

# **Revealing the Molecular Structural Transformation of Hardwood and Softwood in Dilute Acid Flowthrough Pretreatment**

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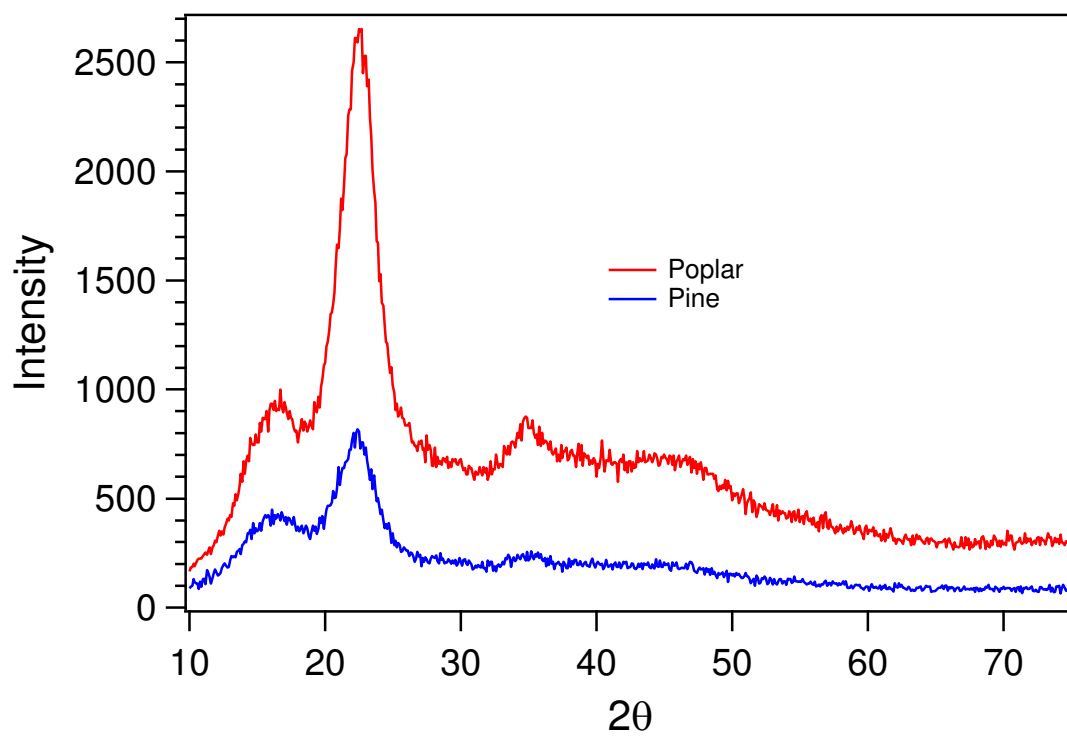
