Electronic Supplementary Material (ESI) for RSC Sustainability.

The effects of lignin source and extraction on the composition and properties of biorefined depolymerization products

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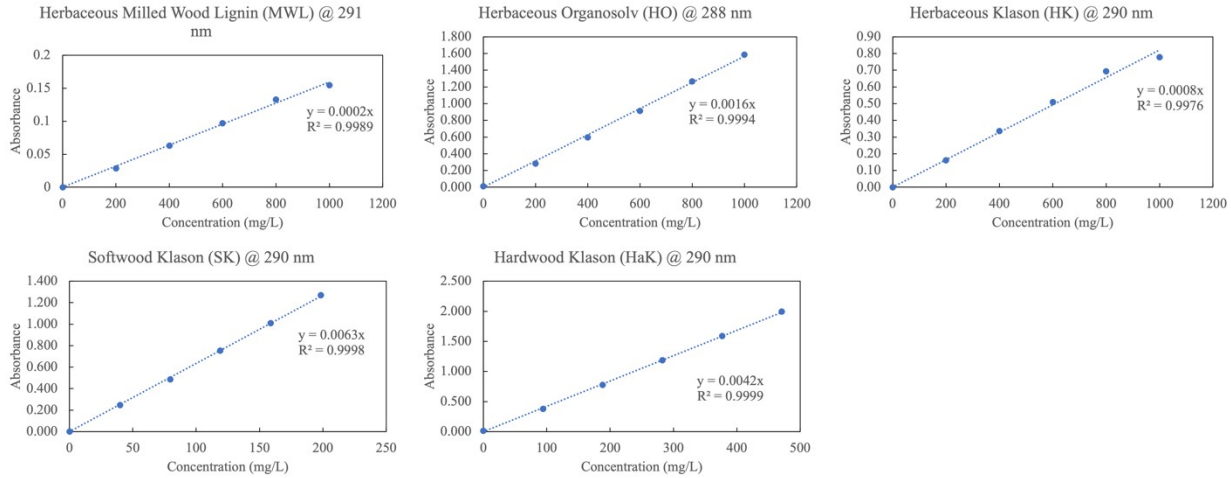

Figure 1. Calibration curves for the Milled Wood, Organosolv, and Klason extraction of herbaceous (corn stover) lignin and the Klason extractions of softwood (white spruce) and hardwood (bitternut hickory) lignin. The MWL and Organosolv calibrations were made with a 20x dilution, the herbaceous Klason was made with a 10x dilution, and the softwood and hardwood were made with a 2x dilution. The softwood and hardwood did not have full dissolution with pH 14 NaOH so the remaining solids were dried and weighed to determine the actual concentration in solution.

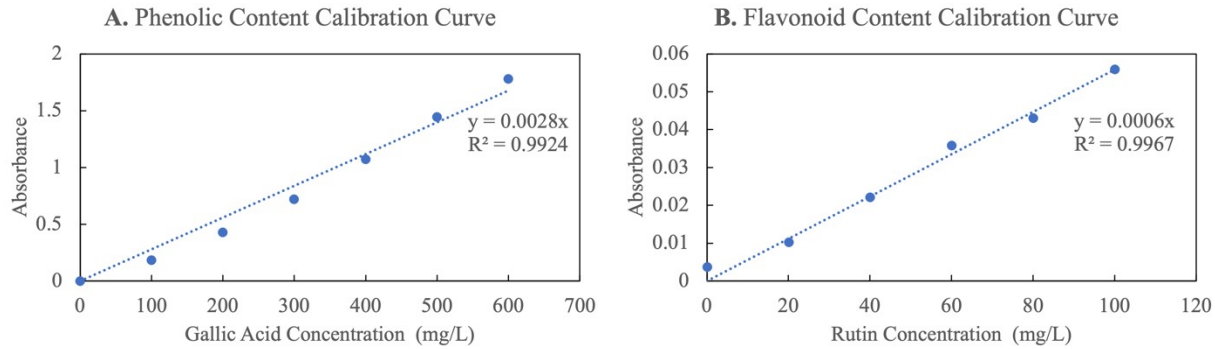

Figure 2. a) The calibration curve for the Folin-Ciolcalteu method, made with gallic acid (3, 4,5-trihydroxybenzoic acid) at 750 nm. b) The calibration curve for the Zhishen method, made with rutin (3',4',5,7-Tetrahydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]flavone) at 510 nm.

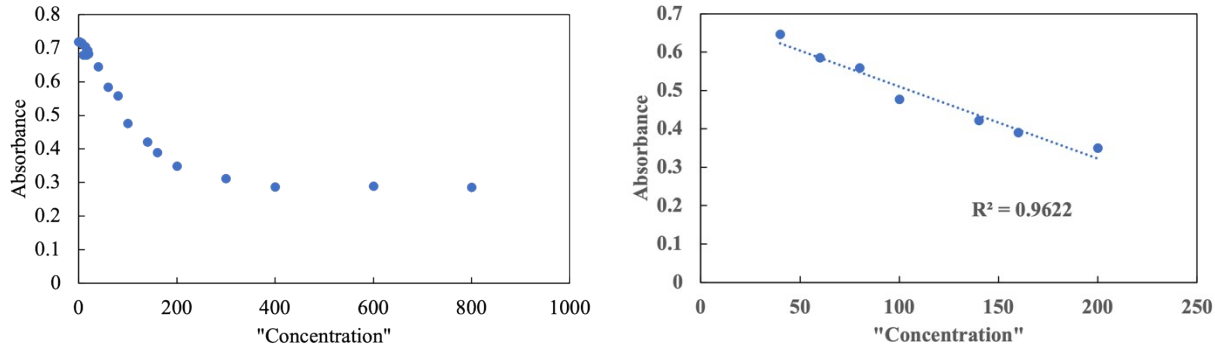

Figure 3. The inhibition of absorbance produced by the phosphate buffer at pH 10.8. Under 20 mg/L of buffer, the inhibition is negligible (SD < 0.01). Between 20-200 mg/L the inhibition increases linearly. Above 200 mg/L the inhibition is constant (SD < 0.01). The correction factors are listed in Table S1.

