# Native lignin extraction from soft- and hardwood by green and benign sub/supercritical fluid extraction methodologies –Supplementary Information (SI)

Federica Nardella [a†], Jens Prothmann [b†] , Margareta Sandahl [b], Peter, Spégel[b], Erika Ribechini [a], and Charlotta Turner *[b]

[1] Department of Chemistry and Industrial Chemistry, University of Pisa, Via G. Moruzzi 13, 56124, Pisa, Italy

[2] Lund University, Department of Chemistry, Centre for Analysis and Synthesis, Lund, Sweden

† Both authors contributed equally

* Corresponding author. Charlotta.Turner@chem.lu.se

**Content**

**Table S1**. Values for the studied variables set (temperature, $CO_2$ ratio and water content in the co-solvent) for the 11 experiments performed as part of the DOE. The run order was randomised.

| | T (°C) | $CO_2$ ratio (vol%) | Water content in co-solvent (vol%) |
|---|---|---|---|
| **Centre point (n=3)** | 60 | 50 | 10 |
| **Experiment 1** | 40 | 10 | 0 |
| **Experiment 2** | 80 | 10 | 0 |
| **Experiment 3** | 40 | 90 | 0 |
| **Experiment 4** | 80 | 90 | 0 |
| **Experiment 5** | 40 | 10 | 20 |
| **Experiment 6** | 80 | 10 | 20 |
| **Experiment 7** | 40 | 90 | 20 |
| **Experiment 8** | 80 | 90 | 20 |

**Table S2.** Settings to create the feature list in MZmine 2.

1. Raw data methods/Peak detection
    1.1 Mass detection
        Settings:
            - Polarity: negative
            - Mass detector: centroid
            - Spectrum type: any
            - Noise level: 1.0E4
    1.2 Chromatogram builder
        Settings:
            - MS level: 1
            - Polarity: negative
            - Spectrum type: any
            - Min time span: 0.03 min
            - Min height: 3.0E4
            - m/z tolerance: 0.01 Da or 5.0 ppm
2. Peak list methods
    2.1 Peak detection/chromatogram deconvolution
        Settings:
            - Algorithm: local minimum search
            - Chromatographic threshold: 30.0%
            - Minimum relative height: 1.0%
            - Minimum absolute height: 1.0E4
            - Min ratio of peak top/edge 2
            - m/z center calculation: median
    2.2 Isotopes/isotopes peak grouper
        Settings:
            - m/z tolerance: 0.01 Da or 10.0 ppm
            - Retention time tolerance: 0.05 min
            - Maximum charge: 1
            - Representative isotope: most intense

    2.3 Identification/adduct search
        Settings:
            - Retention time tolerance: 0.05 min
            - Adducts: [M+NH3], 17.0265 Da; [M+H3PO4], 97.9769 Da; [M+H2SO4], 97.9674; [M+CH2O2], 46.0056
            - m/z tolerance: 0.01 Da or 5 ppm
            - Max relative adduct peak height: 50.0%
    2.4 Identification/formula prediction
        Settings:
            - Charge: 1
            - Ionisation type: [M-H]-
            - m/z tolerance: 0.005 Da or 5.0 ppm
            - Max best formulas per peak: 1
            - Elements: C, O, H, S (for all min: 0, max: 100)
            - Element count heuristics: H/C ratio: yes, multiple element counts: yes
            - RDBE restrictions: RDBE range: -1 to 40, RDBE must be an integer: yes

**Table S3**. Hansen solubility parameters, including dispersive interactions (δD), polar interactions (δP), hydrogen bonds (δH), and relative energy difference related to lignin (RED) for several green solvents (Hansen, 2007).

| | $\delta D$ (MPa$^{1/2}$) | $\delta P$ (MPa$^{1/2}$) | $\delta H$ (MPa$^{1/2}$) | RED | Condition |
|---|---|---|---|---|---|
| $CO_2$ | 13.2 | 4.9 | 5.4 | 1.7 | 25 °C, 350 bar |
| Water | 15.5 | 16.0 | 42.3 | 2.1 | Ambient |
| Methanol | 15.1 | 12.3 | 22.3 | 1.1 | Ambient |
| Ethanol | 15.8 | 8.8 | 19.4 | 1.0 | Ambient |
| 1-butanol | 16.0 | 5.7 | 15.8 | 1.1 | Ambient |
| Ethyl acetate | 15.8 | 5.3 | 7.2 | 1.3 | Ambient |
| Isopropyl acetate | 14.9 | 4.5 | 8.2 | 1.4 | Ambient |
| Ethyl lactate | 16.0 | 7.6 | 12.5 | 1.0 | Ambient |
| Acetone | 15.5 | 10.4 | 7.0 | 1.2 | Ambient |
| Ethylene glycol | 17.0 | 11.0 | 26 | 1.0 | Ambient |
| Anisole | 17.8 | 4.1 | 6.7 | 1.2 | Ambient |
| MEK (methyl ethyl ketone) | 16.0 | 9.0 | 5.1 | 1.3 | Ambient |

**Table S4**. Hansen solubility parameters, including dispersive interactions (δD), polar interactions (δP) and hydrogen bonds (δH), for solvent mixtures at the experimental condition in the DOE design space for ethanol and ethyl lactate. RED =Relative energy difference related to lignin; EtOH = ethanol, ELac = ethyl lactate.

