Electronic Supplementary Material

Supplementary information

Characterisation of organosolv birch lignins – Towards application-specific lignin production

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Section S1: $^{13}$C NMR data

For quantitative $^{13}$C NMR analysis (Table S1 and S2), trioxane was applied as internal standard. The concentration of C$_2$-H$_2$ in G units and C$_{α}$-H$_{α}$ in β-O-4’ sub-structures was used to correlate the $^{13}$C and $^1$H-$^{13}$C NMR spectra (Table S3 and S4) for aromatic and aliphatic structures, respectively.

Table S1. Reported carbon shifts for relevant lignin structures and experimentally determined shifts obtained in the present study.

| Reported shift | Experimental shift | Assignment |
|----------------|--------------------|------------|
| 175–168$^a$    | 174.6–174.5        | -COOH      |
| 154–152$^b$    | 153.2–151.2        | S$_{3,5}$  |
| 150–146$^b$    | 148.5–147.1        | G$_{3,5}$ + S$_{1,5}$ |
Table S2. Occurrence of relevant lignin structures in the various treatments.

| Assignment | B1  | B2  | B3  | B4  | B5  | B6  | B7  |
|------------|-----|-----|-----|-----|-----|-----|-----|
|            | mmol/g | mmol/g | mmol/g | mmol/g | mmol/g | mmol/g | mmol/g |
| -COOH      | 0.10  | 0.09  | 0.04  | 0.06  | 0.09  | 0.06  | 0.08  |
| S_{3,5}    | 1.53  | 1.36  | 1.13  | 1.26  | 1.31  | 0.81  | 1.29  |
| G_{3,5} + S_{3,5} | 0.75  | 0.58  | 0.38  | 0.43  | 0.71  | 1.14  | 0.80  |
| S_{1,4} + G_{1} | 1.26  | 1.12  | 0.87  | 1.28  | 1.03  | 0.41  | 1.26  |
| G_{5}      | 0.62  | 0.81  | 0.52  | 0.60  | 1.00  | 0.36  | 0.77  |
| G_{2}      | 1.08  | 0.90  | 1.16  | 1.48  | 1.69  | 1.20  | 1.33  |
| S_{2,6} + ox. S_{2,6} | 2.18  | 1.74  | 1.69  | 1.69  | 2.07  | 1.81  | 2.40  |
| β_{5} (C_{α}) | 0.60  | 0.64  | 0.46  | 0.45  | 0.73  | 0  | 0.23  |
| C_{β} in β-O-4' + C_{α} in β-5' + β-β' | 0.32  | 0.32  | 0.26  | 0.29  | 0.51  | 0  | 0.12  |
| + C_{α} in β-5' + β-β' | 0.33  | 0.27  | 0.19  | 0.20  | 0.37  | 0  | 0.20  |
| C_{α} in β-O-4' | 1.47  | 1.01  | 0.84  | 1.30  | 1.50  | 0.92  | 1.28  |
| -OCH_{3}   | 5.98  | 5.10  | 4.56  | 5.25  | 7.02  | 4.94  | 6.93  |
| C_{α} + C_{β} in methylene groups | 0.83  | 1.12  | 0.60  | 1.10  | 1.22  | 1.42  | 1.36  |

a Balakshin et al.\textsuperscript{51}
b Hage et al.\textsuperscript{52}
c Holtman et al.\textsuperscript{53}
methylene groups

| CH₃ in acetyl group | 0.06 | 0.09 | 0.05 | 0.06 | 0.10 | 0.06 | 0.11 |
|--------------------|------|------|------|------|------|------|------|
| C/C-H Quat. C/Tert. C | 0.91 | 0.89 | 0.71 | 0.79 | 0.64 | 0.70 | 0.74 |

Section S2: ¹H-¹³C HSQC data

Table S3. Relevant chemical structures, their experimentally determined HSQC shifts, and corresponding reported shifts.

