.1. Alkaline Cookings (Kraft, Soda-AQ, Soda-O<sub>2</sub>).** The feedstock consisted of wood chips from a eucalyptus *E. grandis* × [*E. urophylla* × *E. globulus*] (G1×UGL) hybrid grown in Brazil. Chips were classified according to standard SCAN-CM 40:94, which is based on chip thickness (4–6 mm), and the accepts were stored.

Two series of alkaline cooking experiments were carried out (at Suzano Papel e Celulose, Brazil): (i) pulps intended for paper production by the kraft and soda-AQ processes, at kappa 20 and 15, and (ii) pulps intended for bioethanol and/or biogas production by the soda-AQ and soda-O<sub>2</sub> processes, at kappa 50, 35, and 15, without rejects removal after cooking (see flowchart in Figure 1). Two soda-AQ pulps (from both lines) were available at kappa 15, which were practically identical. In fact, similar results of the soda-AQ residual lignins at kappa 15 were obtained in both lines and the mean values are therefore given for the results of this residual lignin.

The general conditions for the kraft, soda-AQ, and soda-O<sub>2</sub> alkaline cookings are detailed in Table S1 in the Supporting Information. The cooking experiments were carried out in a CRS digester (model CPS 010, recycle digester system) containing two individual reactors of 10 L each, equipped with a forced liquor circulation system and electrically heated with temperature and pressure control. Eight cooking experiments were carried out for every type of process using variable alkali charges in order to

establish the delignification curves for each sample. The kappa number of the pulp was determined according to the TAPPI T236 cm-85 standard.<sup>30</sup> In the soda-O<sub>2</sub> cooking, the oxygen dosage was split into three parts and applied after 50, 70, and 110 min of reaction time from the beginning of cooking, thereby ensuring that the pressure limit (20 kgf/cm<sup>2</sup>) of the digester was not exceeded. At this point, the cooling system was turned on for 30 min, and the temperature of the residual liquor reduced to levels close to 55 °C. After cooling and unloading the digester, the cooked chips were washed with hot water (70 °C) for 3 h and then with water at ambient temperature until the complete removal of residual liquor (~12 h). Fiber individualization was achieved in a “hydrapulper” (15 L capacity), followed by fine screening (Voith laboratory cleaner, equipped with perforated plates with 0.2 mm openings). The material retained on the sieve (rejects) was dried and weighed. The accept pulp was dewatered to a consistency of ~30% and stored for further analysis.

**2.2. MWL Isolation from Wood.** The isolation of MWL from wood of the eucalyptus G1×UGL hybrid was previously described.<sup>31</sup> MWL was extracted from finely ball-milled (15 h) wood, free of extractives and hot water-soluble material, using dioxane–water (9:1, v/v), followed by evaporation of the solvent, and purified as described.<sup>32</sup> The final yield ranged from 15% to 20% of the original Klason lignin content.

**2.3. Residual Lignin Isolation from Pulp.** For the isolation of residual lignin, first, all pulp samples were air-dried at 37–40 °C. Extractives were eliminated by Soxhlet extraction for 9 h with acetone and subsequent extraction with water at 100 °C (three steps). Then, 15 g of dry pulp was subjected to acidolysis, according to the method previously described,<sup>33</sup> using a two-step extraction with 0.1 M HCl in a 1,4-dioxane:water mixture (82:18 v/v) under an inert (argon) atmosphere at 88–92 °C. The solid:liquid ratio for the first and second extraction

step was, respectively, 13.3 and 10.0 mL per gram of dry pulp. After the extractions, the pulp was washed with the same 1,4-dioxane:water mixture, but without HCl. In order to avoid high acid concentration, the washing liquor was added equally to both extracts, which were evaporated separately under reduced pressure until a volume reduction of ~70% was obtained. Both concentrated extracts were added together in 1.5 L of cold distilled water under strong stirring. The lignin was precipitated overnight at 4 °C and then centrifuged (25 min, 9000 rpm, 4 °C). A washing step with cold distilled water was included, and after recovery (30 min, 9000 rpm, 4 °C) the samples were freeze-dried. The extraction yields of the residual lignins ranged from 60% to 82%, based on the theoretical lignin content of pulp (% lignin = 0.15 × kappa number), except for the residual lignins from the pulps with kappa 15, which were in the range of 26%–30%. The residual lignins were submitted to Soxhlet extraction with *n*-pentane during 8 h. After drying with nitrogen, the residual lignin was ready for analysis.

**2.4. Lignin Isolation from Black Liquors.** The lignins from the collected black liquors were precipitated at pH 4.0 and the obtained slurries were air-dried at 40 °C. The homogenized samples were stored in a desiccator until stable humidity was attained. All analytical techniques (SEC, Py-GC/MS, <sup>1</sup>H–<sup>13</sup>C 2D-NMR, and <sup>31</sup>P NMR) were performed on these samples.

**2.5. Size Exclusion Chromatography (SEC).** The SEC analysis were performed on a Waters HPLC system equipped with a UV detector with response set at 280 nm using the following conditions: columns, PSS's MCX 1000 and 100 000 Å; eluent, 0.1 M NaOH; flow rate, 0.5 mL/min; and temperature, 25 °C. The data acquisition and computation was performed with Empower 2 software. Before analysis, ~4 mg of lignin was dissolved overnight in 4 mL of analytical NaOH (0.1 M) and filtered with 0.45 μm PTFE membrane syringe filters. The molecular masses were calculated relative to the sulfonated Na-polystyrene (Na-PSS, 3420–148500 g/mol) standards. The weight-average molecular weights ( $M_w$ ), number-average molecular weights ( $M_n$ ), and the polydispersities ( $M_w/M_n$ ) were calculated.

**2.6. Pyrolysis-Gas Chromatography–Mass Spectrometry (Py-GC/MS).** Pyrolysis of lignins (~100 μg) was performed with an EGA/PY-3030D microfurnace pyrolyzer (Frontier Laboratories, Ltd.) connected to an Agilent 7820A gas chromatograph using a DB-1701 fused-silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness) and an Agilent 5975 mass selective detector (EI at 70 eV). The pyrolysis was performed at 500 °C. The oven temperature was programmed from 45 °C (4 min) to 280 °C (10 min) at 4 °C min<sup>-1</sup>. Helium was the carrier gas (1 mL min<sup>-1</sup>). The compounds were identified by comparing their mass spectra with those of the Wiley and NIST libraries and those reported in the literature.<sup>34</sup> Peak molar areas were calculated for the lignin-degradation products, the summed areas were normalized, and the data for two repetitive analyses were averaged and expressed as percentages.