| | ***δD*** **(MPa$^{1/2}$)** | ***δP*** **(MPa$^{1/2}$)** | ***δH*** **(MPa$^{1/2}$)** | **RED** | **Condition** |
|---|---|---|---|---|---|
| $CO_2$/EtOH/$H_2O$ (50/45/5, v/v/v) | 13.0 | 6.8 | 12.7 | 1.4 | 60 °C, 350 bar |
| $CO_2$/EtOH (10/90, v/v) | 15.1 | 8.2 | 17.6 | 1.1 | 40 °C, 350 bar |
| $CO_2$/EtOH (10/90, v/v) | 13.9 | 7.6 | 16.2 | 1.3 | 80 °C, 350 bar |
| $CO_2$/EtOH (90/10, v/v) | 12.7 | 5.1 | 6.6 | 1.7 | 40 °C, 350 bar |
| $CO_2$/EtOH (90/10, v/v) | 10.4 | 4.7 | 5.8 | 2.0 | 80 °C, 350 bar |
| $CO_2$/EtOH/ $H_2O$ (10/72/18, v/v/v) | 15.1 | 9.5 | 21.6 | 1.1 | 40 °C, 350 bar |
| $CO_2$/EtOH/ $H_2O$ (10/72/18, v/v/v) | 13.9 | 8.9 | 20.3 | 1.2 | 80 °C, 350 bar |
| $CO_2$/EtOH/ $H_2O$ (90/8/2, v/v/v) | 12.7 | 5.3 | 7.0 | 1.7 | 40 °C, 350 bar |
| $CO_2$/EtOH/ $H_2O$ (90/8/2, v/v/v) | 10.4 | 4.9 | 6.3 | 2.0 | 80 °C, 350 bar |
| $CO_2$/EtLac/$H_2O$ (50/45/5, v/v/v) | 13.2 | 6.3 | 9.9 | 1.5 | 60 °C, 350 bar |
| $CO_2$/EtLac (10/90, v/v) | 15.4 | 7.2 | 11.6 | 1.1 | 40 °C, 350 bar |
| $CO_2$/EtLac (10/90, v/v) | 14.4 | 6.8 | 10.9 | 1.3 | 80 °C, 350 bar |
| $CO_2$/EtLac (90/10, v/v) | 12.7 | 5.0 | 5.9 | 1.7 | 40 °C, 350 bar |
| $CO_2$/EtLac (90/10, v/v) | 10.5 | 4.6 | 5.2 | 2.0 | 80 °C, 350 bar |
| $CO_2$/EtLac/ $H_2O$ (10/72/18, v/v/v) | 15.3 | 8.7 | 16.9 | 1.0 | 40 °C, 350 bar |
| $CO_2$/EtLac/ $H_2O$ (10/72/18, v/v/v) | 14.4 | 8.2 | 16.0 | 1.2 | 80 °C, 350 bar |
| $CO_2$/EtLac/ $H_2O$ (90/8/2, v/v/v) | 12.7 | 5.2 | 6.5 | 1.7 | 40 °C, 350 bar |
| $CO_2$/EtLac/ $H_2O$ (90/8/2, v/v/v) | 10.5 | 4.8 | 5.8 | 2.0 | 80 °C, 350 bar |

**Table S5.** Gravimetric yields obtained for both oak and fir extracted with the three investigated solvents. The yield was calculated as % dry extract weight obtained/ initial sample weight, w/w. CP = centre point. Run order was randomised.

| | Ethanol | | Acetone | | Ethyl lactate | |
|---|---|---|---|---|---|---|
| | **Oak (%)** | **Fir (%)** | **Oak (%)** | **Fir (%)** | **Oak (%)** | **Fir (%)** |
| **CP (average)** | 3.1 | 0.9 | 4.3 | 1.3 | 5.3 | 2.2 |
| **Experiment 1** | 5.5 | 0.9 | 5.2 | 1.0 | 9.1 | 5.3 |
| **Experiment 2** | 7.8 | 1.1 | 5.6 | 0.9 | 8.5 | 6.0 |
| **Experiment 3** | 0.3 | 0.7 | 0.3 | 0.7 | 7.4 | 3.0 |
| **Experiment 4** | 0.5 | 0.8 | 0.4 | 0.7 | 0.4 | 3.1 |
| **Experiment 5** | 8.0 | 1.1 | 9.1 | 11.0 | 11.3 | 1.9 |
| **Experiment 6** | 10.0 | 2.6 | 13.1 | 1.7 | 17.5 | 3.7 |
| **Experiment 7** | 3.5 | 0.8 | 0.4 | 0.9 | 0.3 | 1.6 |
| **Experiment 8** | 2.3 | 0.9 | 1.1 | 0.7 | 3.5 | 1.7 |

**Figure S1.** Softwood (fir) coefficient plot and contour plots obtained from the DOE for ethyl lactate, showing the effect of $CO_2$ ratio and water content in the co-solvent on the gravimetric yield. Model obtained for other solvents were invalid.