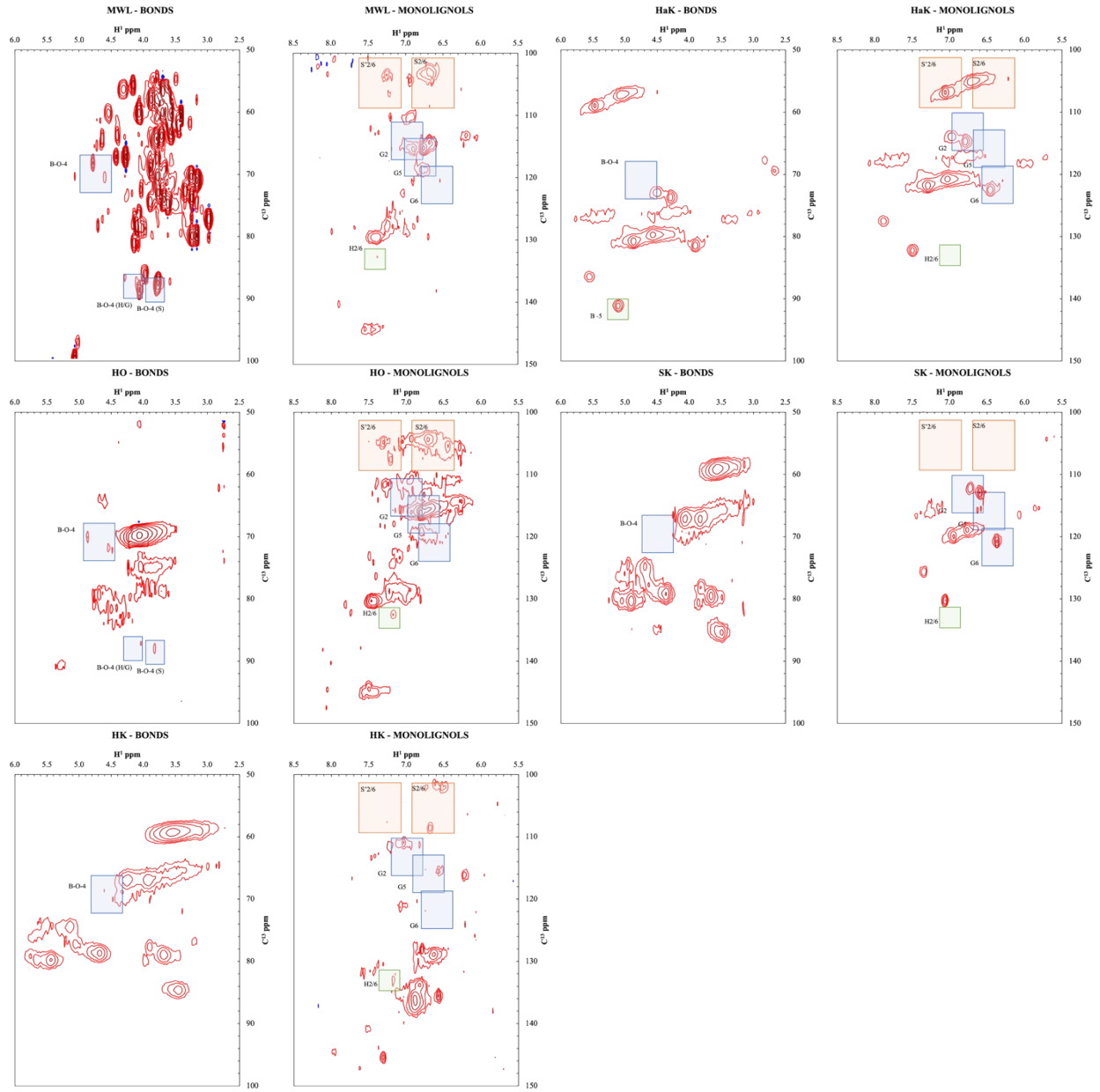

Figure 4. NMR spectra for aromatic and aliphatic regions for all lignin extractions.