**2.7. Two-Dimensional Nuclear Magnetic Resonance (2D-NMR).** 2D-NMR spectra were recorded at 25 °C in a Bruker AVANCE 600 MHz spectrometer, equipped with a cryogenically cooled z-gradient triple resonance probe. Forty milligrams (40 mg) of lignin sample were dissolved in 0.75 mL of dimethylsulfoxide (DMSO)-*d*<sub>6</sub>, and <sup>1</sup>H–<sup>13</sup>C HSQC (heteronuclear single quantum correlation) spectra were recorded. The spectral widths were 5000 and 13200 Hz for the <sup>1</sup>H and <sup>13</sup>C dimensions, respectively. The number of collected complex points was 2048 for the <sup>1</sup>H dimension, with a recycle delay of 1 s. The number of transients was 64, and 256 time increments

were recorded in the <sup>13</sup>C dimension. The <sup>1</sup>J<sub>CH</sub> constant was set to 140 Hz. The *J*-coupling evolution delay was set to 3.2 ms. Squared cosine-bell apodization function was applied in both dimensions. Prior to Fourier transformation, the data matrices were zero-filled up to 1024 points in the <sup>13</sup>C dimension. The central solvent peak was used as an internal reference ( $\delta_C$  39.5 ppm;  $\delta_H$  2.49 ppm). HSQC cross-signals were assigned by comparing with the literature.<sup>23,29,35–40</sup> In the aromatic region, C<sub>2,6</sub>-H<sub>2,6</sub> correlations from S units and C<sub>2</sub>-H<sub>2</sub> correlations from G units were used to estimate the S/G lignin ratios. The abundances of the different interunit linkages were referred to the number of aromatic units (per 100 aromatic units), to obtain a comparative estimation of their removal during pulping and bleaching.

**2.8. <sup>31</sup>P NMR spectroscopy.** Phosphitylation of lignin samples was performed according to the literature.<sup>41</sup> About 40 mg of pure lignin was weighted and dissolved in 1.6 mL of a solvent mixture which consisted of dimethylformamide:pyridine:deuterated chloroform (1:1.70:2.63 v/v) to which 0.01 mmol of endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide was added as internal standard, together with 0.032 mmol of chromium(III)acetylacetonate as a relaxation agent. Then, 200 μL of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (phosphitylating reagent II) was added. The <sup>31</sup>P NMR spectra were recorded on a Bruker Avance III 500 MHz spectrometer at 23 °C using a 90° pulse width. For acquisition, an inverse gated decoupling sequence was used with deuterated chloroform as the locking solvent and 512 scans were recorded with a delay time of 5 s between successive pulses. For processing data, the chemical shifts were calibrated to the sharp signal (132.2 ppm) of the reaction product between water and phosphitylation reagent II and the line broadening was set to 2 Hz, followed by exponential window multiplication, Fourier transformation, automatic/manual phase correction, and automatic baseline correction. The integration areas were expressed relative to the area of the internal standard (IS) signal, which was set to 1. In addition, the peak width at half height of the IS signal was checked to guarantee a similar resolution in all samples and, therefore, an exact quantification. The deconvolutions of the region containing syringyl and C5-substituted guaiacyl hydroxyl group signals were processed on spectra with a line broadening factor of 8 Hz. In order to obtain a satisfactory deconvolution result (as close as possible to the experimental <sup>31</sup>P NMR spectrum), 12–20 signals were selected in the 144.2–141.0 ppm region. To calculate the hydroxyl group content, the impurity of the IS (97%) was taken into account. The results were expressed as millimoles of hydroxyl groups per gram of pure lignin. For the black liquor samples, the purity was based on the sum of the Klason and acid-soluble lignin content. The purity of the MWL and residual lignin samples was assumed to be 100%, as their isolation included purifications steps. Standard deviations were calculated from the results of three independent samples taken from the soda-O<sub>2</sub> residual lignin bulk sample at kappa 50. Their <sup>31</sup>P NMR spectra were recorded and processed independently and the 144.2–141.0 ppm region was deconvoluted with selected signals having similar chemical shifts and widths.

### 3. RESULTS AND DISCUSSION

The residual lignins (isolated from the pulps by acidolysis), together with the lignins precipitated from the black liquors, produced in the different cooking experiments (kraft, soda-AQ, soda-O<sub>2</sub>) were analyzed by different analytical techniques, including SEC, Py-GC/MS, <sup>1</sup>H–<sup>13</sup>C 2D-NMR and <sup>31</sup>P NMR, and their structural characteristics were compared to those of the MWL isolated from the initial raw material.

**Table 1.** Weight-Average ( $M_w$ ) and Number-Average ( $M_n$ ) Molecular Weights, and Polydispersity ( $M_w/M_n$ ) of the MWL from Wood of the Eucalyptus G1XUGL Hybrid, and Residual and Black Liquor Lignins from Kraft, Soda-AQ, and Soda-O<sub>2</sub> Pulping at Different Kappa Numbers

MWL	Residual Lignins									Black Liquor Lignins				
	Kraft			Soda-AQ			Soda-O <sub>2</sub>			Kraft		Soda-AQ		
	kappa 20	kappa 15	kappa 50	kappa 35	kappa 20	kappa 15	kappa 50	kappa 35	kappa 15	kappa 20	kappa 15	kappa 20	kappa 15	
Molecular weight (Da)														
$M_n$	3500	2440	2370	2480	2510	2710	2530	2380	2360	2300	1680	1640	1660	1580
$M_w$	13300	4340	3490	10550	8300	4770	3870	9380	6080	3640	2390	2280	2540	2350
polydispersity, $M_w/M_n$	3.8	1.7	1.5	4.3	3.3	1.8	1.5	3.9	2.6	1.6	1.4	1.4	1.5	1.5

