

MODIFICATION OF THE LIGNIN STRUCTURE OF EUCALYPT FEEDSTOCKS DURING CHEMICAL DECONSTRUCTION BY KRAFT, SODA-AQ AND SODA-O₂ PROCESSING

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ABSTRACT

The modification of the lignin structure in an eucalypt feedstock after alkaline deconstruction by the kraft, soda-AQ and soda-O₂ processes was assessed. For this purpose, the residual lignins isolated from the pulps at kappa levels 50, 35, 20 and 15 were analyzed by different analytical techniques, including 2D-NMR, ³¹P-NMR and SEC (size exclusion chromatography). In addition, dissolved lignins precipitated from the black liquors of the kraft and soda-AQ processes at kappa 20 and 15 were also analyzed. The distribution of the different lignin substructures in the residual lignins was similar to that in the native lignin, although with a drastic reduction in their content. This reduction was more evident in pulps with lower kappa number due to the more drastic pulping conditions.

I. INTRODUCTION

During the deconstruction of the lignin polymer in pulping processes, degradation of preferentially non-condensed moieties is the main reaction, although the accumulation of condensed moieties may also result from condensation reactions. This lowers the delignification rate drastically. Soda processes can induce recalcitrant structures to posterior oxygen bleaching stages. In the production of bioethanol and other fermentation products, the mechanical characteristics of the cellulose pulp fibers are not essential as deconstruction of the whole lignocellulosic matrix is desired for the posterior hydrolysis steps. Therefore the oxygen treatment in alkaline conditions (soda-O₂), which has more drastic consequences for the mechanical fiber properties, is a promising alternative to kraft and soda-AQ as pretreatment for biorefinery processes. In this work, we have studied the modifications of the lignin structure during alkaline processes (kraft, soda-AQ and soda-O₂) at different kappa numbers. For this purpose, the residual lignins were isolated from these pulps and subsequently analyzed by an array of analytical techniques (2D-NMR, ³¹P-NMR and SEC).

II. EXPERIMENTAL

Samples. The pulps and black liquors selected for this study arise from the alkaline pulping of a Eucalypt hybrid *E. grandis* × [*E. urophylla* × *E. globulus*] (G1×UGL) feedstock, from the Brazilian Network of Eucalyptus Genome Research (Grattapaglia, 2004).

Lignin isolation. The residual lignins were isolated from the pulps by acidolysis according to (Evtuguin et al., 2001). A two stage extraction of both 40 minutes under argon atmosphere using a dioxane:water (82:18 v/v) mixture with 0.1 M HCl was carried out, first at 13.3 and then at 10.0 ml/g dry pulp. After freeze-drying, the lignins were extracted with n-pentane during 8 hours in Soxhlet devices. The lignins of the black liquors were precipitated and air dried at 40 °C.

2D-NMR. Around 40 mg of lignin was dissolved in 0.750 mL of DMSO-d₆. NMR spectra were recorded at 25 °C on a Bruker AVANCE 600 MHz instrument equipped with a cryogenically cooled z-gradient triple-resonance probe. The acquisition and processing parameters are described in (del Río et al., 2012).

³¹P-NMR. The phosphorylation of dry lignin samples was performed according to (Granata and Argyropoulos, 1995). The ³¹P-NMR spectra were recorded on a Bruker Avance III 500 MHz

spectrometer using a 90° pulse width and an inverse-gated decoupling sequence. CDCl₃ was the locking solvent and 512 scans were accumulated 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 and the line broadening was set to 2 Hz, followed by exponential window multiplication, Fourier transformation, automatic/manual phase correction and automatic baseline correction. For all samples the gain of 203 scans was attained and the integration of the internal standard (IS) signal was set to 1. Additionally the peak width at half height of the IS signal was checked to guarantee a satisfactory resolution and an exact quantification. After establishing optimal deconvolution parameters used for all samples, the deconvolutions of the syringylic - and condensed hydroxyl group signals were processed with a line broadening factor of 8 Hz. Between 12 and 20 peaks were used in the 144.2 – 141.ppm region to obtain an exact deconvolution result (Bruker topspin software).

SEC. The molar mass measurements are relative measurements. They were performed by Waters HPLC in 0.1M NaOH eluent using PSS's MCX 1000 and 100 000 Å columns with UV detection at 280 nm. The average molar masses (M_n , M_w) and the molar mass distributions were calculated relative to the sulphonated Na-polystyrene (Na-PSS, 3 420-148 500 g/mol) standards using Waters Empower 2 software. For SEC analysis, about 4 mg lignin was dissolved overnight in 4 ml analytical NaOH (0.1 M) and filtered with 0.45 µm PTFE membrane syringe filters (VWR).