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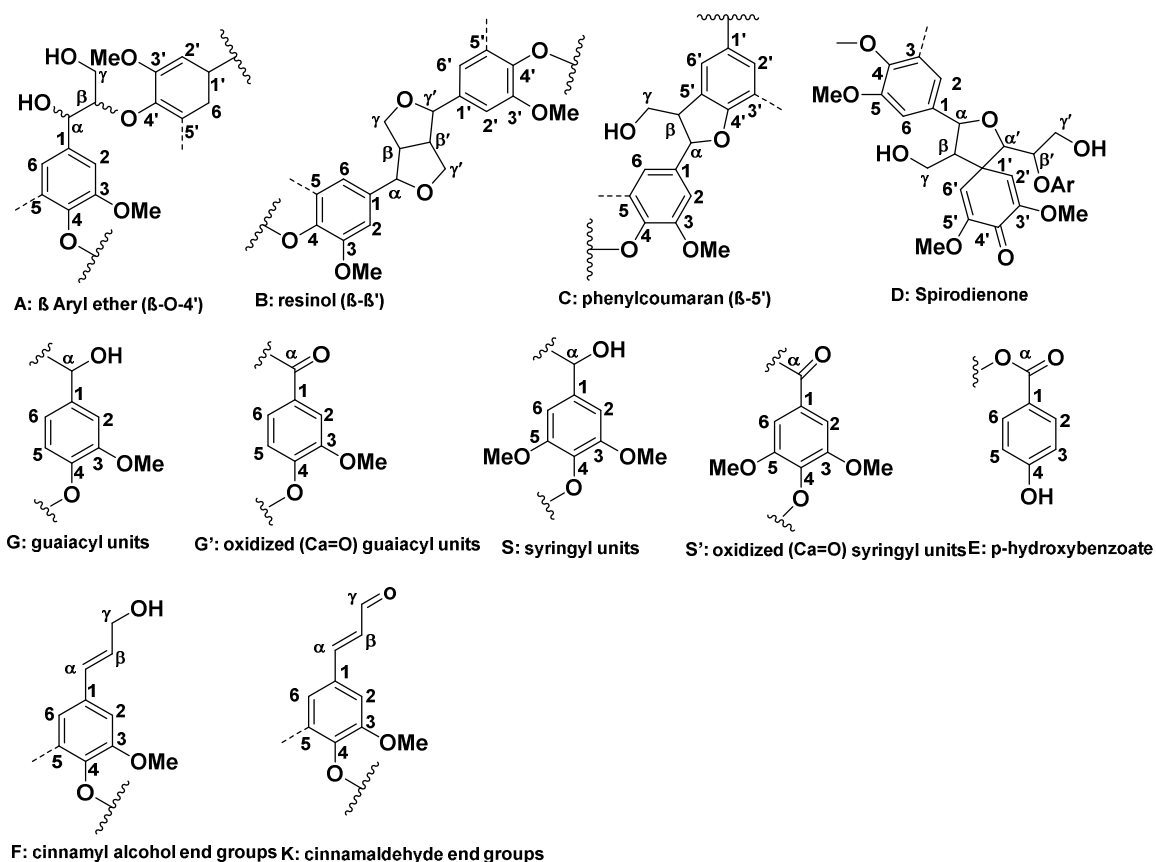
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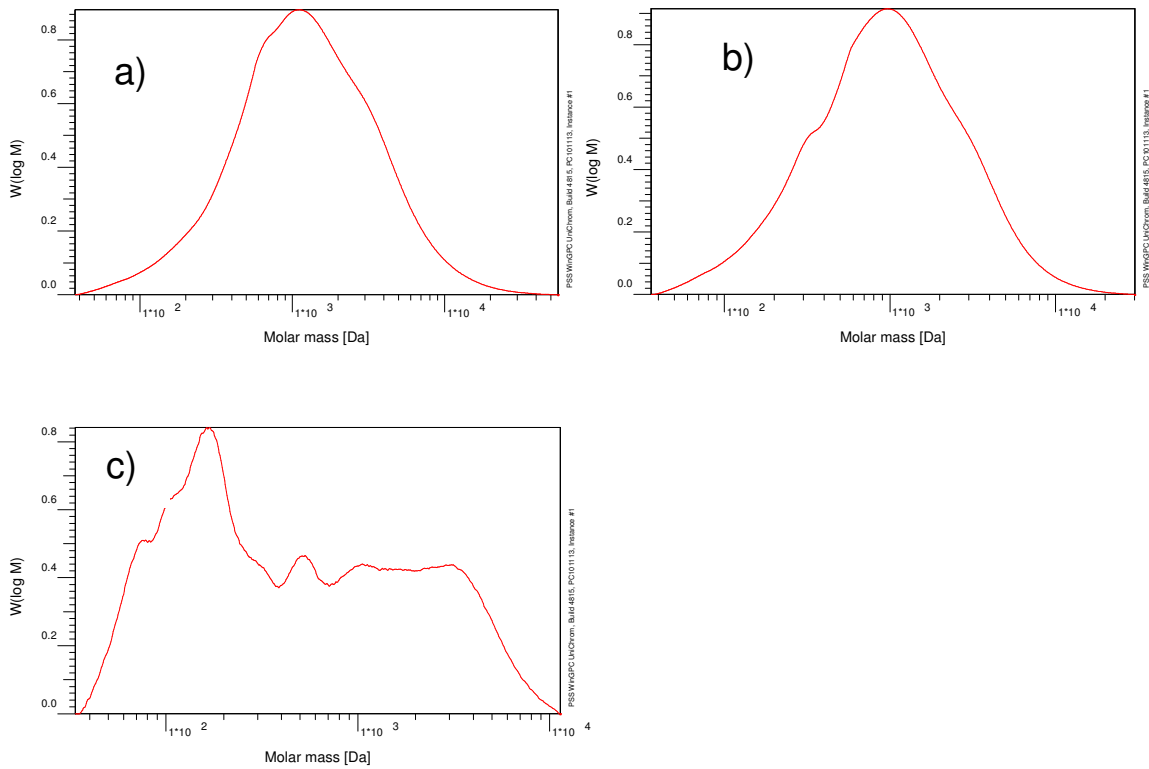
6 pages, 3 figures, and 2 tables