| Lignin sample | Lignin inter-unit bonds | Reported shift | Experimental shift |
|---------------|-------------------------|----------------|-------------------|
| Cα-Hα in β-O-4’ | δH/δC | 4.86/71.8 | 5.15/71.4 |
| | | 5.07/76.6 | |
| Cβ-Hβ in β-O-4’ linked to G/H | δH/δC | 4.29/83.9 | 4.58/80.12 |
| Cα-Hα in ethoxylated (Cα = O) β-O-4’ structure | δH/δC | 5.21/83.1 | 5.23/83.1 |
| Methylene OEt | δH/δC | 3.32/63.6 | 3.40/64.04 |
| Methyl OEt | δH/δC | 1.08/15.0 | 1.07/14.78 |
| Cα in ethoxylated β-O-4’ structure | δH/δC | 4.42/79.8 | 4.58/80.3 |
| Cβ in non- and ethoxylated β-O-4’ structure linked to G | δH/δC | 4.4-4.3/82.1-83.0 | 4.32/83.9 |
| Cβ in non- and ethoxylated β-O-4’ structure linked to S | δH/δC | 4.2-4.0/84.9-86.0 | 4.17/85.60 |
| Cα-Hα in phenylcoumaran | δH/δC | 3.77/50.2 | 3.49/52.84 |
| Cγ-Hγ in phenylcoumaran | δH/δC | 3.73/62.5 | 3.73/62.70 |
| Cα-Hα in phenylcoumaran | δH/δC | 5.49/88.2 | 5.48/86.88 |
| Phenylcoumaran (β-5’’) | δH/δC | - | - |
| Chemical Structure                  | δ_H/δ_C (a)  | δ_H/δ_C (b) |
|-------------------------------------|--------------|------------|
| C\textsubscript{7}-H\textsubscript{7} in resinol | 3.85/70.86   | 3.85/70.86 |
| C\textsubscript{β}-H\textsubscript{β} in resinol | 3.08/54.30   | 3.10/53.36 |
| C\textsubscript{α}-H\textsubscript{α} in resinol | 4.65/84.8     | 4.70/84.82 |
| Pinoresinols (β-β’)                | -            | -          |
| β-1                                | 3.58/50.8     | 3.61/50.68 |
| C\textsubscript{β}-H\textsubscript{β} in spirodienone (D) | 2.98/57.5     | -          |
| C\textsubscript{α}-H\textsubscript{α} in spirodienone (D) | 5.07/81.2     | -          |
| C\textsubscript{β}-H\textsubscript{β} in spirodienone (D) | 4.12/79.5     | -          |
| C2-H2 in spirodienone (D)          | 6.22/113.3    | -          |
| C6-H6 in spirodienone (D)          | 7.60/123.3    | -          |
| Spirodienone (average)             | -            | -          |
| Dibenzodioxocin (5-5'/4-O-β’) C\textsubscript{α}-H\textsubscript{α} | 4.9/83.4      | -          |
| Dibenzodioxocin (5-5'/4-O-β’) C\textsubscript{β}-H\textsubscript{β} | 3.8/85.5      | -          |
| Dibenzodioxocin (5-5'/4-O-β’)      | -            | -          |
| 4-O-5 C2/H2 (overlap)              | 6.8/105.0     | -          |
| 4-O-5 C6/H6                        | 6.4/110.0     | -          |
| 4-O-5 (average)                    | -            | -          |
| Stilbenes C2-H2                    | 7.15/109.0    | -          |
| Stilbenes C\textsubscript{αβ}-H\textsubscript{αβ} | 7.00/126.0   | -          |
| Stilbenes (average)                | -            | -          |
| Trans-Enol ether (G-G; C\textsubscript{α}-H\textsubscript{α}) | 109.1/5.56    | -          |
| Cis-Enol ether (G-G; C\textsubscript{α}-H\textsubscript{α}) | 112.0/6.14    | -          |
| Trans-Enol ether (S-G; C\textsubscript{α}-H\textsubscript{α}) | 112.10/6.10   | -          |
| Cis-Enol ether (S-G; C\textsubscript{α}-H\textsubscript{α}) | 109.45/5.57   | -          |
| Trans-Enol ether (G-S; C\textsubscript{α}-H\textsubscript{α}) | 108.20/5.72   | -          |
| Cis-Enol ether (G-S; C\textsubscript{α}-H\textsubscript{α}) | 106.42/5.27   | -          |
| Trans-Enol ether (S-S; C\textsubscript{α}-H\textsubscript{α}) | 107.70/5.77   | -          |
| Cis-Enol ether (S-S; C\textsubscript{α}-H\textsubscript{α}) | 106.20/5.28   | -          |
| Lignin end-groups - Abundance      | δ_H/δ_C      | δ_H/δ_C    |
| pCA C7-H7                          | 7.51/144.8    | -          |
| pCA C8-H8                          | 6.29/113.9    | -          |
| pCA C3,5-H3,5                      | 6.83/115.8    | -          |
| pCA C2,6-H2,6                      | 7.48/130.2    | -          |
| pCA (average)                      | -            | -          |
| Compound                                         | δ_H / δ_C       | δ_H / δ_C       |
|------------------------------------------------|-----------------|-----------------|
| *p*-hydroxycinnamyl alcohol C<sub>β</sub>-H<sub>β</sub> | 6.25/128.2<sup>a</sup> | 6.45/131.31     |
| *p*-hydroxycinnamyl alcohol C<sub>α</sub>-H<sub>α</sub> | 6.44/128.2<sup>a</sup> | 6.49/131.40     |
| *p*-hydroxycinnamyl alcohol C<sub>γ</sub>-H<sub>γ</sub> | 4.10/61.4<sup>a</sup> | 4.12/61.46      |
| *p*-hydroxycinnamyl alcohol a                   | -               | -               |
| pBA C<sub>2.6-H2.6</sub>                         | 7.67/131.2<sup>b</sup> | -               |
| Cinnamaldehyde C<sub>α</sub>-H<sub>α</sub>        | 7.60/153.5<sup>b</sup> | 7.61/153.50     |
| Cinnamaldehyde C<sub>β</sub>-H<sub>β</sub>        | 6.76/126.1<sup>b</sup> | 6.77/125.90     |
| Cinnamaldehyde                                   | -               | -               |
| α methylene                                      | 3.6/44.3<sup>c</sup> | 3.67/44.57      |
| Hibberts ketone, H<sub>γ</sub>                   | 4.2/67.0<sup>c</sup> | 4.18/66.70      |