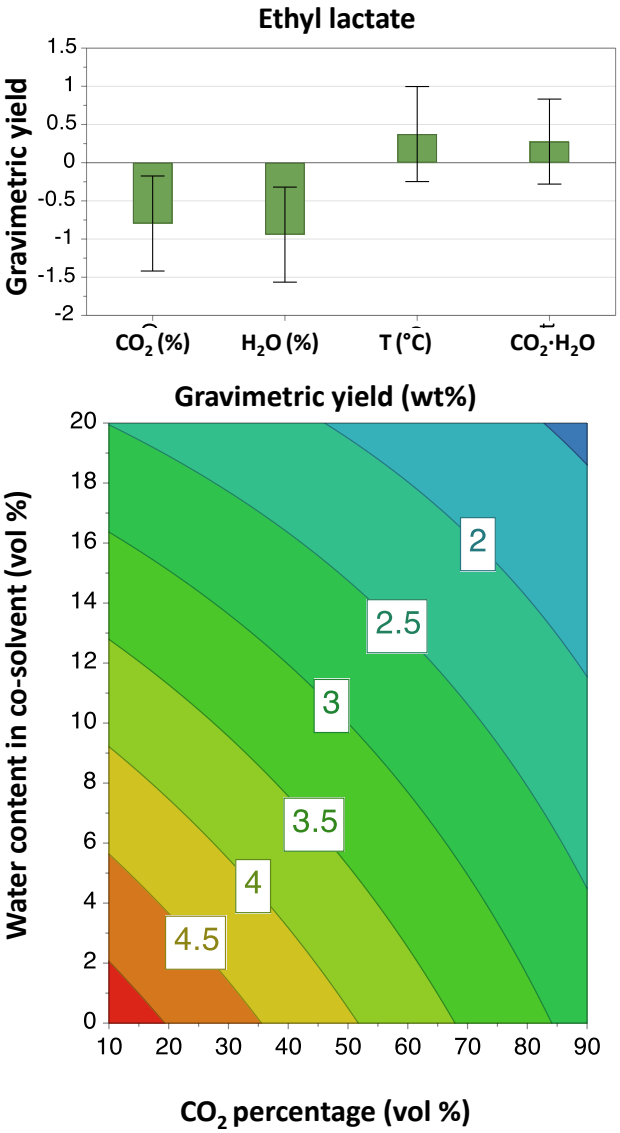


*Figure S1 - Coefficient plots from the interaction model with a full factorial design showing the influence of the investigated factors and two-factor interactions on the gravimetric yield and contour plots showing the influence of the $CO_2$ percentage and water content in the co-solvent on the gravimetric yield of fir wood using ethyl lactate as co-solvent. $CO_2$ (%): $CO_2$ percentage; $H_2O$ (%): water content in co-solvent. Temperature was 60 °C and pressure 350 bar.*

**Table S6.** Model fit of the data obtained for all the DOE performed and different responses. $R^2$ is the percent of the variation of the response explained by the model. $Q^2$ is the percent of the variation of the response predicted by the model according to cross validation.

| | Gravimetric yield (wt%) | | | | | |
|---|---|---|---|---|---|---|
| | Ethanol | | Acetone | | Ethyl lactate | |
| | Oak | Fir | Oak | Fir | Oak | Fir |
| $R^2$ | 0.86 | 0.64 | 0.97 | 0.63 | 0.91 | 0.82 |
| $Q^2$ | 0.60 | 0.23 | 0.73 | 0.07 | 0.48 | 0.58 |
| $R^2$-$Q^2$ | 0.26 | 0.41 | 0.24 | 0.56 | 0.43 | 0.24 |
| Reproducibility | 0.78 | 0.54 | 1.00 | 1.00 | 0.97 | 0.94 |
| | Number of identified compounds | | | | | |
| | Ethanol | | Acetone | | Ethyl lactate | |
| | Oak | Fir | Oak | Fir | Oak | Fir |
| $R^2$ | 0.46 | 0.55 | 0.22 | 0.20 | 0.66 | 0.64 |
| $Q^2$ | -0.19 | 0.30 | -0.13 | -0.20 | 0.39 | 0.39 |
| $R^2$-$Q^2$ | 0.66 | 0.24 | 0.35 | 0.40 | 0.26 | 0.26 |
| Reproducibility | 0.43 | -0.20 | 0.60 | 0.42 | 0.97 | 0.08 |
| | Vanillin concentration (µg/g) | | | | | |
| | Ethanol | | Acetone | | Ethyl lactate | |
| | Oak | Fir | Oak | Fir | Oak | Fir |
| $R^2$ | 0.50 | 0.85 | 0.33 | 0.24 | 0.94 | 0.70 |
| $Q^2$ | -0.20 | 0.18 | -0.11 | -0.20 | 0.46 | 0.01 |
| $R^2$-$Q^2$ | 0.70 | 0.68 | 0.44 | 0.44 | 0.49 | 0.69 |
| Reproducibility | 0.97 | 0.83 | -0.20 | 0.95 | 0.94 | 0.53 |
| | Vanillic acid concentration (µg/g) | | | | | |
| | Ethanol | | Acetone | | Ethyl lactate | |
| | Oak | Fir | Oak | Fir | Oak | Fir |
| $R^2$ | 0.59 | 0.69 | 0.42 | 0.67 | 0.87 | 0.72 |
| $Q^2$ | -0.01 | 0.17 | 0.06 | -0.07 | 0.28 | 0.13 |
| $R^2$-$Q^2$ | 0.60 | 0.52 | 0.35 | 0.74 | 0.59 | 0.59 |
| Reproducibility | 0.88 | 0.68 | -0.20 | 0.89 | 0.73 | 0.92 |
| | Coniferyl aldehyde concentration (µg/g) | | | | | |
| | Ethanol | | Acetone | | Ethyl lactate | |
| | Oak | Fir | Oak | Fir | Oak | Fir |
| $R^2$ | 0.74 | 0.46 | 0.65 | 0.23 | 0.91 | 0.86 |
| $Q^2$ | 0.20 | 0.26 | 0.10 | -0.20 | 0.41 | 0.37 |
| $R^2$-$Q^2$ | 0.54 | 0.20 | 0.56 | 0.43 | 0.50 | 0.49 |
| Reproducibility | 0.39 | -0.20 | 0.83 | 0.92 | 0.55 | 0.37 |
| | Ferulic acid concentration (µg/g) | | | | | |
| | Ethanol | | Acetone | | Ethyl lactate | |
| | Oak | Fir | Oak | Fir | Oak | Fir |
| $R^2$ | 0.36 | 0.35 | 0.20 | 0.93 | 0.52 | 0.81 |
| $Q^2$ | 0.30 | -0.16 | -0.20 | 0.28 | -0.03 | 0.27 |
| $R^2$-$Q^2$ | 0.07 | 0.51 | 0.40 | 0.65 | 0.55 | 0.53 |
| Reproducibility | -0.20 | -0.20 | 1.00 | 1.00 | 0.17 | 0.80 |