**3.1. Size Exclusion Chromatography (SEC).** The values of the weight-average ( $M_w$ ) and number-average ( $M_n$ ) molecular weights and the polydispersity ( $M_w/M_n$ ) of the analyzed samples are listed in Table 1. The strong decrease observed in the molecular weights of the isolated residual lignins, compared to the MWL, clearly indicates the degradation and depolymerization of the lignin macromolecule during pulping. Interestingly, the molecular weights of the residual lignins decreased concomitantly with progressing delignification (with decreasing kappa number), in agreement with previous works.<sup>24,42</sup> At high kappa numbers (kappa 50 and 35), the molecular weights ( $M_w$ ) of the residual lignins from soda-O<sub>2</sub> ( $M_w = 9380$  and  $6080$  g mol<sup>-1</sup>) were lower than those of the residual lignins from soda-AQ ( $M_w = 10550$  and  $8300$  g mol<sup>-1</sup>), clearly indicating a higher lignin degradation of the residual lignins of the soda-O<sub>2</sub> cooking process. However, at the lowest kappa numbers (kappa 15), the molecular weights of the residual lignins were rather similar, with the residual lignins from kraft and soda-O<sub>2</sub> processes having the lowest values ( $M_w = 3490$  and  $3640$  g mol<sup>-1</sup>), compared to those of the residual lignin from soda-AQ ( $M_w = 3870$  g mol<sup>-1</sup>). Polydispersity also progressively decreased with increasing delignification, as previously observed.<sup>42</sup> The degradation of the lignin polymer occurring during the delignification process results in residual lignin fragments with smaller molecular weights, which were more uniformly distributed. No condensation reactions were observed to occur during the cooking processes from the SEC chromatographs, as also reported in previous papers.<sup>24,42</sup> On the other hand, the molecular weights of the lignins precipitated from the black liquors from kraft and soda-AQ processes were lower than their respective residual lignins ( $M_w = 2280$ – $2540$  g mol<sup>-1</sup>). These results indicate the higher degree of degradation of the lignin fragments dissolved in the alkaline cooking media.

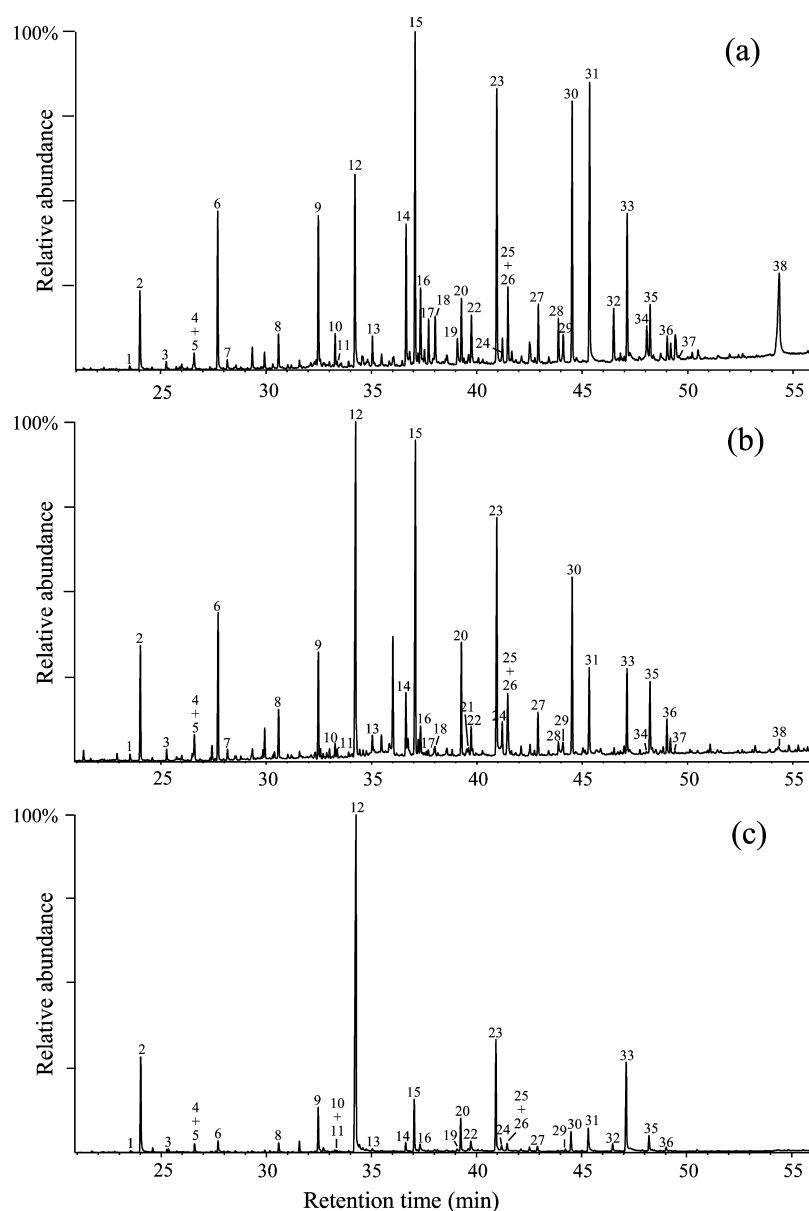
**3.2. Pyrolysis Gas Chromatography–Mass Spectrometry (Py-GC/MS).** Information about the composition of the lignins was obtained from Py-GC/MS. The pyrograms of a selected pulp residual lignin from eucalyptus G1XUGL hybrid (from kraft pulp at kappa 20) and the lignin precipitated from the corresponding black liquor are shown in Figure 2. The Py-GC/MS of the MWL from eucalyptus G1XUGL hybrid is also shown for comparison. The identities and relative molar abundances of the identified lignin compounds are shown in Table S2 in the Supporting Information. The main lignin structural characteristics obtained from the Py-GC/MS data (percentage of H, G, and S units, and S/G ratio, ratio of lignin markers bearing side-chains of 0–2 carbon atoms, with respect to those bearing side-chains of 3 carbon atoms, and percentage of lignin markers bearing a carboxyl/carbonyl group at the  $\alpha$ -carbon) of the residual lignins and black liquors from the

kraft, soda-AQ and soda-O<sub>2</sub> processes at different kappa numbers are shown in Table 2.