**Sugar units - Abundance**

| Compound                             | δ_H / δ_C       | δ_H / δ_C       |
|--------------------------------------|-----------------|-----------------|
| β-D-xylapyranoside                   | 4.20/103.2<sup>b</sup> | 4.31/101.4 (197.32) |
| C2-H2 in β-D-xylapyranoside          | 3.02/72.5<sup>b</sup> | 3.09/72.3 (152.40)  |
| C3-H3 in β-D-xylapyranoside          | 3.22/73.7<sup>b</sup> | 3.29/73.6 (256.11)  |
| C4-H4 in β-D-xylapyranoside          | 3.60/75.4<sup>b</sup> | 3.54/75.17 (253.16) |
| C5-H5 in β-D-xylapyranoside          | 62.6/3.40<sup>b</sup>-3.72<sup>b</sup> | 3.72/62.70 (Overlap region) |
| PhGlc1                               | 4.90/98.4<sup>b</sup> | -               |
| PhGlc2                               | 4.65/100.6<sup>b</sup> | -               |
| PhGlc3                               | 4.79/101.5<sup>b</sup> | -               |
| Benzyl ether<sub>α</sub>             | 4.65/81.3<sup>b</sup> | 4.64/82.54 (24.09) |
| (1->4)α-D-xylapyranoside             | 4.88/92.2<sup>b</sup> | -               |
| (1->4)-β-D-xylapyranoside            | 4.26/97.4<sup>b</sup> | -               |

**Lignin aromatic units - Abundance**

| Compound                            | δ_H / δ_C       | δ_H / δ_C       |
|-------------------------------------|-----------------|-----------------|
| C2.6-H2.6 in H units                | 7.05/128.8<sup>a</sup> | 6.97/128.69     |
| C2-H2 in G units                    | 6.98/110.9<sup>b</sup> | 6.93/111.05     |
| C5-H5 in G units (overlap with H3,5)| 6.80/116.0<sup>a</sup> | 6.84/115.35     |
| C6-H6 in G units                    | 6.87/120.1<sup>a</sup> | 6.84/119.06     |
| G units                             | -               | -               |
| C2.6-H2.6 in S units                | 6.59/104.1<sup>a</sup> | 6.69/104.27     |
| Methoxy groups                      | 3.77/55.38<sup>a</sup> | 3.73/55.60      |

<sup>a</sup> Wen et al.<sup>84</sup>
<sup>b</sup> Zhang and Gellerstedt.<sup>55</sup>
<sup>c</sup> Brandt et al.<sup>56</sup>
<sup>d</sup> Wen et al.<sup>57</sup>
<sup>e</sup> Zeng et al.<sup>58</sup>
<sup>f</sup> Li et al.<sup>59</sup>
Table S4. Structures evaluated in the various treatments; those not detected are indicated by (-).