| | Syringaldehyde concentration (μg/g) | | |
|---|---|---|---|
| | **Ethanol** | **Acetone** | **Ethyl lactate** |
| | **Oak** | **Oak** | **Oak** |
| **$R^2$** | 0.34 | 0.77 | 0.77 |
| **$Q^2$** | -0.20 | 0.57 | 0.45 |
| **$R^2$-$Q^2$** | 0.54 | 0.20 | 0.32 |
| **Reproducibility** | -0.14 | 0.90 | 1.00 |
| | **Syringic acid concentration (μg/g)** | | |
| | **Ethanol** | **Acetone** | **Ethyl lactate** |
| | **Oak** | **Oak** | **Oak** |
| **$R^2$** | 0.64 | 0.28 | 0.09 |
| **$Q^2$** | 0.20 | -0.19 | -0.20 |
| **$R^2$-$Q^2$** | 0.44 | 0.47 | 0.29 |
| **Reproducibility** | 0.74 | 0.09 | -0.20 |
| | **Sinapaldehyde concentration (μg/g)** | | |
| | **Ethanol** | **Acetone** | **Ethyl lactate** |
| | **Oak** | **Oak** | **Oak** |
| **$R^2$** | 0.01 | 0.77 | 0.73 |
| **$Q^2$** | -0.14 | 0.44 | 0.33 |
| **$R^2$-$Q^2$** | 0.15 | 0.32 | 0.40 |
| **Reproducibility** | -0.20 | 0.81 | 0.81 |

**Table S7.** ANOVA results comparing the gravimetric yields obtained by the center points of each DoE, which were performed as triplicates.

| Gravimetric yield (wt%) | Ethanol | Acetone | Ethyl lactate |
|---|---|---|---|
| **Oak** | 1.4 | 4.48 | 4.35 |
| | 3.4 | 4.29 | 5.89 |
| | 4.4 | 4.16 | 5.62 |
| **Fir** | 0.77 | 1.31 | 2.48 |
| | 0.55 | 1.21 | 1.8 |
| | 1.28 | 1.31 | 2.34 |

| SUMMARY | Ethanol | Acetone | Ethyl lactate | Total |
|---|---|---|---|---|
| ***Oak*** | | | | |
| **Count** | 3 | 3 | 3 | 9 |
| **Sum** | 9.2 | 12.93 | 15.86 | 37.99 |
| **Average** | 3.066667 | 4.31 | 5.286667 | 4.221111 |
| **Variance** | 2.333333 | 0.0259 | 0.676233 | 1.687386 |
| | | | | |
| ***Fir*** | | | | |
| **Count** | 3 | 3 | 3 | 9 |
| **Sum** | 2.6 | 3.83 | 6.62 | 13.05 |
| **Average** | 0.866667 | 1.276667 | 2.206667 | 1.45 |
| **Variance** | 0.140233 | 0.003333 | 0.128933 | 0.4217 |
| | | | | |
| ***Total*** | | | | |
| **Count** | 6 | 6 | 6 | |
| **Sum** | 11.8 | 16.76 | 22.48 | |
| **Average** | 1.966667 | 2.793333 | 3.746667 | |
| **Variance** | 2.441427 | 2.772027 | 3.167987 | |