Interestingly, there is a similarity in the pyrograms of the residual lignins isolated from kraft, soda-AQ, and soda-O<sub>2</sub> pulps and the native lignin (as reflected by the MWL). However, the residual lignins are comparatively depleted in S-lignin units and enriched in G- and H-lignin units, with decreasing kappa number (with increasing the extent of delignification), resulting in a decrease of the S/G ratio, as a consequence of the preferential removal of S-lignin during alkaline delignification, as previously observed.<sup>16</sup> A small increase in the relative amounts of pyrolysis compounds with shorter chain (as indicated by the ratio of lignin phenolic markers bearing side-chains of 0–2 carbon atoms respect to lignin phenolic markers bearing side-chains of 3 carbon atoms, Ph–C0–2/Ph–C3) indicates the side chain degradation of the residual lignins, which is more evident at lower kappa numbers.

Interestingly, at earlier stages of delignification (kappa numbers 50 and 35), the S/G ratios of the residual lignins from the soda-O<sub>2</sub> process were significantly lower than those of the soda-AQ residual lignins. Therefore, the soda-O<sub>2</sub> process seems to produce higher lignin degradation than soda-AQ, at least at higher kappa numbers. However, at low kappa numbers (kappa 20 and 15), the S/G ratios of the kraft, soda-AQ, and soda-O<sub>2</sub> residual lignins were rather similar. However, no major structural oxidation was observed in the residual lignins isolated from the kraft, soda-AQ, and soda-O<sub>2</sub> pulps, compared to the MWL, as shown by the relatively low amounts of  $\alpha$ -oxidized lignin compounds (i.e., peak 16, vanillin; peak 22, acetoguaiacone; peak 31, syringaldehyde; peak 33, acetosyringone; peak 37, propiosyringone) in their pyrograms. The content of carbonyl compounds decreased in the residual lignin until kappa 35–20, with respect to the MWL (Table 2), indicating that native oxidized units were removed during delignification and that new oxidized units were cleaved rapidly. Then, at kappa 15, the content was higher again in all residual lignins. This could be the result of a slower degree of removal during the terminal phase. The slower delignification at the final stage has been explained to be due to the recalcitrance of the residual lignin structure and/or its binding with carbohydrates.<sup>43</sup>

On the other hand, the pyrograms of the lignins precipitated from the black liquors are completely different from the native lignin and from the residual lignins in the pulps. The lignins in black liquors are highly enriched in S-lignin units (due to the preferential solubilization of S-lignin units) and depleted in G-units. In addition, the higher amounts of pyrolysis compounds with shorter chain and/or oxidized, indicates that this lignin is heavily degraded and oxidized. Similar results have been

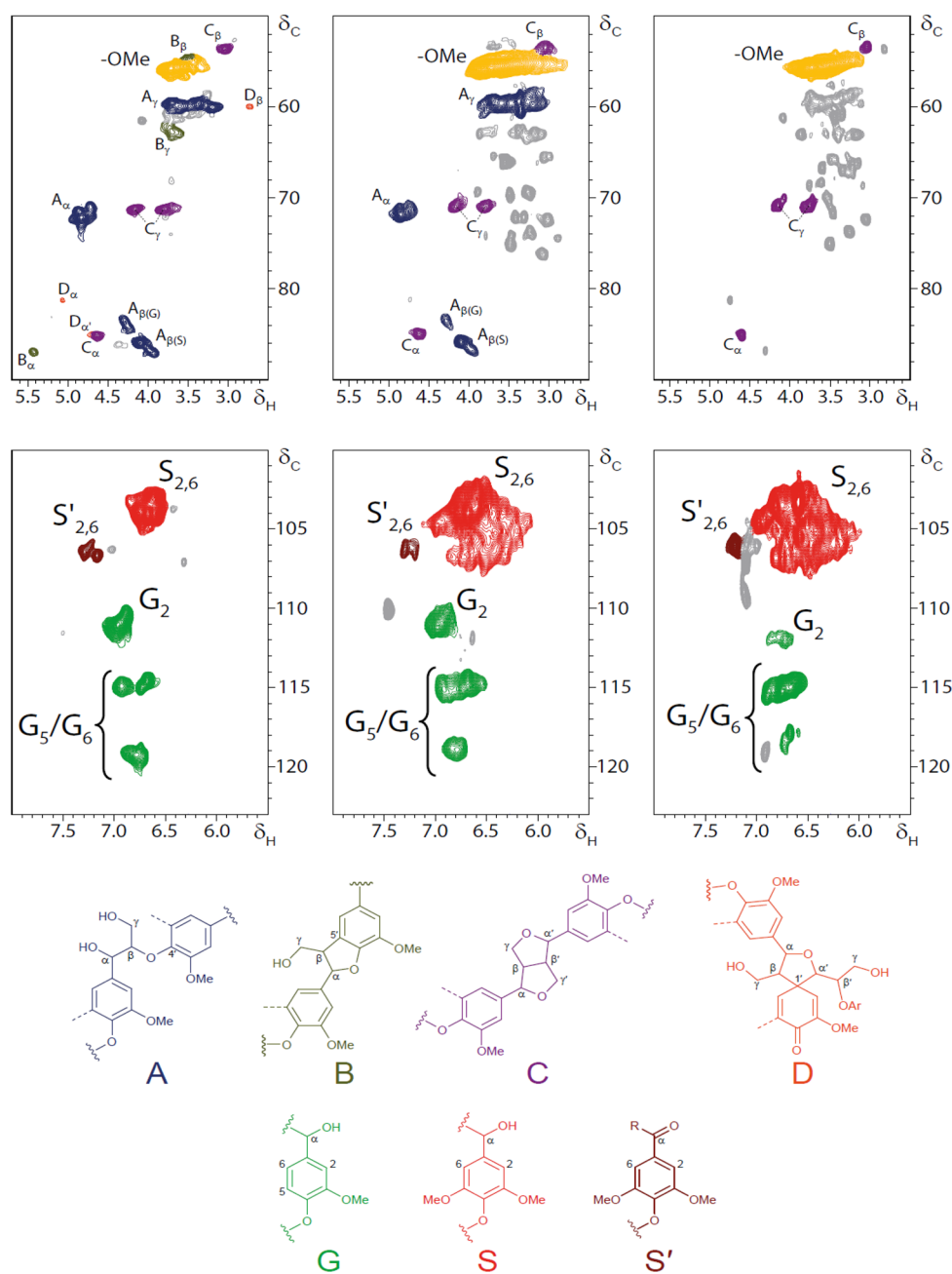


**Figure 2.** Py-GC/MS chromatograms of (a) MWL isolated from wood of the eucalyptus G1xUGL hybrid; (b) kraft residual lignin at kappa number 20, and (c) lignin from the corresponding black liquor. The numbers refer to the compounds listed in Table S2 in the Supporting Information.