β-O-4 percentage and S/G ratio was calculated based on the regions and equations reported by Zijlstra et al [1] and Mansfield et al [2].
The following regions were used for the determination of the aromatic ratios:
S2/6: [(6.48-6.90)(104-109)]
S'2/6: [(7.17-7.50)(105-109)]
G2: [(6.78-7.14)(111.5-116)]
G5: [(6.48-7.06)(115-120.5)]
G6: [(6.65-6.96)(120.5-124.5)]
H2/6: [(7.05-7.29)(128.5-133)]

The H/G/S ratios were calculated with the following equations:
Equation S1:Total aromatic =(((S2/6 + S'2/6)/ 2)) + ((G2 + G5 + G6 – H2/6) / 3) + (H2/6 / 2)
Equation S2: Ratio S = (((S2/6 + S'2/6) / 2): total aromatic x 100%
Equation S3: Ratio G = ((G2 + G5 + G6 – H2/6) / 3) : total aromatic x 100%
Equation S4: Ratio H = (H2/6 / 2) : total aromatic x 100%

The following regions were used for the determination of the linking motifs:
β-O-4α [(4.76-5.10)(73-77.5)]
β'-O-4α [(4.44-4.84)(81.5-86)]

The number of linking motifs were calculated with the following equations:
Equation S5: # β-O-4 linkages = ((β-O-4α + β'-O-4α) / total aromatic x 100)

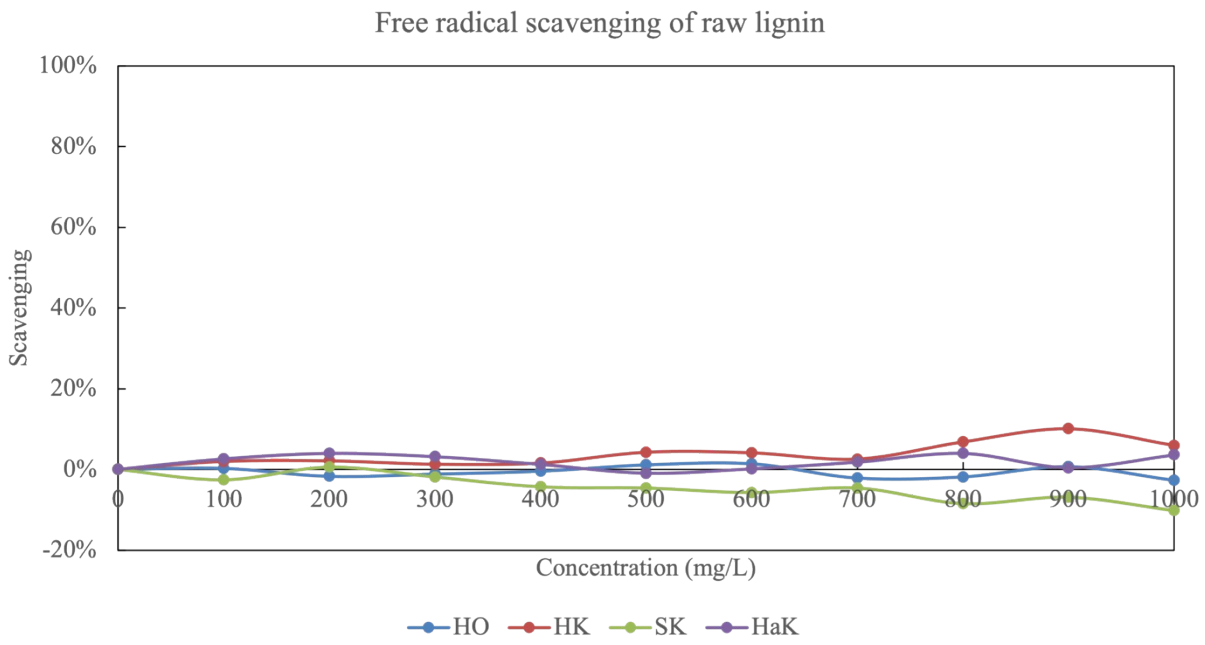

Figure 5. ABTS scavenging for herbaceous Organosolv (HO) and the Klason extractions of herbaceous (HK), softwood (SK), and hardwood (HaK)lignin in water. We see no antioxidant activity.

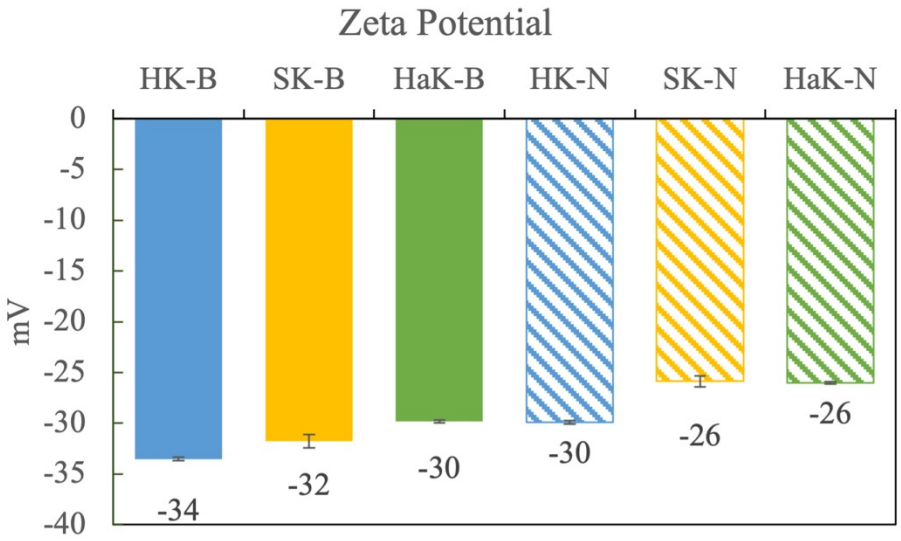

Figure 6. The zeta potential in mV for HK, SK, and HaK in basic and neutral conditions. Zeta potential indicates moderate stability in basic conditions.