**ANOVA**

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| **Wood types** | 34.55576 | 1 | 34.55576 | 62.67733 | 4.183E-06 | 4.7472253 |
| **Solvents** | 9.521244 | 2 | 4.760622 | 8.634831 | 0.0047488 | 3.8852938 |
| **Interaction** | 0.735511 | 2 | 0.367756 | 0.667036 | 0.5312643 | 3.8852938 |
| **Within** | 6.615933 | 12 | 0.551328 | | | |
| | | | | | | |
| **Total** | 51.42844 | 17 | | | | |

**Table S8.** ANOVA results comparing the number of detected compounds obtained by the center points of each DoE, which were performed as triplicates.

| Number of identified compounds | Ethanol | Acetone | Ethyl lactate |
|---|---|---|---|
| **Oak** | 17 | 24 | 18 |
| | 18 | 39 | 20 |
| | 11 | 34 | 20 |
| **Fir** | 20 | 13 | 15 |
| | 5 | 24 | 7 |
| | 9 | 20 | 18 |

| SUMMARY | Ethanol | Acetone | Ethyl lactate | Total |
|---|---|---|---|---|
| ***Oak*** | | | | |
| **Count** | 3 | 3 | 3 | 9 |
| **Sum** | 46 | 97 | 58 | 201 |
| **Average** | 15.33333 | 32.33333 | 19.33333 | 22.33333 |
| **Variance** | 14.33333 | 58.33333 | 1.333333 | 77.75 |
| ***Fir*** | | | | |
| **Count** | 3 | 3 | 3 | 9 |
| **Sum** | 34 | 57 | 40 | 131 |
| **Average** | 11.33333 | 19 | 13.33333 | 14.55556 |
| **Variance** | 60.33333 | 31 | 32.33333 | 42.77778 |
| ***Total*** | | | | |
| **Count** | 6 | 6 | 6 | |
| **Sum** | 80 | 154 | 98 | |
| **Average** | 13.33333 | 25.66667 | 16.33333 | |
| **Variance** | 34.66667 | 89.06667 | 24.26667 | |

| **ANOVA** | | | | | | |
|---|---|---|---|---|---|---|
| ***Source of Variation*** | ***SS*** | ***df*** | ***MS*** | ***F*** | ***P-value*** | ***F crit*** |
| **Wood types** | 272.2222 | 1 | 272.2222 | 8.263069 | 0.0139711 | 4.7472253 |
| **Solvents** | 496.4444 | 2 | 248.2222 | 7.53457 | 0.00759 | 3.8852938 |
| **Interaction** | 72.44444 | 2 | 36.22222 | 1.099494 | 0.3643704 | 3.8852938 |
| **Within** | 395.3333 | 12 | 32.94444 | | | |
| **Total** | 1236.444 | 17 | | | | |

**Table S9.** Results from comparison of the slopes obtained by plotting predicted vs observed values with the slope of 1 for each DoE of gravimetric yield.

| Gravimetric yield | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ethanol | | | | Acetone | | | | Ethyl lactate | | | |
| | Oak | | Fir | | Oak | | Fir | | Oak | | Fir | |
| | Pred | Obs | Pred | Obs | Pred | Obs | Pred | Obs | Pred | Obs | Pred | Obs |
| **Exp 1** | -0.47 | 0.30 | 0.44 | 0.73 | -0.38 | 0.27 | -1.00 | 0.94 | 1.13 | 0.26 | 0.98 | 1.64 |
| **Exp 2** | 0.36 | 0.50 | 0.58 | 0.83 | 0.54 | 0.41 | -0.91 | 0.74 | 1.60 | 3.54 | 1.82 | 1.75 |
| **Exp 3** | 2.03 | 3.50 | 0.72 | 0.89 | 0.77 | 0.39 | -0.41 | 0.65 | 3.09 | 7.37 | 2.07 | 1.90 |
| **Exp 4** | 2.86 | 2.30 | 0.91 | 0.83 | 1.69 | 1.14 | 1.92 | 0.96 | 3.56 | 0.35 | 2.40 | 2.99 |
| **Exp 5** | 4.28 | 1.40 | 1.05 | 0.90 | 4.38 | 4.48 | 1.95 | 1.31 | 6.71 | 4.35 | 2.91 | 3.72 |
| **Exp 6** | 4.28 | 3.40 | 1.06 | 0.77 | 4.38 | 4.29 | 1.95 | 1.21 | 6.71 | 5.89 | 3.00 | 2.48 |
| **Exp 7** | 4.28 | 4.40 | 1.06 | 0.55 | 4.38 | 4.16 | 1.95 | 1.31 | 6.71 | 5.62 | 3.00 | 1.80 |
| **Exp 8** | 5.78 | 5.50 | 1.06 | 1.28 | 4.39 | 5.23 | 2.01 | 0.74 | 8.05 | 9.11 | 3.00 | 2.34 |
| **CP1** | 6.61 | 7.80 | 1.20 | 1.13 | 5.53 | 5.58 | 2.51 | 0.92 | 8.52 | 8.53 | 3.24 | 3.11 |
| **CP2** | 8.13 | 8.00 | 1.55 | 1.13 | 10.66 | 9.11 | 4.28 | 1.66 | 13.61 | 11.27 | 4.87 | 5.27 |
| **CP3** | 8.96 | 10.00 | 2.03 | 2.61 | 11.81 | 13.08 | 7.20 | 11.00 | 14.08 | 17.48 | 5.71 | 6.00 |
| **Slope** | 1.000000 | | 0.999999 | | 1.000002 | | 1.000000 | | 0.999997 | | 1.000000 | |
| **n** | 11 | | 11 | | 11 | | 11 | | 11 | | 11 | |
| **SE (reg)** | 1.28155138 | | 0.353123537 | | 0.794891708 | | 1.98170723 | | 2.553638349 | | 0.656485923 | |
| **SE(slope)** | 0.135543709 | | 0.252758419 | | 0.064121878 | | 0.265557111 | | 0.186672217 | | 0.15660464 | |
| **Difference** | 1.1E-07 | | 1.4E-06 | | 2.3E-06 | | 2.4E-07 | | 2.8E-06 | | 3.2E-07 | |
| **SE (diff)** | 0.135543709 | | 0.252758419 | | 0.064121878 | | 0.265557111 | | 0.186672217 | | 0.15660464 | |
| **t-stat** | 7.9E-07 | | 5.6E-06 | | 3.5E-05 | | 9.1E-07 | | 1.5E-05 | | 2.0E-06 | |
| **df** | 14 | | 14 | | 14 | | 14 | | 14 | | 14 | |
| **p** | 0.99999938 | | 0.999995597 | | 0.999972439 | | 0.999999284 | | 0.999988147 | | 0.999998423 | |