**Figure S1.** XRD analysis of poplar and pine wood



**Figure S2.** Assigned interunit linkages of lignin, including different side-chain linkages, and aromatic units: (A)  $\beta$ -O-4 aryl ether linkages; (B) resinol substructures ( $\beta$ - $\beta'$ ,  $\alpha$ -O- $\gamma'$ , and  $\gamma$ -O- $\alpha'$  linkages); (C) phenylcoumaran substructures ( $\beta$ -5' and  $\alpha$ -O-4' linkages); (D) spirodienone substructures ( $\beta$ -1' and  $\alpha$ -O- $\alpha'$  linkages); (G) guaiacyl units; (G') oxidized guaiacyl units with an C $\alpha$ -ketone; (S) syringyl units; (S') oxidized syringyl units with a C $\alpha$  ketone; (H) hydroxyphenyl groups; (E) p-hydroxybenzoate substructures; (K) cinnamaldehyde end groups.



**Figure S3.** GPC analysis of lignin molecular weight of a) poplar flowthrough pretreatment recovered insoluble lignin, b) lodgepole pine wood flowthrough pretreatment recovered insoluble lignin, c) lodgepole pine wood flowthrough derived residual lignin under 240°C with 0.05% (w/w)  $H_2SO_4$  for 10min.

**Table S1.** Enzymatic hydrolysis of flowthrough pretreated lodgepole pine wood whole slurries.

Pretreatment conditions				Enzyme loading (mg/g glucan + xylan)	Glucose yield (% of original glucose <sup>a</sup> )
Temp.	t	H <sub>2</sub> SO <sub>4</sub>	LogR <sub>0</sub>		72h
220	2	0.05	3.83	20	70.9
				100	73.6
220	4	0.05	4.14	20	75.5
				100	80.1
220	6	0.05	4.31	20	85.7
				100	91.7
220	8	0.05	4.44	20	87.7
				100	92.6
220	10	0.05	4.53	20	85.9
				100	94.6
240	2	0.05	4.61	20	85.8
				100	93.7
240	4	0.05	4.72	20	87.4
				100	90.8
240	6	0.05	4.90	20	90.4
				100	92.7
240	8	0.05	5.03	20	90.9
				100	96.7
240	10	0.05	5.12	20	92.1
				100	97.2
270	2	0.05	5.20	20	82.4
				100	90.7
270	4	0.05	5.61	20	80.7
				100	89.5
270	6	0.05	5.78	20	79.4
				100	88.9
270	8	0.05	5.91	20	80.4
				100	86.7
270	10	0.05	6.01	20	75.8
				100	82.1

<sup>a</sup>based on original glucose content in loaded biomass; Temp. refers to the pretreatment temperature (°C), t refers to the pretreatment time (min), and H<sub>2</sub>SO<sub>4</sub> refers to the concentration of dilute acid, % (w/w). 20 mg protein Ctec2 (12 FPU) with 4 mg protein Htec2/g glucan + xylan; 100 mg protein Ctec 2 (60 FPU) with 20mg Htec2/g glucan + xylan.

**Table S2.** Assignments of main lignin  $^1\text{H}$ - $^{13}\text{C}$  cross-peaks in the HSQC Spectra of the pretreatment recovered insoluble lignin <sup>2-7</sup>.

Lignin linkages and monolignol	Chemical shift
-OCH <sub>3</sub>	55.47(C) 3.70(H)
A: $\beta$ -O-4	71.70(S-C $\alpha$ ) 4.84(S-H $\alpha$ ) 59.50-59.70(C $\gamma$ ) 3.40-3.63(H $\gamma$ ) 83.49(G/H-C $\beta$ ) 4.28(G/H-H $\beta$ )
B: resinol	84.85(C $\alpha$ ) 4.62(H $\alpha$ ) 53.30(C $\beta$ ) 3.05(H $\beta$ ) 70.85(C $\gamma$ ) 4.14/3.78(H $\gamma$ )
C: phenylcoumaran	86.79(C $\alpha$ ) 5.41(H $\alpha$ ) 53.3(C $\beta$ ) 3.46(H $\beta$ ) 62.52(C $\gamma$ ) 3.68(H $\gamma$ )
G: guaiacyl	111.02(C2) 6.95(H2) 115.05(G5) 6.74(H5) 119.01(G6) 6.78(H6)
G': oxidized (C $\alpha$ =O) guaiacyl	111.56(C2) 7.50(H2) 123.55(C6) 7.54(H6)
S: syringyl	103.95(C2/6) 6.67(H2/6)
S': oxidized (C $\alpha$ =O) syringyl	106.52(C2/6) 7.29(H2/6)
E: <i>p</i> -hydroxybenzoate	131.33(C2/6) 7.62(H2/6)
K: cinnamaldehyde	126.23(C $\beta$ ) 6.74(H $\beta$ )

<sup>a</sup>Note: G, S, H-C or G, S, H-H refers to C and H in the lignin sub-units, guaiacyl,

syringyl and *p*-hydroxyphenyl.

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