| Lignin sample | B1  | B2  | B3  | B4  | B5  | B6  | B7  |
|---------------|-----|-----|-----|-----|-----|-----|-----|
| **Lignin inter-units - Abundance (mmol/g)** |     |     |     |     |     |     |     |
| \( C_{\alpha}\)-H\( \alpha \) in \( \beta-O-4' \) structures (excl. \( \alpha\)-OEt) | 1.47 | 1.01 | 0.84 | 1.30 | 1.49 | 0.92 | 1.28 |
| \( C_{\beta}\)-H\( \beta \) in \( \beta-O-4' \) linked to G/H (incl. \( \alpha\)-OEt) | 0.25 | 0.16 | 0.08 | 0.21 | 0.30 | 0.29 | 0.25 |
| \( C_{\beta}\)-H\( \beta \) in \( \beta-O-4' \) linked to S (incl. \( \alpha\)-OEt) | 0.95 | 0.79 | 0.64 | 0.97 | 0.91 | 0.43 | 0.88 |
| Total \( C_{\beta}\)-H\( \beta \) in \( \beta-O-4' \) | 1.20 | 0.95 | 0.72 | 1.18 | 1.21 | 0.72 | 1.13 |
| Total \( C_{\alpha}\)-H\( \alpha \) in \( \beta-O-4' \) (incl. \( \alpha\)-OEt) | 1.75 | 1.30 | 0.99 | 1.53 | 1.99 | 1.95 | 1.68 |
| \( \beta-O-4' \) linked to S:G/H | 3.80 | 4.94 | 8.00 | 4.62 | 3.03 | 1.48 | 3.52 |
| \( C_{\gamma}\)-H\( \gamma \) in \( \beta-O-4' \) | 3.03 | 2.18 | 1.66 | 2.48 | 3.92 | 6.44 | 3.34 |
| C2-H2 ox. G units | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.00 | 0.04 |
| C6-H6 ox. G units | 0.02 | 0.01 | 0.01 | 0.02 | 0.03 | 0.00 | 0.03 |
| Ox. G units (average) | 0.02 | 0.02 | 0.02 | 0.02 | 0.03 | 0.00 | 0.03 |
| C2.6-H2.6 in ox. S units | 0.18 | 0.16 | 0.34 | 0.35 | 0.31 | 0.32 | 0.25 |
| \( C_{\beta}\)-H\( \beta \) in oxidized (\( C_{\alpha} = O \)) \( \beta-O-4' \) | 0.01 | 0.01 | 0.01 | 0.00 | 0.03 | 0.12 | 0.00 |
| **Oxidised \( C_{\alpha}\) S (ox. \( C_{\alpha}\) aromatic S cont.)** | 0.04 | 0.05 | 0.07 | 0.07 | 0.05 | 0.07 | 0.05 |
| **Oxidised \( C_{\alpha}\) G (ox. \( C_{\alpha}\) aromatic G cont.)** | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | - | 0.03 |
| Methylene OEt | 1.11 | 0.74 | 0.68 | 0.92 | 2.09 | 9.81 | 2.73 |
| Methyl OEt | 1.00 | 0.74 | 0.65 | 0.92 | 2.21 | 9.38 | 2.62 |
| \( C_{\alpha}\) in ethoxylated \( \beta-O-4' \) structure | 0.28 | 0.29 | 0.15 | 0.23 | 0.49 | 1.03 | 0.40 |
| \( C_{\beta}\) in non- and ethoxylated \( \beta-O-4' \) structure linked to G | 0.22 | 0.16 | 0.08 | 0.21 | 0.30 | 0.29 | 0.25 |
| \( C_{\beta}\) in non- and ethoxylated \( \beta-O-4' \) structure linked to S | 0.95 | 0.79 | 0.64 | 0.97 | 0.91 | 0.43 | 0.88 |
| \( C_{\beta}\)-H\( \beta \) in phenylcoumaran | 0.11 | 0.07 | 0.05 | 0.10 | 0.11 | 0.31 | - |
| \( C_{\gamma}\)-H\( \gamma \) in phenylcoumaran | 0.25 | 0.18 | 0.16 | 0.19 | 0.34 | 1.41 | 0.23 |
| \( C_{\alpha}\)-H\( \alpha \) in phenol | 0.12 | 0.08 | 0.06 | 0.10 | 0.14 | 0.45 | - |
| Phenylcoumaran (\( \beta-5' \)) | 0.12 | 0.08 | 0.06 | 0.10 | 0.12 | 0.38 | - |
| \( C_{\gamma}\)-H\( \gamma \) in resinol | 0.24 | 0.16 | 0.16 | 0.21 | 0.29 | 0.73 | 0.26 |
| \( C_{\beta}\)-H\( \beta \) in resinol | 0.21 | 0.14 | 0.13 | 0.18 | 0.26 | 0.86 | 0.04 |
| Bond                     | pCA C7-H7 | pCA C8-H8 | pCA C3,5-H3,5 | pCA C2,6-H2,6 | pCA | p-hydroxycinnamyl alcohol C_{\beta}-H_\beta | p-hydroxycinnamyl alcohol C_{\alpha}-H_\alpha | p-hydroxycinnamyl alcohol C_{\gamma}-H_{\gamma} | p-hydroxycinnamyl alcohol |
|--------------------------|-----------|-----------|---------------|---------------|-----|---------------------------------------------|---------------------------------------------|---------------------------------------------|--------------------------|
| C_\alpha-H_\alpha in resinol | 0.27      | 0.22      | 0.15          | 0.25          | 0.36| 0.93                                        | 0.29                                        | 0.04                                        | 0.02         |
| Pinoresinols (\beta-\beta') | 0.24      | 0.17      | 0.15          | 0.21          | 0.30| 0.84                                        | 0.28                                        | 0.03                                        | 0.02         |
| \beta-1'                 |           |           |               |               |     |                                             |                                             |                                             |             |
| C_\beta-H_\beta in spirodienone (D) |         |           |               |               |     |                                             |                                             |                                             |             |
| C_\alpha-H_\alpha in spirodienone (D) |         |           |               |               |     |                                             |                                             |                                             |             |
| C_\beta'-H_\beta' in spirodienone (D) |         |           |               |               |     |                                             |                                             |                                             |             |
| C2'-H2' in spirodienone (D) |           |           |               |               |     |                                             |                                             |                                             |             |
| C6'-H6' in spirodienone (D) |           |           |               |               |     |                                             |                                             |                                             |             |
| Spirodienone (average)   |           |           |               |               |     |                                             |                                             |                                             |             |
| Dibenzodioxocin (5-5'/4-O-\beta') C_\alpha-H_\alpha |         |           |               |               |     |                                             |                                             |                                             |             |
| Dibenzodioxocin (5-5'/4-O-\beta') C_\beta-H_\beta |         |           |               |               |     |                                             |                                             |                                             |             |
| Dibenzodioxocin (5-5'/4-O-\beta') |         |           |               |               |     |                                             |                                             |                                             |             |
| 4-O-5' C2/H2 (overlap)   |           |           |               |               |     |                                             |                                             |                                             |             |
| 4-O-5' C6/H6             |           |           |               |               |     |                                             |                                             |                                             |             |
| Stilbenes C2-H2          | 0.04      | 0.02      | 0.02          | 0.04          | 0.04| 0.04                                        |                                             |                                             |             |
| Stilbenes C_{\alpha\beta}-H_{\alpha\beta} | 0.03      | 0.02      | 0.01          | 0.02          | 0.02| 0.00                                        |                                             |                                             |             |
| Stilbenes                | 0.03      | 0.02      | 0.01          | 0.03          | 0.03| 0.02                                        |                                             |                                             |             |
| Trans-Enol ether (G-G)   |           |           |               |               |     |                                             |                                             |                                             |             |
| Cis-Enol ether (G-G)     |           |           |               |               |     |                                             |                                             |                                             |             |
| Trans-Enol ether (S-G)   |           |           |               |               |     |                                             |                                             |                                             |             |
| Cis-Enol ether (S-G)     |           |           |               |               |     |                                             |                                             |                                             |             |
| Trans-Enol ether (G-S)   |           |           |               |               |     |                                             |                                             |                                             |             |
| Cis-Enol ether (G-S)     |           |           |               |               |     |                                             |                                             |                                             |             |
| Trans-Enol ether (S-S)   |           |           |               |               |     |                                             |                                             |                                             |             |
| Cis-Enol ether (S-S)     |           |           |               |               |     |                                             |                                             |                                             |             |