**Table S10.** Results from comparison of the slopes obtained by plotting predicted vs observed values with the slope of 1 for each DoE of number of identified compounds.

| Number of identified compounds | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ethanol | | | | Acetone | | | | Ethyl lactate | | | |
| | Oak | | Fir | | Oak | | Fir | | Oak | | Fir | |
| | Pred | Obs | Pred | Obs | Pred | Obs | Pred | Obs | Pred | Obs | Pred | Obs |
| **Exp 1** | 13.70 | 8.00 | 6.37 | 5.00 | 14.42 | 8.00 | 7.91 | 2.00 | 10.27 | 9.00 | 5.43 | 4.00 |
| **Exp 2** | 9.20 | 8.00 | 5.25 | 8.00 | 8.52 | 2.00 | 4.99 | 2.00 | 4.77 | 3.00 | 4.83 | 3.00 |
| **Exp 3** | 19.20 | 21.00 | 13.42 | 12.00 | 23.67 | 17.00 | 14.26 | 13.00 | 21.27 | 18.00 | 17.40 | 19.00 |
| **Exp 4** | 17.20 | 21.00 | 7.53 | 8.00 | 10.42 | 7.00 | 12.33 | 8.00 | 10.77 | 8.00 | 9.31 | 11.00 |
| **Exp 5** | 12.95 | 18.00 | 13.72 | 17.00 | 19.36 | 13.00 | 7.80 | 7.00 | 13.27 | 10.00 | 7.31 | 8.00 |
| **Exp 6** | 8.45 | 9.00 | 12.60 | 9.00 | 13.46 | 8.00 | 4.88 | 1.00 | 7.77 | 5.00 | 6.71 | 4.00 |
| **Exp 7** | 18.45 | 16.00 | 20.77 | 22.00 | 28.61 | 24.00 | 14.15 | 9.00 | 24.27 | 23.00 | 19.28 | 16.00 |
| **Exp 8** | 16.45 | 12.00 | 14.88 | 15.00 | 15.36 | 8.00 | 12.22 | 9.00 | 13.77 | 12.00 | 11.19 | 7.00 |
| **CP1** | 14.45 | 17.00 | 11.82 | 20.00 | 16.73 | 24.00 | 9.82 | 13.00 | 13.27 | 18.00 | 10.18 | 15.00 |
| **CP2** | 14.45 | 18.00 | 11.82 | 5.00 | 16.73 | 39.00 | 9.82 | 24.00 | 13.27 | 20.00 | 10.18 | 7.00 |
| **CP3** | 14.45 | 11.00 | 11.82 | 9.00 | 16.73 | 34.00 | 9.82 | 20.00 | 13.27 | 20.00 | 10.18 | 18.00 |
| **Slope** | 1.000005 | | 1.000002 | | 1.000005 | | 1.000000 | | 1.000001 | | 1.000000 | |
| **n** | 11 | | 11 | | 11 | | 11 | | 11 | | 11 | |
| **SE (reg)** | 3.86188 | | 4.19377 | | 11.21952 | | 6.92611 | | 4.20558 | | 3.96305 | |
| **SE(slope)** | 0.3578 | | 0.3064 | | 0.6259 | | 0.6730 | | 0.2416 | | 0.2746 | |
| **Difference** | 4.55E-06 | | 2.17E-06 | | 5.16E-06 | | 5.00E-07 | | 9.36E-07 | | 2.48E-07 | |
| **SE (diff)** | 0.3578 | | 0.3064 | | 0.6259 | | 0.6730 | | 0.2416 | | 0.2746 | |
| **t-stat** | 1.27E-05 | | 7.09E-06 | | 8.25E-06 | | 7.42E-07 | | 3.87E-06 | | 9.03E-07 | |
| **df** | 14 | | 14 | | 14 | | 14 | | 14 | | 14 | |
| **p** | 0.999990 | | 0.999994 | | 0.999994 | | 0.999999 | | 0.999997 | | 0.999999 | |