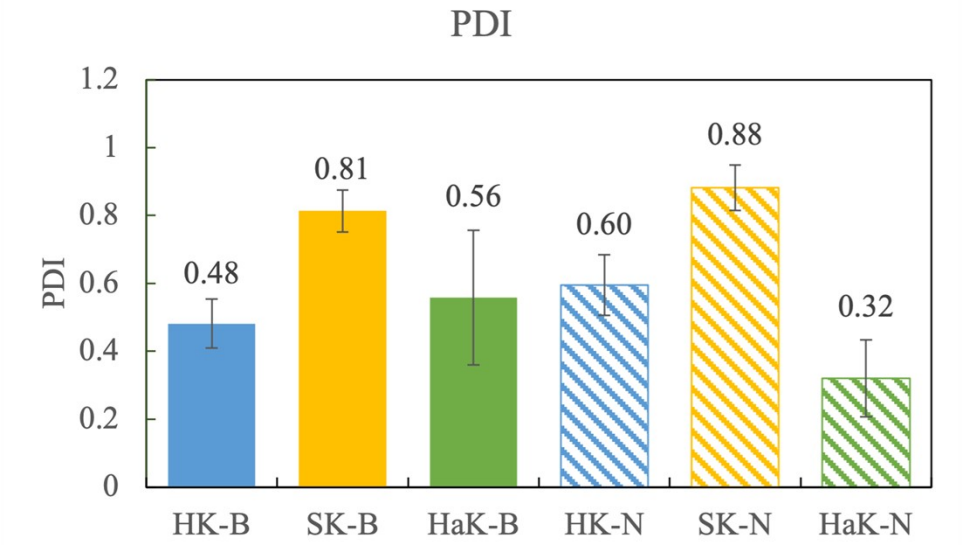

Figure 7. The PDI for HK, SK, and HaK in basic and neutral conditions. PDI of <0.05 indicates a monodisperse sample. PDI > 0.7 indicates highly polydisperse sample.

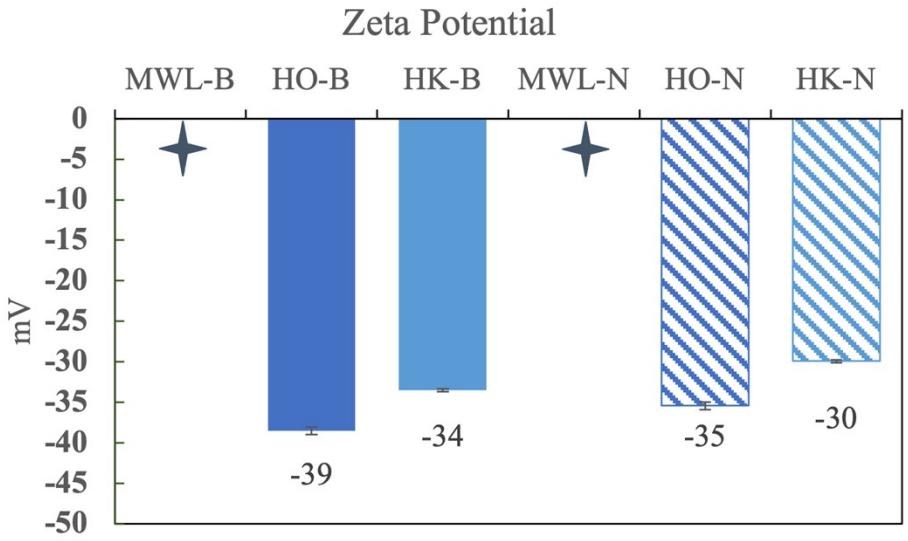

Figure 8. The zeta potential in mV for HO and HK in basic and neutral conditions. Zeta potential indicates moderate stability in basic conditions. A reading could not be done for MWL.

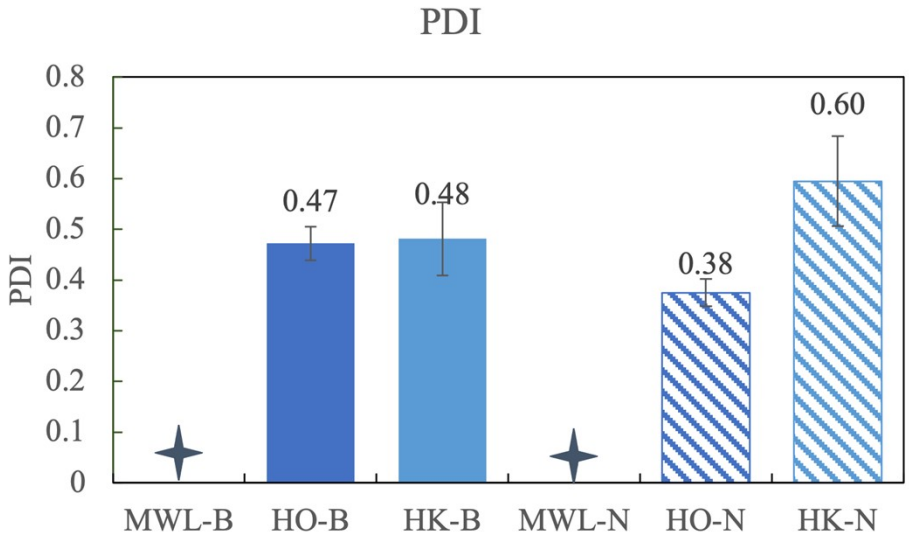

Figure 9. The PDI for HO and HK in basic and neutral conditions. PDI of <0.05 indicates a monodisperse sample. PDI > 0.7 indicates highly polydisperse sample. A reading could not be done for MWL.