**Lignin endgroups - Abundance (mmol/g)**
### Section S3: FT-IR data

FT-IR spectra of lignins isolated after the various treatments are presented in Figure S1. The three peaks associated with aromatic skeletal vibrations are found at 1600, 1510, and 1420 cm$^{-1}$, and are all readily visible in Figure S1, indicating that the isolated lignins were of high purity. Several signals can be used to distinguish G and S units, which are the dominant lignin monomers in hardwood. G

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**Lignin aromatic units - Abundance (mmol/g)**

|                          | 0.00 | 0.01 | 0.03 | 0.03 | 0.00 | 0.07 | 0.02 |
|--------------------------|------|------|------|------|------|------|------|
| C2.6-H2.6 in H units     |      |      |      |      |      |      |      |
| C2-H2 in G units         | 1.08 | 0.90 | 1.16 | 1.48 | 1.70 | 1.20 | 1.33 |
| C5-H5 in G units (overlap with H3,5) | 1.57 | 1.38 | 1.75 | 2.03 | 2.74 | 2.42 | 1.74 |
| C6-H6 in G units         | 1.04 | 0.88 | 1.08 | 1.24 | 1.72 | 1.10 | 1.20 |
| G units (average of C6 and C2) | 1.06 | 0.89 | 1.12 | 1.36 | 1.71 | 1.15 | 1.27 |
| C2.6-H2.6 in S units     | 4.01 | 2.95 | 4.27 | 4.90 | 5.85 | 4.34 | 4.79 |
| Sum S2,6 + G2 (mmol/g)   | 5.07 | 3.84 | 5.39 | 6.26 | 7.56 | 5.49 | 6.06 |
| S/G (S2,6 + S2,6ox)/(G2+G2ox) | 3.88 | 3.42 | 4.04 | 3.80 | 3.54 | 4.05 | 3.88 |
| Methoxy groups           | 7.19 | 5.24 | 4.23 | 6.33 | 9.02 | 13.04 | 7.98 |
| S/G ratio Pyr-GC/MS      | 1.85 | 1.69 | 2.27 | 2.23 | 1.62 | 1.09 | 1.82 |