**Table S11**. Comparison and statistics of standard addition and external calibration curve.

| | Slope | Slope error | Intercept | Intercept error | t calculated | t listed (α=0.05) |
|---|---|---|---|---|---|---|
| **External** | 0.10827 | 0.00154 | -0.00629 | 0.00825 | 1.04 | 2.31 |
| **Standard addition** | 0.09927 | 0.01937 | 0.43518 | 0.11545 | | |

**Table S12**. Calibration curves obtained by linear regression using standards of LMs.

| Compound | Slope | Slope error | Intercept | Intercept error | $R^2$ |
|---|---|---|---|---|---|
| Vanillin | 0.108 | 0.00154 | -0.006 | 0.00825 | 0.999 |
| Vanillic acid | 0.001 | 0.00003 | 0.001 | 0.00062 | 0.998 |
| Coniferyl aldehyde | 0.039 | 0.00112 | 0.008 | 0.02412 | 0.996 |
| Ferulic acid | 0.053 | 0.00266 | -0.078 | 0.06154 | 0.992 |
| Syringaldehyde | 0.010 | 0.00026 | -0.008 | 0.00537 | 0.998 |
| Syringic acid | 0.001 | 0.00003 | -0.001 | 0.00072 | 0.998 |
| Sinapaldehyde | 0.049 | 0.00180 | -0.054 | 0.03922 | 0.996 |

**Table S13**. Concentration of vanillin, vanillic acid, coniferyl aldehyde, ferulic acid, syringaldehyde, syringic acid and sinapaldehyde obtained in the investigated extracts. Being a softwood, fir wood does not contain syringaldehyde, syringic acid and sinapaldehyde. (<LOD): the concentration was below the LOD of method.

| | **Vanillin** | | | | | |
|---|---|---|---|---|---|---|
| | **Ethanol** | | **Acetone** | | **Ethyl lactate** | |
| | **Oak (µg/g)** | **Fir (µg/g)** | **Oak (µg/g)** | **Fir (µg/g)** | **Oak (µg/g)** | **Fir (µg/g)** |
| **CP (average)** | 3.0 ± 1 | 9.0 ± 3 | 16.0 ± 6 | 23.0 ± 2 | 8.0 ± 2 | 15.0 ± 6 |
| **Experiment 1** | 6.0 | <LOD | 6.6 | <LOD | <LOD | 6.2 |
| **Experiment 2** | 11.0 | 12.5 | 9.5 | <LOD | 7.3 | <LOD |
| **Experiment 3** | 1.9 | 4.8 | <LOD | <LOD | <LOD | <LOD |
| **Experiment 4** | 7.0 | 16.9 | <LOD | <LOD | <LOD | 3.0 |
| **Experiment 5** | 14.3 | <LOD | 5.6 | 17.6 | 5.3 | 3.3 |
| **Experiment 6** | 14.0 | 42.5 | 16.3 | <LOD | 29.7 | 37.2 |
| **Experiment 7** | 83.5 | 7.2 | 5.5 | 8.7 | <LOD | 6.3 |
| **Experiment 8** | 10.8 | 17.0 | 5.9 | 7.9 | 4.7 | 12.6 |
| | **Vanillic acid** | | | | | |
| | **Ethanol** | | **Acetone** | | **Ethyl lactate** | |
| | **Oak (µg/g)** | **Fir (µg/g)** | **Oak (µg/g)** | **Fir (µg/g)** | **Oak (µg/g)** | **Fir (µg/g)** |
| **CP (average)** | 3.0 ± 1 | 5.0 ± 2 | 6.0 ± 2 | 9.0 ± 2 | 6.0 ± 2 | 20.0 ± 3 |
| **Experiment 1** | 4.0 | <LOD | <LOD | <LOD | <LOD | <LOD |
| **Experiment 2** | 4.5 | 22.2 | 3.5 | 0.9 | 8.2 | <LOD |
| **Experiment 3** | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| **Experiment 4** | 6.2 | 39.6 | <LOD | <LOD | 8.8 | <LOD |
| **Experiment 5** | 22.6 | 25.7 | 3.7 | 18.8 | 7.4 | 15.4 |
| **Experiment 6** | 18.6 | 22.1 | 6.3 | <LOD | 5.4 | 32.5 |
| **Experiment 7** | 9.7 | 18.2 | <LOD | 5.0 | 4.4 | 12.7 |
| **Experiment 8** | 3.4 | 37.9 | 2.0 | 4.7 | 8.2 | 11.3 |
| | **Coniferyl aldehyde** | | | | | |
| | **Ethanol** | | **Acetone** | | **Ethyl lactate** | |
| | **Oak (µg/g)** | **Fir (µg/g)** | **Oak (µg/g)** | **Fir (µg/g)** | **Oak (µg/g)** | **Fir (µg/g)** |
| **CP (average)** | 12.0 ± 6 | 14.0 ± 7 | 8.0 ± 1 | 17.0 ± 3 | 3.0 ± 2 | 7.0 ± 5 |
| **Experiment 1** | <LOD | 4.7 | 3.1 | <LOD | <LOD | <LOD |
| **Experiment 2** | 9.8 | 22.6 | 8.1 | 4.1 | <LOD | <LOD |
| **Experiment 3** | <LOD | 2.3 | <LOD | <LOD | <LOD | <LOD |
| **Experiment 4** | 3.9 | 16.1 | <LOD | <LOD | <LOD | <LOD |
| **Experiment 5** | 6.8 | 12.8 | 4.2 | 4.2 | 4.3 | 12.7 |
| **Experiment 6** | 6.8 | 24.5 | 9.2 | <LOD | 7.5 | 18.5 |
| **Experiment 7** | 13.4 | 15.2 | 2.3 | <LOD | 2.3 | 6.9 |
| **Experiment 8** | <LOD | 17.6 | 7.8 | 16.2 | 5.1 | 9.2 |
| | **Ferulic acid** | | | | | |
| | **Ethanol** | | **Acetone** | | **Ethyl lactate** | |
| | **Oak (µg/g)** | **Fir (µg/g)** | **Oak (µg/g)** | **Fir (µg/g)** | **Oak (µg/g)** | **Fir (µg/g)** |
| **CP (average)** | 7.0 ± 3 | 8.0 ± 4 | 3.3 ± 0.2 | 5.9 ± 0.03 | 7.0 ± 2 | 7.0 ± 2 |
| **Experiment 1** | 5.9 | 5.9 | 9.1 | <LOD | 4.7 | <LOD |
| **Experiment 2** | 6.0 | 6.0 | 9.3 | 8.9 | 8.9 | <LOD |
| **Experiment 3** | 6.0 | 5.9 | <LOD | <LOD | <LOD | <LOD |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Experiment 4** | 6.0 | 5.9 | 12.0 | <LOD | 8.9 | <LOD |
| **Experiment 5** | 9.4 | <LOD | 9.4 | 8.9 | 6.0 | 6.0 |
| **Experiment 6** | 9.0 | 9.0 | 9.3 | 8.9 | 6.2 | 6.0 |
| **Experiment 7** | 6.2 | 6.0 | 8.9 | 8.9 | 8.9 | 8.9 |
| **Experiment 8** | 6.3 | 5.9 | 9.1 | 8.9 | 8.9 | 9.0 |