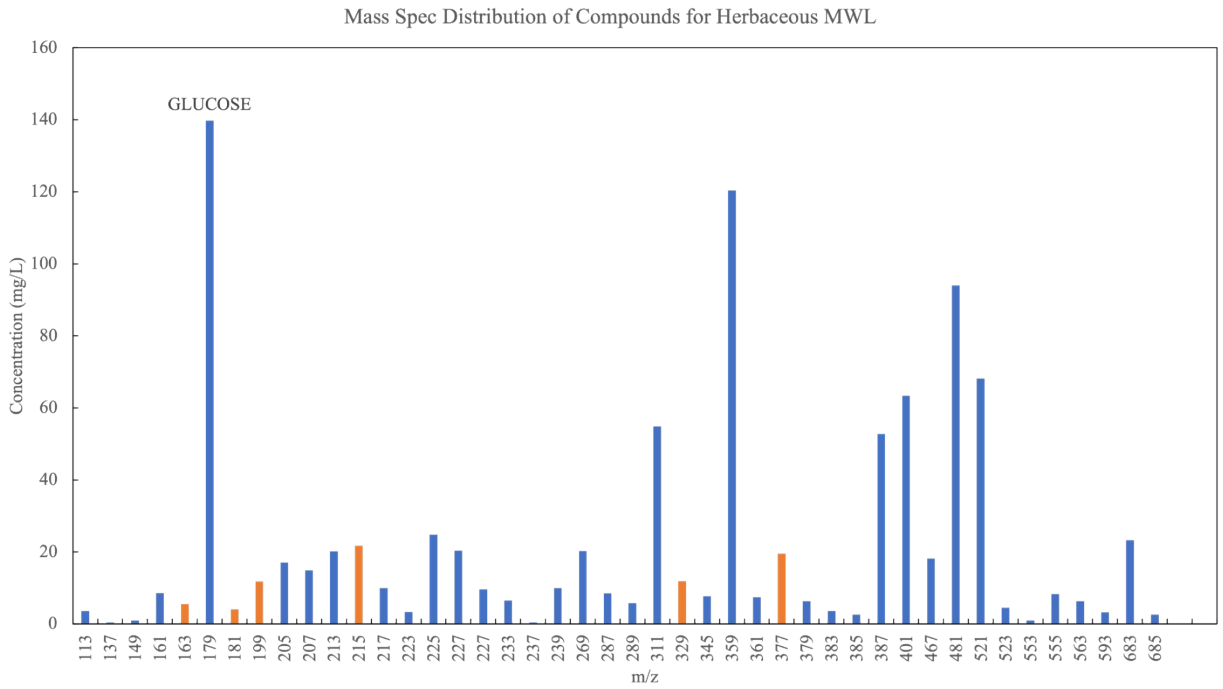

Figure 10. The distribution of compounds from solubilized MWL. The orange indicate compounds that are aromatic.