**Table 2. Structural Characteristics (Percentages of the H-, G-, and S-Lignin Units, S/G Ratios, Ratios of Lignin Markers Bearing Side-Chains of 0–2 Carbon Atoms, with Respect to Those Bearing Side-Chains of 3 Carbon Atoms, and Percentages of Lignin Markers Bearing a Carboxyl/Carbonyl Group at the  $\alpha$ -Carbon) from the Py-GC/MS of the Residual Lignins and Black Liquor Lignins from Kraft, Soda-AQ, and Soda-O<sub>2</sub> Cookings at Different Kappa Numbers, and the Respective MWL**

MWL	Residual Lignins									Black Liquor Lignins				
	Kraft			Soda-AQ			Soda-O <sub>2</sub>			Kraft		Soda-AQ		
	kappa 20	kappa 15	kappa 50	kappa 35	kappa 20	kappa 15	kappa 50	kappa 35	kappa 15	kappa 20	kappa 15	kappa 20	kappa 15	
Lignin composition														
% H	1.7	2.7	3.6	1.6	1.8	2.6	4.1	1.8	2.2	2.8	1.1	1.9	2.0	2.3
% G	33.1	30.3	33.5	27.5	27.6	31.3	35.6	33.3	35.3	36.2	22.6	24.4	25.7	25.8
% S	65.2	67.0	62.8	70.9	70.5	66.1	60.4	64.9	62.5	61.0	76.3	73.7	72.4	71.9
S/G	2.0	2.1	1.9	2.6	2.5	2.1	1.7	2.0	1.8	1.7	3.4	3.1	2.8	2.8
Ph-C <sub>0-2</sub> /Ph-C <sub>3</sub> <sup>a</sup>	1.7	3.2	5.7	2.3	2.6	4.6	5.3	2.6	3.2	5.3	15	19	13	13
% Ph-C=O <sup>b</sup>	18	9	11	14	12	10	12	14	12	13	12	16	10	12

<sup>a</sup>Ratio of lignin phenolic markers bearing side-chains of 0–2 carbon atoms, with respect to lignin phenolic markers bearing side-chains of 3 carbon atoms. <sup>b</sup>Percentage of lignin phenolic markers bearing a carboxyl/carbonyl group at the  $\alpha$ -carbon.



**Figure 3.** HSQC NMR spectra of (left) MWL isolated from wood of eucalyptus G1×UGL hybrid; (middle) residual lignin isolated from kraft pulp at kappa 20; and (right) the corresponding black liquor lignin, showing (upper) the oxygenated aliphatic ( $\delta_C/\delta_H$  50–90/2.5–5.7) and (lower) the aromatic region ( $\delta_C/\delta_H$  100–123/5.5–8.0). The main lignin substructures identified are **A**,  $\beta$ -O-4' substructures; **B**, phenylcoumarane substructures formed by  $\beta$ -5'/ $\alpha$ -O-4' linkages; **C**, resinol substructures formed by  $\beta$ - $\beta$ '/ $\alpha$ -O- $\gamma$ '/ $\gamma$ -O- $\alpha$ ' linkages; **D**, spirodienone substructures formed by  $\beta$ -1'/ $\alpha$ -O- $\alpha$ ' linkages; **G**, guaiacyl units; **S**, syringyl units; and **S'**, oxidized syringyl units bearing a carbonyl group at  $C_{\alpha}$ .

previously observed during kraft cooking of eucalyptus wood.<sup>22,23</sup>

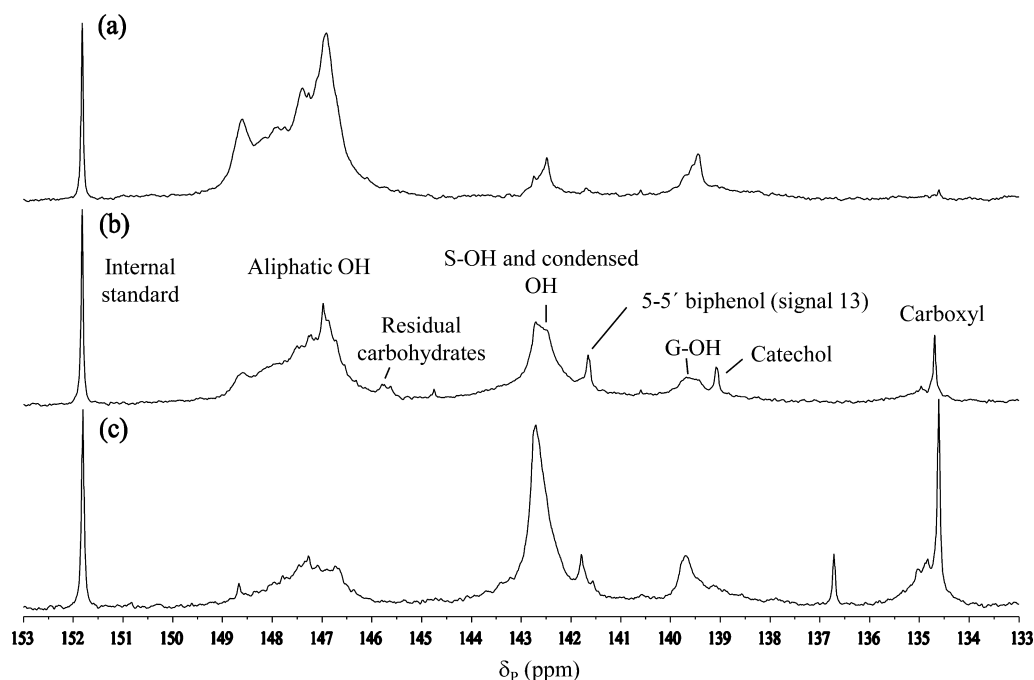
**3.3. 2D-NMR.** Additional information regarding the structure of these lignins was obtained from 2D-NMR. The HSQC spectra of a representative residual lignin (isolated from the eucalyptus G1×UGL hybrid kraft pulp at kappa 20), and the precipitated lignin from its respective black liquor, are shown in Figure 3. The spectrum of the MWL isolated from G1×UGL is also shown for comparison. The main substructures found are also depicted in Figure 3. Quantitation of the abundance of the main lignin interunit linkages, as well as the abundance of the G and S lignin units, present in the different residual lignins

from the eucalyptus pulps produced from the kraft, soda-AQ, and soda-O<sub>2</sub> processes, was performed by integration of the volume contours of their cross-signals and was referenced as per 100 aromatic units (see Table 3).