III. RESULTS AND DISCUSSION

Lignin isolation

Table 1 shows that the extraction yield was higher at higher kappa levels, and higher for the soda-AQ than the Soda-O₂ pulp. The yield at kappa 15 is similar for the three different pulps.

Table 1: Extraction yield (%) of lignin isolation by acidolysis of different pulps of G1×UGL eucalyptus based on theoretical content of: % lignin in pulp = 0.15 × K (K = kappa number).

Process	Kraft		Soda-AQ				Soda-O ₂		
Kappa number	K20	K15	K50	K35	K20	K15	K50	K35	K15
	35	26	82	63	28	28	60	41	28

¹³C - ¹H HSQC 2D-NMR

The detailed structural characteristics of the MWL of G1×UGL eucalyptus from HSQC data has been reported in (Prinsen et al., 2012). From **Table 2** it can be seen that the distributions of the inter-unit linkages in the residual lignins are similar to that in the native lignin, although with a drastic reduction in their content. This reduction was more evident in the pulps with lower kappa number due to the more drastic pulping conditions.

Table 2: Content of lignin inter-unit linkages (expressed as linkages per 100 unmodified aromatic units) and S/G ratio in MWL of G1×UGL eucalypt wood, and in residual - and black liquor lignin from kraft, soda-AQ and soda-O₂ processes at different kappa levels.

Inter-unit linkage	MWL	Residual lignin									Black liquor lignin			
		Kraft			Soda-AQ			Soda-O ₂			Kraft		Soda-AQ	
		K20	K15	K50	K35	K20	K15	K50	K35	K15	K20	K15	K20	K15
β-O-4' ether	84	35	24	77	68	35	23	48	44	19	2	2	1	0
β-β' resinol	11.3	10.6	13.9	14.9	12.6	12.2	14.5	17.0	15.7	13.3	14.0	14.0	25.3	25.3
β-5' phenylcoumaran	4.3	0.8	0.2	1.4	1.2	0.9	0.8	1.0	1.6	1.7	0.0	0.0	0.0	0.0
S/G ratio	2.8	4.4	3.1	4.5	4.4	4.5	3.6	4.2	3.6	3.0	13.5	21.0	18.2	21.9

At kappa number 20 and 15, the content of β-O-4' ether linkages in the residual lignins was almost identical for the soda-AQ - as for the kraft process. The lignins precipitated from the respective black liquors were enriched in S-units, which are predominantly forming β-O-4' ether structures. This indicates that they are preferentially removed during pulping. Interestingly, the black liquor lignins from soda-AQ were more enriched in S-lignin units than those from the kraft process, suggesting that the soda-AQ is more efficient to depolymerize eucalypt lignin, although differences were not significant. From higher to lower kappa numbers the estimated S/G ratios of

the residual lignins decreased as their respective β -O-4' linkages content decreased. At kappa 15 the degradation of β -O-4' ether - and β - β' resinol linkages was similar in the kraft - and soda-AQ pulps. The soda-O₂ process seems to be more efficient in the degradation of β -O-4' ether linkages.

³¹P-NMR

The hydroxyl group (OH) content of lignins can be determined with quantitative ³¹P-NMR (Granata and Argyropoulos, 1995). When using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane as phosphitylating reagent, the aliphatic -, phenolic - and carboxylic OH signals are well resolved. In the case of hardwoods, the syringylic OH signals overlap with the condensed guayacyl OH signals. The same is observed in grasses, which show an additional *p*-hydroxyphenol OH signal. The aim of this work was the accurate separation of syringylic - and total condensed OH signals, although some condensed structures like 4-O-5' may overlap completely with the syringylic OH (high resolution needed for deconvolution). The deconvolution was carried out for all samples to quantify syringylic - and condensed OH content. The results are shown in **Table 3**.

Table 3: Aliphatic -, phenolic - (syringylic + guayacylic + condensed + catechol) and carboxylic hydroxyl content determined by quantitative ³¹P-NMR (mmol OH /g pure lignin) in G1xUGL eucalypt wood, residual - and black liquor lignin from kraft, soda-AQ and soda-O₂ processes at different kappa levels. The standard deviation was calculated from 3 independent samples of one bulk sample + phosphitylation + ³¹P-NMR recording + integration + deconvolution for syringylic OH, total condensed OH and 5-5' biphenylic content.