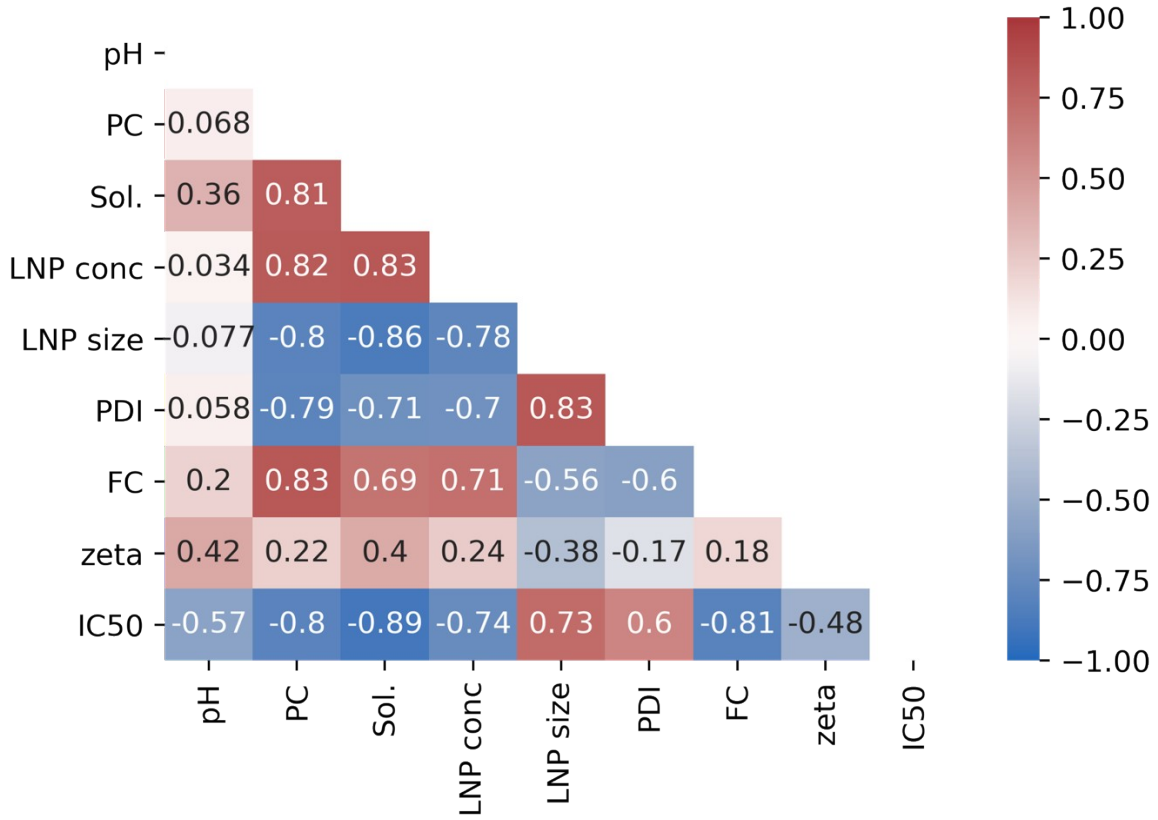

Figure 11. The Spearman correlation between various parameters. The higher the value (and darker the shade), the stronger the correlation. Red indicates a positive relationship and blue indicates a negative relationship. Note that IC50 is inverse to antioxidant capacity. Therefore a blue value with IC50 indicates a positive correlation with antioxidant capacity.

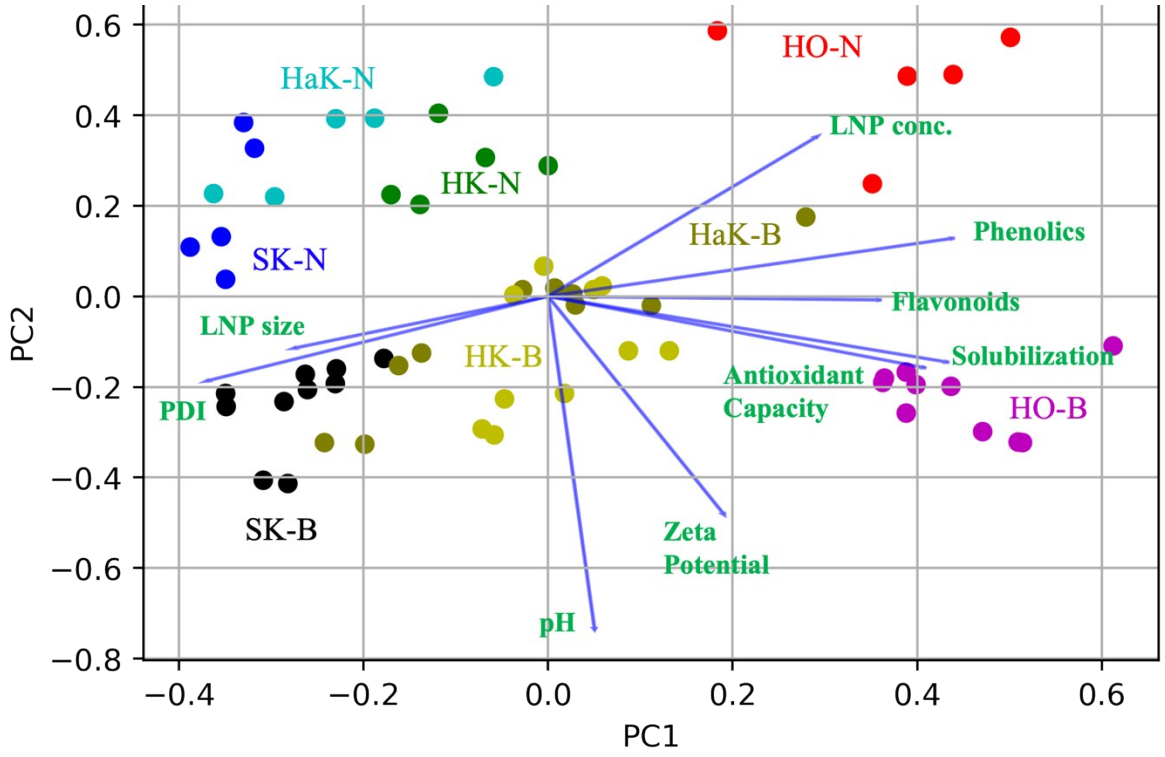

Figure 12. PCA of all depolymerization experiment of HO, HK, SK, and HaK in neutral and basic conditions. The arrows represent various product characteristics and the dots represent individual experiments.

Table 1. Alternative calculations for product properties. Solubilization (sol), LNP conc. (concentration), size, PDI, PC (phenolic content), FC (flavonoid content), zeta (potential), and IC50 data along with its associated error for Klason extractions of herbaceous (HK), softwood (SK), and hardwood (HaK) lignin at basic (B) and neutral (N) conditions for a 14 day time average, the time at which max solubilization occurs, and the time at which minimum IC50 occurs. In the case of the Klason extractions, maximum solubilization and minimum IC50 occur at the same time – day 14.