The detailed structural characteristics of the MWL of eucalyptus G1×UGL from HSQC data has been described previously.<sup>31</sup> The main interunit linkages observed in the residual lignins from the kraft, soda-AQ, and soda-O<sub>2</sub> pulps were  $\beta$ -O-4' alkyl-aryl ether,  $\beta$ - $\beta$  resinol, and  $\beta$ -5 phenylcoumaran structures. Interestingly, no oxidized lignin side chains were observed by 2D-NMR in the residual lignins, as already seen by Py-GC/MS. The distributions of the different interunit linkages in the pulp

**Table 3. Abundance of Lignin Interunit Linkages (Expressed as Linkages per 100 Aromatic Units) and S/G Ratio in MWL from Wood of the Eucalyptus G1×UGL Hybrid, and in Residual Lignins and Black Lignin Lignins from Kraft, Soda-AQ, and Soda-O<sub>2</sub> Pulping at Different Kappa Numbers**

	Residual Lignins										Black Lignin Lignins				
	MWL	Kraft		Soda-AQ				Soda-O <sub>2</sub>				Kraft		Soda-AQ	
		kappa 20	kappa 15	kappa 50	kappa 35	kappa 20	kappa 15	kappa 50	kappa 35	kappa 15	kappa 20	kappa 15	kappa 20	kappa 15	
$\beta$ -O-4' alkyl-aryl ether	84	16	14	56	47	18	12	41	29	12	1	0	0	0	
$\beta$ - $\beta'$ resinol	11	7	7	11	11	7	7	11	10	8	10	9	9	8	
$\beta$ -5' phenylcoumaran	4	0	0	1	1	0	0	1	1	1	0	0	0	0	
$\beta$ -1' spirodienone	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
S/G ratio	2.8	5.1	4.4	5.9	5.9	5.2	4.8	4.6	4.5	4.6	8.6	8.2	7.9	8.5	



**Figure 4.** <sup>31</sup>P NMR spectra of (a) MWL isolated from wood of the eucalyptus G1×UGL hybrid, (b) kraft residual lignin at kappa 20, and (c) the corresponding black liquor lignin, processed with a line-broadening factor of 8 Hz.

residual lignins are similar to those observed in the native lignin in wood, with a predominance of  $\beta$ -O-4' alkyl-aryl ether linkages, followed by lower amounts of resinols and phenylcoumarans, but with a significant reduction in their content, especially at low kappa numbers. However, the structure of the lignins precipitated from the black liquors are completely different, being highly enriched in  $\beta$ - $\beta'$  resinol substructures, while the other linkages ( $\beta$ -O-4' alkyl-aryl ethers and  $\beta$ -5' phenylcoumarans), if present, are in much lower abundance. In addition, and as already observed by Py-GC/MS, the lignins from the black liquors show a S/G ratio higher than the residual lignins, which indicates that S-lignin units, which are predominantly forming  $\beta$ -O-4' alkyl-aryl ether structures, are preferentially removed from the eucalyptus during pulping and are being enriched in the black liquors. It is known that eucalyptus lignin resinol structure is made almost exclusively by syringaresinol.<sup>40,44</sup>

Interestingly, the pulps with higher kappa number (kappa 50 and 35) still present a high content of  $\beta$ -O-4' alkyl-aryl ether linkages (up to 56 linkages per 100 aromatic units in the residual lignin from soda-AQ pulp of kappa 50), which were drastically reduced in the pulps with low kappa numbers (12–14 linkages per 100 aromatic units) due to the increasing extent of delignification, as already shown in previous studies.<sup>16,25,42</sup>

The residual lignins from kraft and soda-AQ pulps showed similar abundances of linkages, while the residual lignins from soda-O<sub>2</sub> pulps showed significant differences. At kappa 50 and 35, the abundance of  $\beta$ -O-4' ether linkages (A) in the residual lignin from soda-O<sub>2</sub> pulps (41 and 29 linkages per 100 aromatic units, respectively) was significantly lower than in the corresponding soda-AQ residual lignins (56 and 47 linkages per 100 aromatic units, respectively). At kappa 15, the amount of  $\beta$ -O-4' ether linkages was similar in all residual lignins (12–14 linkages per 100 aromatic units). Therefore, it is clear that soda-O<sub>2</sub> produces higher lignin degradation than kraft and soda-AQ processes, at least at high kappa number levels, in agreement with the SEC and Py-GC/MS results shown above.

**3.4. <sup>31</sup>P NMR Spectroscopy.** Additional information about the occurrence of hydroxyl groups was obtained from <sup>31</sup>P NMR spectroscopy. Figure 4 shows the <sup>31</sup>P NMR spectra of a representative residual lignin (isolated from kraft pulp at kappa 20), and the corresponding black liquor lignin. The <sup>31</sup>P NMR of the MWL isolated from wood of the eucalyptus G1×UGL hybrid is also shown for comparison. The contents of aliphatic hydroxyl groups were calculated from the integration area in the 149.4–145.0 ppm region and are shown in Table 4. At kappa 15, the removal of aliphatic hydroxyl groups in the

**Table 4.** Aliphatic, Phenol (Syringyl + Guaiacyl + Total Condensed + Catechol) and Carboxyl Hydroxyl Group Content (mmol OH/G Pure Lignin) Determined by Quantitative  $^{31}\text{P}$ -NMR in MWL Isolated From Wood of the Eucalyptus G1×UGL Hybrid, Residual Lignins, and Black Liquor Lignins Isolated from Alkaline Pulping at Different Kappa Numbers