**Sugar units - Abundance (mmol/g)**

|                          | 0.04 | -    | -    | -    | -    | -    | -    |
|--------------------------|------|------|------|------|------|------|------|
| β-D-xylapyranoside       |      |      |      |      |      |      |      |
| C2-H2 in β-D-xylapyranoside | 0.03 | -    | -    | -    | -    | -    | -    |
| C3-H3 in β-D-xylapyranoside | 0.05 | -    | -    | -    | -    | -    | -    |
| C4-H4 in β-D-xylapyranoside | 0.05 | -    | -    | -    | -    | -    | -    |
| C5-H5 in β-D-xylapyranoside | -   | -    | -    | -    | -    | -    | -    |
| PhGlc1                   | -    | -    | -    | -    | -    | -    | -    |
| PhGlc2                   | -    | -    | -    | -    | -    | -    | -    |
| PhGlc3                   | -    | -    | -    | -    | -    | -    | -    |
| Benzyl etherα            | 0.01 | -    | -    | -    | -    | -    | -    |
| (1->4)α-D-xylapyranoside | -    | -    | -    | -    | -    | -    | -    |
| (1->4)-β-D-xylapyranoside | -   | -    | -    | -    | -    | -    | -    |
units can be identified by bands at 1267, 1218 (both C-O of the G ring), 850 cm\(^{-1}\); whereas S units are characterised by a band at 1315 cm\(^{-1}\) (C-O of the S ring). In addition, a band shared by the two monomers (G and S ring C-H) is located at 1113 cm\(^{-1}\). The peak at 1215 cm\(^{-1}\) was assigned to the C-C stretch, C-O stretch, and C=O stretch, which makes it non-unique for either of the two lignin monomers. An evaluation of unique bands revealed that changing the ethanol content caused only minor modifications, which were in line with the few changes in S/G ratio obtained by HSQC (Table 2). Increasing the treatment time from 15 to 30 and then 60 min seemingly enhanced the G band at 850 cm\(^{-1}\) and the S band at 1267 cm\(^{-1}\), while significantly augmenting both overlapping bands at 1215 and 1113 cm\(^{-1}\). Greater absorbance in this region could also be attributed to C-O stretching in alcohols and ethers or carboxylic acids from degradation products. This was relevant also for lignin extracted by acid treatments. When increasing the concentration of acid from 0.2 to 1% (B4, B5, B6), the peak assigned to the C-O of the S ring at 1315 cm\(^{-1}\) also increased, as did the overlapping peaks for the G and S units (1218 and 1113 cm\(^{-1}\)). However, the peak corresponding to the G monomer at 850 cm\(^{-1}\) seemed to be smaller and more compressed. The peak observed at 3440 cm\(^{-1}\) was attributed to O-H stretching, while peaks at 2935 and 2855 cm\(^{-1}\) were assigned to methyl and methylene (C-H) bond stretching (CH\(_2\) and CH\(_3\)). The larger hydroxyl peak seen by FT-IR supported earlier TGA results suggesting significant levels of loosely bound water in B3 lignin. While there were few major changes associated with the bands of CH\(_2\) and CH\(_3\) structures, increasing the acid concentration from 0.2 to 1% lowered the signal associated with the hydroxyl group, and increased methyl and methylene signals. This switch could be attributed to the formation of aliphatic hydroxyls through cleavage of aryl ether linkages, followed by subsequent condensation reactions to resinol or other structures. Indeed, results obtained by both HSQC and P NMR suggest that a rise from 0.2 to 1.0% acid caused the aliphatic hydroxyl concentration to drop from 3.54 to 1.34 mmol/g, while the resinol content (HSQC) increased from 0.276 to 0.602 mmol/g. Similar results were observed when evaluating the effect of time. An increase from 30 to 60 min did not lead to major changes in the overall hydroxyl content, neither by FT-IR nor P NMR. Only the aliphatic hydroxyl (P NMR; Table 2) content decreased slightly alongside an increase in resinol content (HSQC; Table 2), possibly explaining the stronger signals at 2935 and 2855 cm\(^{-1}\), while that at 3440 cm\(^{-1}\) remained unaffected (O-H stretch).
Figure S1. FT-IR spectra for the various treatments at wavenumbers ranging from 4000 to 2500 cm\(^{-1}\) (left) and 1750 to 750 cm\(^{-1}\) (right).

Section S4: Visual representation of the various isolated lignins

The various lignins and their visual appearance after isolation is presented in Figure S2.

Figure S2: Visual of the various lignins isolated in the present work. Photograph courtesy of Alok Patel (Luleå University of Technology). Copyright 2020.
Section S5: Quantitative $^{13}$C NMR and $^1$H-$^{13}$C HSQC spectra.

(A)

Figure S3: Quantitative $^{13}$C NMR (A) and $^1$H-$^{13}$C HSQC spectra of B1 (B).
Figure S4: Quantitative $^{13}$C NMR (A) and $^{1}$H-$^{13}$C HSQC spectra of B2 (B).
Figure S5: Quantitative 13C NMR (A) and 1H-13C HSQC spectra of B3 (B).
Figure S6: Quantitative 13C NMR (A) and 1H-13C HSQC spectra of B4 (B).
Figure S7: Quantitative 13C NMR (A) and 1H-13C HSQC spectra of B5 (B).
Figure S8: Quantitative $^{13}$C NMR (A) and $^{1}$H-$^{13}$C HSQC spectra of B6 (B).
Figure S9: Quantitative 13C NMR (A) and 1H-13C HSQC spectra of B7 (B).
Section S6: Background for discussion on specific lignin applications

Section S6.1: Lignin as a lubricant component
The application of lignin in lubricants is similar to that as a filler in composites, where sufficient filler-filler and filler-matrix interactions ensure efficient energy absorption through secondary bond-breakage, chain movement, and disentanglement. In a lubricant, lignin often co-exists with another component in a liquid mixture. Hydrogen-bonding between the components in the lubricant and high PDI ensure efficient stress transfer and energy absorption through the entire liquid. The choice of lignin thus depends on the nature of the other lubricant components. For example lubricants containing ethylene glycol or polyethylene glycol rely on hydrogen bonding,\textsuperscript{S16,S17} which is mediated by hydroxyl groups.