			value								RSE							
		time (days)	Sol. (mg/L)	LNP conc. (mg/L)	Size (nm)	PDI	PC (mg/L)	FC (mg/L)	Zeta (-mV)	IC50 (g/L)	Sol.	LNP conc.	Size	PDI	PC	FC	Zeta	IC50
HK-B	AVG	-----	503.7	144.9	1495.3	0.68	146.0	50.6	31.0	113.9	11%	18%	32%	8%	9%	14%	-4%	6%
	Max depol.	14	620.9	212.9	3417.1	0.48	186.1	52.3	33.5	107.4	6%	9%	36%	15%	6%	22%	-1%	2%
	lowest IC50	14	620.9	212.9	3417.1	0.48	186.1	52.3	33.5	107.4	6%	9%	36%	15%	6%	22%	-1%	2%
SK-B	AVG	-----	26.1	9.1	3760.8	0.88	66.3	73.8	31.4	834.5	14%	30%	18%	6%	18%	15%	-2%	16%
	Max depol.	14	33.4	18.6	6252.8	0.81	109.8	110.5	31.8	505.3	46%	7%	24%	8%	23%	14%	-2%	14%
	lowest IC50	14	33.4	18.6	6252.8	0.81	109.8	110.5	31.8	505.3	46%	7%	24%	8%	23%	14%	-2%	14%
HaK-B	AVG	-----	263.0	117.3	2364.0	0.82	212.3	138.3	29.0	148.9	22%	42%	12%	9%	17%	25%	-4%	34%
	Max depol.	14	345.1	283.9	3379.8	0.56	318.6	231.0	29.8	72.3	1%	16%	15%	36%	2%	4%	0%	2%
	lowest IC50	14	345.1	283.9	3379.8	0.56	318.6	231.0	29.8	72.3	1%	16%	15%	36%	2%	4%	0%	2%
HK-N	AVG	-----	260.9	106.3	1303.7	0.76	137.8	82.1	29.0	201.1	6%	29%	11%	6%	10%	16%	-2%	8%
	Max depol.	14	295.4	109.3	751.0	0.60	155.7	107.2	29.9	167.2	6%	9%	36%	15%	6%	22%	-1%	2%
	lowest IC50	14	295.4	109.3	751.0	0.60	155.7	107.2	29.9	167.2	6%	9%	36%	15%	6%	22%	-1%	2%
SK-B	AVG	-----	12.0	9.4	3035.1	0.94	39.0	31.8	29.2	995.9	16%	30%	5%	4%	17%	52%	-5%	8%
	Max depol.	14	19.3	14.0	3296.7	0.88	49.4	87.4	25.9	729.8	46%	7%	24%	8%	23%	14%	-2%	14%
	lowest IC50	14	19.3	14.0	3296.7	0.88	49.4	87.4	25.9	729.8	46%	7%	24%	8%	23%	14%	-2%	14%
HaK-B	AVG	-----	32.9	16.9	2042.9	0.73	133.2	49.2	27.3	569.5	7%	48%	11%	17%	17%	41%	-2%	13%
	Max depol.	14	36.2	46.8	2043.3	0.32	191.0	123.1	26.0	385.6	1%	16%	15%	36%	2%	4%	0%	2%
	lowest IC50	14	36.2	46.8	2043.3	0.32	191.0	123.1	26.0	385.6	1%	16%	15%	36%	2%	4%	0%	2%

Table 2. Alternative calculations for product properties Solubilization (sol), LNP conc. (concentration), size, PDI, PC (phenolic content), FC (flavonoid content), zeta (potential), and IC50 data along with its associated error for Milled wood lignin (MWL), Organosolv (HO) and Klason(HK) extractions of herbaceous lignin at basic (B) and neutral (N) conditions for a 14 day time average, the time at which max solubilization occurs, and the time at which minimum IC50 occurs.

		Value									RSE							
		Time (days)	Sol. (mg/L)	LNP conc. (mg/L)	Size (nm)	PDI	PC (mg/L)	FC (mg/L)	Zeta (- mV)	IC50 (g/L)	Sol.	LNP conc.	Size	PDI	PC	FC	Zeta	IC50
MWL-B	AVG	-----	627.5	61.5	1849.0	0.95	48.6	28.2	43.0	316.5	7%	25%	4%	1%	2%	47%	8%	27%
	Max depol.	0	671.4	45.9	1771.7	0.96	47.5	15.0	46.6	230.3	4%	12%	13%	4%	6%	6%	2%	15%
	lowest IC50	0	671.4	45.9	1771.7	0.96	47.5	15.0	46.6	230.3	4%	12%	13%	4%	6%	6%	2%	15%
HO-B	AVG	-----	826.4	209.4	474.5	0.49	349.4	191.1	34.6	33.5	4%	31%	10%	5%	3%	17%	3%	5%
	Max depol.	7	935.9	465.3	446.4	0.47	373.3	295.1	38.5	34.4	10%	3%	3%	7%	2%	0%	1%	7%
	lowest IC50	0	720.2	138.8	424.4	0.47	349.8	234.0	34.8	29.7	4%	6%	3%	5%	3%	4%	2%	4%
HK-B	AVG	-----	503.7	144.9	1495.3	0.68	146.0	50.6	31.0	113.9	11%	18%	32%	8%	9%	14%	4%	6%
	Max depol.	14	620.9	212.9	3417.1	0.48	186.1	52.3	33.5	107.4	6%	9%	36%	15%	6%	22%	1%	2%
	lowest IC50	14	620.9	212.9	3417.1	0.48	186.1	52.3	33.5	107.4	6%	9%	36%	15%	6%	22%	1%	2%
MWL-N	AVG	-----	335.7	86.7	7521.8	0.78	48.3	35.3	35.4	475.8	2%	84%	37%	17%	4%	55%	11%	25%
	Max depol.	0	342.0	13.7	10282.3	0.92	46.6	15.9	39.4	357.1	1%	50%	7%	9%	0%	44%	3%	2%
	lowest IC50	0	342.0	13.7	10282.3	0.92	46.6	15.9	39.4	357.1	1%	50%	7%	9%	0%	44%	3%	2%
HO-N	AVG	-----	423.1	640.8	538.8	0.45	364.7	203.0	31.8	53.8	1%	27%	14%	12%	5%	14%	6%	6%
	Max depol.	7	431.7	996.5	504.7	0.38	390.8	310.0	35.5	58.1	11%	8%	5%	7%	2%	0%	1%	7%
	lowest IC50	0	441.3	165.3	767.0	0.56	350.8	215.0	24.5	50.0	3%	5%	4%	5%	3%	3%	2%	4%
HK-N	AVG	-----	260.9	106.3	1303.7	0.76	137.8	82.1	29.0	201.1	6%	29%	11%	6%	10%	16%	2%	8%
	Max depol.	14	295.4	109.3	751.0	0.60	155.7	107.2	29.9	167.2	6%	9%	36%	15%	6%	22%	1%	2%
	lowest IC50	14	295.4	109.3	751.0	0.60	155.7	107.2	29.9	167.2	6%	9%	36%	15%	6%	22%	1%	2%