	MWL	Residual Lignin									Black Liquor Lignin			
		Kraft			Soda-AQ			Soda-O <sub>2</sub>			Kraft		Soda-AQ	
		kappa 20	kappa 15	kappa 50	kappa 35	kappa 20	kappa 15	kappa 50	kappa 35	kappa 15	kappa 20	kappa 15	kappa 20	kappa 15
aliphatic OH	4.69	2.20	1.69	3.33	2.80	2.20	1.73	2.81	2.55	1.75	1.67	0.92	1.66	1.43
total phenolic OH	0.67	1.72	2.05	1.47	1.56	2.13	2.25	1.64	1.71	1.82	3.91	4.32	3.99	4.21
syringyl	0.21	0.75	0.85	0.86	0.69	1.01	1.13	0.87	0.79	0.79	2.38	2.67	2.35	2.39
guaiacyl	0.38	0.27	0.36	0.25	0.21	0.35	0.39	0.32	0.33	0.37	0.71	0.87	0.71	0.74
total condensed	0.07	0.64	0.78	0.34	0.62	0.72	0.69	0.43	0.57	0.64	0.82	0.78	0.93	1.08
total 5–5' biphenol	0.04	0.20	0.28	0.10	0.14	0.20	0.19	0.12	0.18	0.20	0.26	0.41	0.28	0.25
5–5' biphenol (signal 13)	0	0.10	0.10	0.05	0.09	0.07	0.07	0.05	0.04	0.03	0	0	0	0
catechol	0	0.06	0.07	0.02	0.03	0.04	0.04	0.02	0.02	0.02	0	0	0	0
carboxyl OH	0.005	0.25	0.27	0.09	0.09	0.20	0.26	0.14	0.18	0.24	1.04	0.83	1.02	0.93
total OH	5.37	4.17	4.01	4.89	4.44	4.40	4.24	4.59	4.44	3.81	6.62	6.07	6.67	6.57
S/G	0.6	2.7	2.4	3.5	3.2	2.9	2.9	2.7	2.4	2.1	3.4	3.1	3.3	3.2

residual lignin side chain is similar in kraft, soda-AQ, and soda-O<sub>2</sub> (62%–63%). At earlier stages (kappa 50), the soda-O<sub>2</sub> residual lignin was 11% more depleted in aliphatic hydroxyl groups than the soda-AQ residual lignin. In the black liquor lignins, the removal was only different at kappa 15 (80% removal in kraft lignin while 70% in soda-AQ lignin). This difference may be related to the higher  $\alpha$ -carbonyl content in kraft lignin, as observed by Py-GC/MS, and can influence in the chemical reactivity and physical characteristics of lignin for further industrial applications. The use of phosphitylating reagent I (2-chloro-1,3,2-dioxaphospholane) could give more-detailed information about the side-chain oxidation pattern.<sup>45</sup>

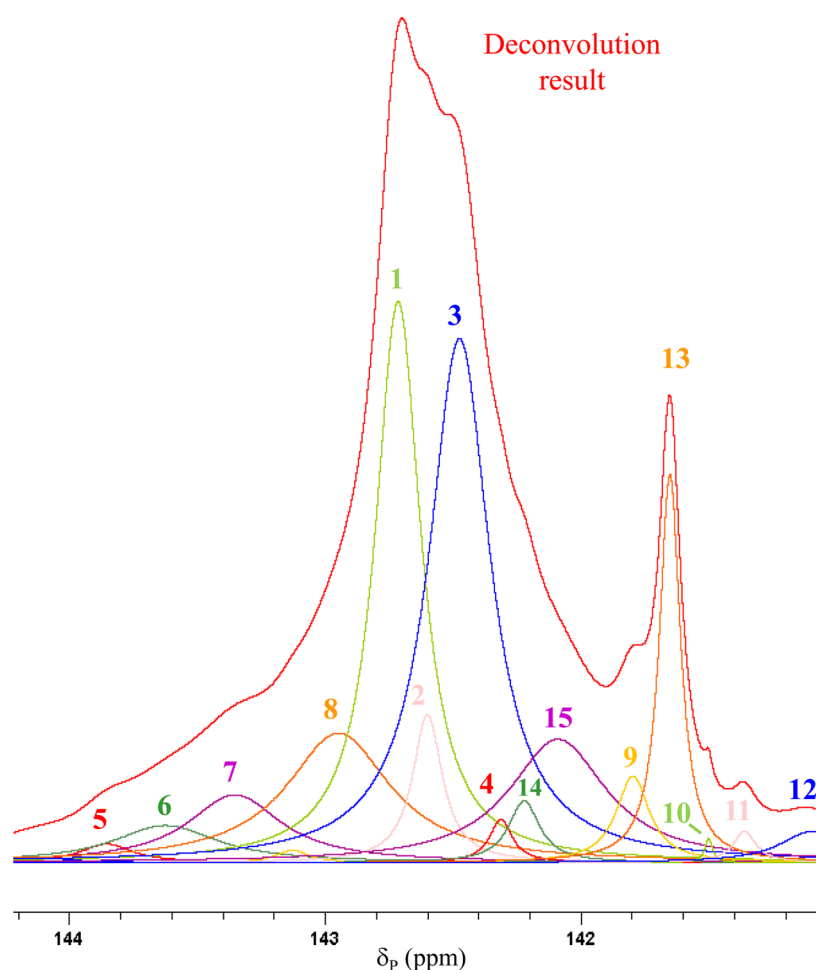
During alkaline pulping, the content of carboxyl groups in the lignins increases (Table 4). At kappa 50, the formation of carboxyl groups in soda-O<sub>2</sub> residual lignin was 60% higher than in soda-AQ residual lignin, because of the oxidative conditions prevailing in soda-O<sub>2</sub>. At kappa 15, the carboxyl content was very similar in kraft, soda-AQ, and soda-O<sub>2</sub> residual lignin. The black liquor lignins recuperated from the kraft and soda-AQ cookings contained 200%–400% more carboxyl groups, with respect to their respective residual lignins. The active alkali demand was higher in the soda-O<sub>2</sub> cookings than in the kraft and soda-AQ cookings (data from Suzano Papel e Celulose), which supports the belief that more carboxyl acid groups were formed during soda-O<sub>2</sub> cooking, both from lignin and carbohydrate moieties.