Section S6.2: Lignin as precursor for production of free phenolics/bio-oil
Extracted lignins may also be used for the production of phenolics through thermal depolymerisation. The aryl ether linkages present in lignins are often thermally labile, and abundant β-aryl ether linkages promote the formation of free phenolics, as opposed to condensed, carbon-carbon structures.\textsuperscript{S18}

Section S6.3: Lignin for antioxidant applications
The antioxidant activity of lignins has been linked mainly to the phenol motif.\textsuperscript{S19} This property is directly affected by the chemical nature of the aliphatic side chain, the presence of methoxy groups in meta and/or ortho positions, and the molecular weight distribution.\textsuperscript{S20} The ability of lignin derivatives to donate protons seems to depend largely on whether the aromatic oxygen receives sufficient electronegativity; if not, the pK\textsubscript{a} decreases leading to increased radical scavenging.\textsuperscript{S21} This electronegativity originates from the delocalised π-bonds, which may or may not be depleted of electrons depending on the chemical nature of the side-chain. The closer and more oxygen species (and aromatic methoxy groups) exist on the side-chain, the more electrons are drawn there, draining the π-electron system. This, in turn, lowers the pK\textsubscript{a} of the aromatic hydroxyl increasing its proton-donating ability. The movement of negative charge along the side-chain is further stabilised through hydrogen bonding between the solvent and the charged side-chain species, explaining why solvents not prone to hydrogen bonds hinder the withdrawal of π-electrons and thus increase pK\textsubscript{a} (poorer antioxidant activity) of the aromatic hydroxyl group.

Thus, introduction of condensed or dehydrated inter-unit linkages (higher content of CH\textsubscript{3}/CH\textsubscript{2} structures relative to oxygen species in the side-chain) could benefit the antioxidant activity of the phenolic hydroxyl in an organic environment. Specifically, electron-dense oxygen species of the hydroxyl can disperse their negative charge easier in the absence of a stabilising solvent (H bond donor) when the proton is released. The opposite would be the case in a solvent with better proton-donating ability, such as water, as this can support electron displacement from the aromatic ring to the
side-chain oxygen species. Thus, an aqueous environment favours lignins carrying an oxidised side-chain with numerous aromatic hydroxyls; whereas an organic solution favours a side-chain containing few oxidised species.

**Section S6.4: Lignin for adsorbent in aqueous environment**

The application of pyrolysed lignin (biochar) for adsorption purposes in aqueous environments benefits from a high specific surface area (proportional to porosity) and thus the presence of certain components in the original lignin that can be volatilised during pyrolysis while leaving behind the non-volatile porous biochar framework. Elevated porosity can be achieved through dispersion with various activating materials during pyrolysis, or by applying lignins with thermally liable structures (e.g., hemicellulose). Oxygen species present in the biochar are important for adsorption of aromatics and heavy metals in aqueous systems (see previous references in this section). They may interact directly with specific components set for adsorption onto the biochar, or promote interaction between the solvent and biochar. The latter prevents lignin-lignin association and ensures a highly exposed adsorbent surface area. An abundance of oxygen species, alongside an intermediate content of aromatics and more volatile aliphatic structures may offer a favourable starting point for remediation in an aqueous environment.

**Section S6.5: Lignin in hydrophobic composite/rubber**

Biochar based on pyrolysis of lignins can replace carbon black as filler in rubber composites. As with traditional styrene-butadiene rubber, it is crucial that the lignin filler displays a graphitic structure to enable interaction with the rubber mesh rich in aromatic conjugated systems. Simultaneously, the lignin-based filler must also enable filler-filler interaction to absorb energy through secondary bond breakage before it is absorbed through deformation of the surrounding mesh. To this end, the lignin-based filler must be compatible with the surrounding matrix; this can be achieved by lignin with fewer chargeable groups. Finally, energy absorption through chain entanglement is another strategy.

**Section S6.6: Lignin in carbon fibre production**

During the first part of carbon fibre production, i.e., stabilisation phase, reactive/volatile species are liberated while oxygen is incorporated at relatively low temperatures. During the subsequent carbonization stage, temperature increases and the incorporated oxygen species contribute to the formation of cross-links (including C-C bonds) in the lignin material. A high content of volatiles is likely to lower the threshold for lignin relaxation processes and promote chain mobility before cross-linking can occur within each individual fibre and these can fuse. Thus, a lower content of structures forming volatile components seems beneficial during the various thermal treatments. Sufficient stabilization is crucial to avoid fibre fusion and rupture at the carbonization stage.
If cross-linking within individual fibres is insufficient, the rapid release of volatiles increases chain mobility. To avoid this scenario, a high content of condensed structures seem desirable. Whereas carbon fibre production itself may benefit from a more condensed structure; this is not necessarily beneficial for downstream applications. For example, carbon fibres used as electrodes or supercapacitors benefit from a graphitic structure. Its high conductivity arises from an elevated aromatic content associated with $sp^2$ orbitals, as opposed to higher aliphatic content ($sp^3$ hybrids) formed at temperatures near and above 850 °C.\textsuperscript{530} Low oxygen content is also beneficial for materials with high conductivity.\textsuperscript{531,532} Graphitic structures and large surface area are more easily obtained through pyrolysis of carbohydrates, even though their volatile nature reduces the biochar yield, as they promote high mobility and adaptation of crystalline sheets.\textsuperscript{533} A highly condensed, high-molecular weight lignin is not necessarily the one most easily transformed into a graphite-like material due to the higher content of carbon-carbon structures. Production of carbon fibres solely from lignin has suggested that lignins with high PDI such as Kraft lignin with branching could further decrease mobility\textsuperscript{534} during adaptation of the crystalline structure. This generates thicker carbon fibres with smaller graphite crystals compared to linear lignins of lower molecular weight and PDI. The latter, form an overall more ordered carbon arrangement with better graphitic structure.\textsuperscript{535} Hence, treatments producing low-molecular weight lignins (alongside low PDI) are suitable for generating materials benefiting from high strength and stiffness, as well as the electrical properties of graphite. Meanwhile, greater variance in molecular weight distributions could be, at least partially, responsible for the greater range of crystallite domains and larger fibre size. This would allow a more heterogeneous morphology, with potentially greater surface area and a less ordered carbon structure.\textsuperscript{535}