Table 3. The correction factors needed for ABTS scavenging calculations at basic pH. These values were used by subtracting the absorbance from Figure S3 from the initial absorbance of the ABTS without any buffer.

Concentration	Absorbance Correction
Between 0-20 mg/L	0.000
40 mg/L	0.054
60 mg/L	0.115
80 mg/L	0.141
100 mg/L	0.223
140 mg/L	0.278
160 mg/L	0.310
Over 200 mg/L	0.405

Table 4. FTIR assignments. The values in bold correspond to those that are highlighted in red in Figure 2 in the manuscript.

MWL	HO	HK	SK	HaK	
3507	3377	3294	3462	3416	O-H stretching
	3086	3086	3005	3005	C=H stretching
2963	2963	2963	2943	2943	C-H stretching (CH2 asymmetric vibration (guaiacyl-syringyl))
	2938	2920	2872		C-H stretching (methyl and methylene groups)
2885	2843	2851	2839	2839	C-H stretching
			2045		
1705	**1707**	**1711**	**1721**	**1707**	**C=O stretching unconjugated**
1602	**1628**	**1619**	**1629**	**1619**	**aromatic skeletal vibration + C=O stretching (conjugated)**
1509	**1512**	**1508**	**1505**	**1506**	**aromatic skeletal vibration**
1459	1459	1458	1460	1462	C-H deformation (methyl and methylene)
1422	1425	1425	1421	1422	C-H in-plane deformation with aromatic ring stretching
1325	1330	1329		1323	C-O of the syringyl ring
1267	1266	1267	1269	1259	C-O of the guiacyl ring
1217	**1235**	**1231**	**1223**	**1233**	**C-C + C-O stretch (phenolic O-H plus ether C-O)**
1163	1170	1165	1156	1163	C-O stretching in aliphatic groups

1135	1123	1124	1124	1122	aromatic C-H in plane deformation of guaiacyl ring
1074	**1086**	**1091**	**1087**	**1087**	**C-O deformations at C_β and aliphatic ethers**
1046	**1033**	**1027**	**1032**	**1027**	**aromatic C-H in plane deformation (G>S), C-O deformation at C_α and aliphatic ethers**
920					=CH out-of-plane deformation in aromatic ring
885	837	856	880	880	C-H out of plane vibrations in 2,5,6 of guaiacyl units

Table 5. The R^2 values between various parameters and products in basic conditions. The higher the value and the darker the shade indicate stronger relationships.

	B-O-4	S/G	Sol.	C-Sol.	NTA conc	DLS size	PDI	phenolics	flavonoids	zeta	IC50
B-O-4											
S/G	0.00										
Sol.	0.59	0.13									
C-Sol.	0.82	0.00	0.80								
NTA conc	0.34	0.22	0.82	0.50							
DLS size	0.74	0.03	0.30	0.51	0.11						
PDI	0.89	0.01	0.68	0.75	0.52	0.22					
phenolics	0.83	0.15	0.72	0.68	0.53	0.27	0.62				
flavonoids	0.46	0.27	0.31	0.16	0.27	0.01	0.25	0.61			
zeta	0.60	0.43	0.00	0.09	0.00	0.02	0.18	0.05	0.00		
IC50	0.38	0.31	0.67	0.56	0.49	0.26	0.37	0.53	0.18	0.00	

Table 6 The R^2 values between various parameters and products in neutral conditions. The higher the value and the darker the shade indicate stronger relationships.

	B-O-4	S/G	Sol.	C-Sol.	NTA conc	DLS size	PDI	phenolics	flavonoids	zeta	IC50
B-O-4											
S/G	0.00										
Sol.	0.65	0.03									
C-Sol.	0.78	0.04	0.88								
NTA conc	0.98	0.03	0.44	0.59							
DLS size	0.75	0.02	0.78	0.81	0.47						
PDI	0.92	0.06	0.45	0.45	0.49	0.59					
phenolics	0.97	0.01	0.62	0.74	0.58	0.75	0.69				
flavonoids	0.96	0.01	0.62	0.70	0.45	0.64	0.71	0.78			
zeta	0.57	0.46	0.07	0.12	0.28	0.05	0.00	0.06	0.00		
IC50	0.59	0.03	0.75	0.77	0.35	0.87	0.55	0.66	0.69	0.01	

References

1. Zijlstra, D.S., et al., *Mild Organosolv Lignin Extraction with Alcohols: The Importance of Benzylic Alkoxylation.* ACS Sustainable Chemistry & Engineering, 2020. **8**(13): p. 5119-5131.
2. Mansfield, S.D., et al., *Whole plant cell wall characterization using solution-state 2D NMR.* Nat Protoc, 2012. **7**(9): p. 1579-89.