During the chemical delignification of the eucalyptus G1×UGL, the residual lignin was enriched with phenolic groups (Table 4). In hardwoods, the phenolic groups in C<sub>5</sub>-substituted guaiacyl units have similar chemical environments as the phenolic groups in syringyl units, and their corresponding signals partially overlap. All previous works reporting the phenolic groups of hardwoods by  $^{31}\text{P}$  NMR expressed both types of phenol groups as their sum, except for Rovio et al.<sup>8</sup> In our work, the separation of both signals was achieved with an appropriate deconvolution technique. Figure 5 shows the deconvolution result of the residual kraft lignin sample from Figure 4. Deconvoluted signals 1–4 represent syringyl phenolic groups,<sup>45</sup> and signals 5–7 and 9–15 correspond to C<sub>5</sub>-condensed phenolic groups.<sup>8,41,45,46</sup> Signal 8 might represent both types of phenolic groups. At the final stage (kappa 15), the aliphatic hydroxyl groups were removed to an almost equal extent in all alkaline cookings, but the enrichment of phenolic groups was different (Table 4). The content of phenolic groups in residual kraft lignin at kappa 20 was 157% higher, with

respect to the MWL. The black liquor lignins are even more enriched in phenolic groups. Between kappa number 50 and 15, syringyl phenolic groups were still enriched in the kraft and soda-AQ residual lignins, but in soda-O<sub>2</sub> residual lignins, they were slightly depleted (Table 4). The further enrichment of syringyl phenolic groups in soda-O<sub>2</sub> residual lignin is masked by their reactivity toward active oxygen species, since an increase in the content of phenolic groups implies that cleavage of  $\beta$ -aryl ether linkages in the residual lignin is still occurring.<sup>11,20</sup>

In contrast to residual kraft lignin from pine wood,<sup>20,47</sup> no enrichment of noncondensed guaiacyl phenolic groups was observed in eucalyptus residual lignin. They were depleted until kappa 50–35 and then again reached the same content as in native lignin at kappa 15. By comparing the content of total and C<sub>5</sub>-condensed phenolic groups in residual kraft lignin from softwood,<sup>11,20</sup> and our eucalyptus wood, it comes clear that the enrichment of C<sub>5</sub>-condensed phenolic groups in hardwoods residual lignins is drastically reduced as a consequence of the preferential cleavage of syringyl units, which lowers the abundance of noncondensed guaiacyl phenolic groups (the MWL has a phenolic S/G ratio of 0.6!), preventing condensation reactions from occurring. Condensed phenolic groups show a strong recalcitrance toward oxygen bleaching.<sup>19,26,48</sup> One of the main condensed phenolic groups found in kraft residual lignins of softwood are diarylmethane and *o,p'*-dihydroxystilbene structures.<sup>12</sup> Some signals were observed from *Ca*–C<sub>5'</sub> aryl and C<sub>5</sub>–C<sub>5'</sub> diarylmethane structures (signals 5–7), and *C $\beta$* –C<sub>5'</sub> dihydroxystilbene structures (signal 8, Figure 5). *Ca*–C<sub>5'</sub> aryl structures result from the attack of C<sub>5</sub> carbon ions to the *Ca* position of quinone methide intermediates. C<sub>5</sub>–C<sub>5'</sub> diarylmethane structures are formed by condensation between a noncondensed guaiacyl phenolic unit and formaldehyde released from quinone methide intermediates;<sup>12,45,46</sup> however, we concluded that the abundance of diarylmethane structures in the residual lignins was relatively low, since their corresponding CH<sub>2</sub> cross-signals were not observed in any HSQC spectrum, in agreement with previous works.<sup>47</sup> *C $\beta$* –C<sub>5'</sub> dihydroxystilbene structures result from formaldehyde elimination in  $\beta$ -5' phenylcoumarane structures. However, signal 8 might also present syringyl phenol groups in *C $\beta$* –C<sub>1'</sub> dihydroxystilbene structures. The signals 14 and 15 most probably represented 4-O-5' structures.<sup>46</sup> Other condensed phenolic structures are





**Figure 5.** Deconvolution results of experimental  $^{31}\text{P}$  NMR spectrum of residual kraft lignin at kappa 20, as the sum of individual deconvoluted signals. Signals 1–4 are syringyl phenolic groups (signal 1 represents syringyl groups in syringaresinol); signals 5–7 are condensed phenolic groups in  $C\alpha$ - $C5'$  aryl and  $C5$ - $C5'$  diarylmethane structures; signal 8 represents condensed phenolic groups in  $C\beta$ - $C5'$  dihydroxystilbene structures and/or syringyl phenolic groups in  $C\beta$ - $C1'$  dihydroxystilbene structures; signals 9–13 are condensed phenolic groups in 5–5' biphenol structures; signals 14 and 15 are condensed phenolic groups in 4-O-5' structures.

5–5' biphenols, which represent an important fraction of the recalcitrant structures to posterior bleaching stages.<sup>49</sup> These 5–5' biphenol structures are mainly formed from alkaline cleavage of dibenzodioxocin structures.<sup>35,50</sup> Figures 4 and 5 (signals 9–13) clearly show the presence of these structures in residual and black liquor lignin from the kraft cooking.<sup>41,45</sup> In all residual lignins, two sharp signals were present at 141.61 and 141.66 ppm. However, they were observable as only one signal when the line-broadening factor was increased (signal 13, Figure 5). We suggest that these signals might correspond to a biphenyl catechol structure, based on the experimental data and correlations from previous works,<sup>45</sup> and indirect evidence from our experimental data.

#### 4. CONCLUSIONS

The structural characteristics of the residual lignins isolated from kraft, soda-AQ, and soda- $\text{O}_2$  eucalyptus pulps at different kappa numbers, and the lignins precipitated from the corresponding black liquors, were investigated and compared with the MWL isolated from the same eucalyptus feedstock. At high kappa numbers (kappa 50 and 35), the residual lignins from soda- $\text{O}_2$  pulps presented significantly lower S/G ratios, lower  $\beta$ -O-4' ether linkages, and lower aliphatic and higher carboxylic hydroxyl groups than the kraft and soda-AQ residual lignins.

These results indicate that the soda- $\text{O}_2$  process has more deconstructive character, compared to kraft and soda-AQ processes. Therefore, it is a potential and effective alkaline pretreatment method for use in lignocellulose biorefineries.

#### ■ ASSOCIATED CONTENT

##### 📄 Supporting Information

The general conditions for the kraft, soda-AQ, and soda- $\text{O}_2$  alkaline cooking of eucalyptus feedstock, are shown in Table S1. The identification and relative molar abundance of the compounds identified in the Py-GC/MS of MWL from eucalyptus wood, and residual and black liquor lignins from kraft, soda-AQ, and soda- $\text{O}_2$  pulping at different kappa numbers, are listed in Table S2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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##### Notes

The authors declare no competing financial interest.

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