**Section S6.7: Lignin for nanoparticle production**

A common method applied for the production of lignin nanoparticles is by gradually introducing an anti-solvent, such as water, in an organic solution containing the fully dissolved lignin.\textsuperscript{536,537} The introduction of an anti-solvent gradually reduces their solubility through an increased drive toward hydrophobic interactions\textsuperscript{536,538} which further can be stabilised through $\pi$-$\pi$ interactions.\textsuperscript{539,540} The extent of such interactions for lignins and hydrophobic surfaces has been found to directly correlate with the area of interaction, and could thus be thought to increase with increased lignin chain length, but will also depend on the polymer chain conformational adaptability.\textsuperscript{541} Interestingly, it has been found that the first sites of nucleation during nanoparticle production originates from the largest lignin structures acting as starting-points, and is further followed by the precipitation of lower molecular mass lignins\textsuperscript{542} indicating the importance of hydrophobic interactions in their formation. In addition, this proceeding is likely related to the inherently higher threshold for translational movement and thus lower threshold for entering the glassy polymer state,\textsuperscript{543} which generally occur at lower levels of hydration or temperatures for larger lignins,\textsuperscript{544} therefore likely generating fixed sites for particle
growth first. Particles originating from large lignin structures has further been reported to form compact lignin nanoparticles formed through an aggregation process dominated by hydrophobic interactions. Such systems would likely favour precipitation of the largest structures first, while smaller structures having lower hydrophobic drive, but with a higher content of polar species, likely precipitating later in order to stabilise the particles interaction with the increasingly aqueous environment when the surface area of the hydrophobic particle increases, thereby contributing to generating a charged surface. For increased aggregation through hydrophobic interactions, acidified water is applied by introducing additional protons which hydration is highly favoured contributing to an increasingly shorter hydrogen bond lengths in the bulk water along with an increased proton transfer suggest a more stable and overall coherent water structure. This is not the case under alkaline conditions where nanoparticles often are found less stable likely due to the introduction of distinct/separated hyper-coordinated water clusters existing in a less coherent bulk solvent presenting less overall coherency thus less drive toward hydrophobic interactions which is also indicated by its slower than neutral-water proton transfer. The hydrated hydroxide also present an increased hydrophobic characteristic suitable for the amphiphilic nature of the lignins upon dissolution. The formation of hollow nanoparticles is also reported to occur. Interestingly, such structures seem to form due to non-ideal mixing between the organic solvent and water, and the fact that the dissolved lignins are able to stabilise an induced separation of the components in the solvent (interestingly, a bulk solvent not mixing ideally could be the driving-force behind the lignin dissolution process). For example, non-ideal mixing is reported for both THF and ethanol, and certain lignins are found to allow formation of hollow spheres when water is used to induce nanoparticle formation in THF and ethanol. This would require lignins able to compromise between the two solvent components displaying non-ideal mixing behaviour, thus would a certain content of hydroxyl and carboxylic groups seem favourable for introducing a link with the aqueous phase, while non-charged components of lignin could associate with the organic solvent phase. Therefore, certain chain depolymerisation is required to introduce more hydroxyl groups, but also to reduce the drive toward hydrophobic interactions generating a precipitation not taking the ordering of charged species into consideration (too high nucleation rate).

Thus, there seem to exist two distinct aims, the first being compact nanoparticles in for example composite materials where high elastic modulus is sought after, while the second being hollow lignin potentially applicable for transport/carrier utilisations. For the first nanoparticle application, it would seem desirable to ensure a high number of lignin nanoparticles generating distinct nucleation sites such that a high effective surface area can be obtained. Thus, it would be appropriate to apply a lignin of high molecular weight offering a stable site for hydrophobic aggregation. Further promoting a rapid nucleation rate could, for example, be achieved through a quick addition of anti-solvent, which thereafter could be proceeded by a controlled addition of anti-solvent for suitable particle growth.
depending on the actual usage. Intuitively, such a scheme would benefit from the presence of high molecular weight lignins, while also smaller lignins with higher charged species could be beneficial for appropriate particle surface properties. Regarding formation of hollow spheres, smaller lignin chains containing oxygen species such as hydroxyls, which efficiently can mediate tension-reduction between the aqueous and organic phase seem beneficial.

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