OH group	MWL	Residual lignin									Black liquor lignin				
		Kraft			Soda-AQ				Soda-O ₂			Kraft		Soda-AQ	
		K20	K15	K50	K35	K20	K15	K50	K35	K15	K20	K15	K20	K15	
Aliphatic (± 0.023)	4.69	2.20	1.69	3.33	2.80	2.07	1.73	2.81	2.55	1.75	1.07	0.60	1.06	0.83	
Phenolic (± 0.010)	0.67	1.72	2.05	1.47	1.56	2.13	2.25	1.64	1.71	1.82	2.50	2.83	2.57	2.42	
Syringylic (± 0.034)	0.21	0.75	0.85	0.86	0.69	1.01	1.13	0.87	0.79	0.79	1.52	1.74	1.51	1.37	
Total condensed (± 0.041)	0.07	0.64	0.78	0.34	0.62	0.72	0.69	0.43	0.57	0.64	0.52	0.51	0.60	0.62	
5-5' biphenylic (± 0.003)	0	0.096	0.098	0.052	0.090	0.072	0.066	0.048	0.036	0.033	0	0	0	0	
Guayacylic (± 0.002)	0.38	0.27	0.36	0.25	0.21	0.35	0.39	0.32	0.33	0.37	0.46	0.58	0.46	0.43	
Catechol (± 0.001)	0	0.057	0.065	0.019	0.033	0.044	0.037	0.016	0.023	0.022	0	0	0	0	
Carboxylic (± 0.002)	0.005	0.25	0.27	0.09	0.09	0.20	0.26	0.14	0.18	0.24	0.66	0.54	0.66	0.53	
Total OH (± 0.034)	5.37	4.17	4.01	4.89	4.44	4.40	4.24	4.59	4.43	3.81	4.24	3.97	4.29	3.77	
S/G ratio (± 0.11)	0.6	2.7	2.4	3.5	3.2	2.9	2.9	2.7	2.4	2.1	3.3	3.0	3.3	3.3	

A negative linear correlation ($r^2 = 0.99$) was observed between the aliphatic - and phenolic OH content for both MWL/kraft and MWL/soda-AQ data. In the case of MWL/soda-O₂ the phenolic OH content remained more constant ($r^2 = 0.94$ for linear correlation). The higher phenolic content in the residual lignin at K15 of the soda-AQ process was not a consequence of a higher condensed OH content, but mainly resulted from a higher syringylic OH content. Identical condensed OH contents were calculated from samples of two independent but similar soda-AQ pulping processes and were in contrast with the kraft residual lignin (higher condensed OH content at K15). The carboxylic OH content in the black liquor lignins was 2-3 times higher than in the corresponding residual lignins, although it remained unclear if the signals at 134.61 (black liquors) and 134.69 (residual lignins) ppm represented carboxylic groups present in lignin or in fatty acids (Dyer et al., 2002). Interestingly, a linear correlation ($r^2 = 0.91$) was observed between the content of 5-5' biphenyl structures and catechol structures. This indicates that both signals might come from one structure. Further investigation is needed to confirm this.

SEC

From the results in **Table 2 and 4** an exponential correlation ($r^2 > 0.99$) was found between the β -O-4' linkages content and the molar mass M_w of the residual lignins of the soda-AQ process. For

the soda-O₂ process the exponential fit was less appropriate ($r^2 = 0.88$), because the β -O-4' linkages content was significant lower at kappa 50 and 35. At kappa 20 and 15 the molar masses of the residual lignins were similar for the different processes. The lowest molar mass at kappa 15 was found in the kraft residual lignin. The black liquor lignins had a molar mass of 55 and 65 % of the corresponding residual lignins at respectively kappa 20 and 15. Similar results were observed for the soda-AQ black liquor lignins (53 and 61 %).

Table 4: Molar masses M_n and M_w (Daltons) and polydispersity of MWL of G1xUGL eucalypt wood, and residual - and black liquor lignin from kraft, soda-AQ and soda-O₂ processes at different kappa numbers.

	MWL	Residual lignin									Black liquor lignin			
		Kraft			Soda-AQ			Soda-O ₂			Kraft		Soda-AQ	
		K20	K15	K50	K35	K20	K15	K50	K35	K15	K20	K15	K20	K15
M_n	1891	2440	2370	2480	2510	2710	2530	2380	2360	2300	1680	1640	1660	1580
M_w	4430	4340	3490	10550	8300	4770	3870	9380	6080	3640	2390	2280	2540	2350
Polydispersity	2.34	1.78	1.47	4.25	3.30	1.76	1.53	3.93	2.58	1.58	1.42	1.39	1.53	1.48

IV. CONCLUSIONS

The residual lignins of G1*UGL eucalypt isolated by acidolysis and the black liquor lignins from the kraft, soda-AQ and soda-O₂ processes at different kappa levels showed rather similar results for the interunit linkages distribution, hydroxyl group content and molar mass. The residual lignin of the soda-O₂ process showed more significant differences: lower β -O-4' linkages at similar molar mass (or lower molar mass at the same kappa number), more constant phenolic hydroxyl group content at different kappa levels and lower total hydroxyl group content.

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