| | **Syringaldehyde** | | |
|---|---|---|---|
| | **Ethanol** | **Acetone** | **Ethyl lactate** |
| | **Oak (µg/g)** | **Oak (µg/g)** | **Oak (µg/g)** |
| **CP (average)** | 25.0 ± 10 | 35.0 ± 5 | 28.5 ± 0.6 |
| **Experiment 1** | 12.4 | 16.5 | 5.6 |
| **Experiment 2** | 38.3 | 37.6 | 11.6 |
| **Experiment 3** | 5.5 | 6.2 | 4.7 |
| **Experiment 4** | 15.4 | 7.9 | 5.6 |
| **Experiment 5** | 35.3 | 21.9 | 21.9 |
| **Experiment 6** | 25.4 | 36.1 | 42.9 |
| **Experiment 7** | 48.8 | 14.0 | 16.0 |
| **Experiment 8** | 22.7 | 31.7 | 36.5 |

| | **Syringic acid** | | |
|---|---|---|---|
| | **Ethanol** | **Acetone** | **Ethyl lactate** |
| | **Oak (µg/g)** | **Oak (µg/g)** | **Oak (µg/g)** |
| **CP (average)** | 25.0 ± 1 | 35.0 ± 5 | 76.0 ± 2 |
| **Experiment 1** | 5.3 | 14.6 | 7.6 |
| **Experiment 2** | 44.3 | 18.2 | 16.4 |
| **Experiment 3** | 8.7 | <LOD | 4.2 |
| **Experiment 4** | 22.2 | <LOD | 5.0 |
| **Experiment 5** | 48.2 | 31.1 | 24.2 |
| **Experiment 6** | 43.4 | 23.2 | 28.9 |
| **Experiment 7** | 28.0 | 7.7 | 17.8 |
| **Experiment 8** | 65.8 | 17.1 | 19.4 |

| | **Sinapaldehyde** | | |
|---|---|---|---|
| | **Ethanol** | **Acetone** | **Ethyl lactate** |
| | **Oak (µg/g)** | **Oak (µg/g)** | **Oak (µg/g)** |
| **CP (average)** | 12.1 ± 0.1 | 9.2 ± 0.9 | 8.0 ± 2 |
| **Experiment 1** | 5.3 | 7.9 | 6.6 |
| **Experiment 2** | 10.4 | 12.1 | <LOD |
| **Experiment 3** | 5.0 | 8.8 | <LOD |
| **Experiment 4** | 7.3 | 8.9 | <LOD |
| **Experiment 5** | 15.8 | 11.9 | 8.3 |
| **Experiment 6** | 11.1 | 14.1 | 11.2 |
| **Experiment 7** | 14.1 | 7.4 | 7.5 |
| **Experiment 8** | 8.5 | 11.9 | 7.5 |

**Figure S2.** Coefficient plots and respective contour plots showing the influence of the investigated factors on the sinapaldehyde concentration (µg/g) of oak and fir wood.

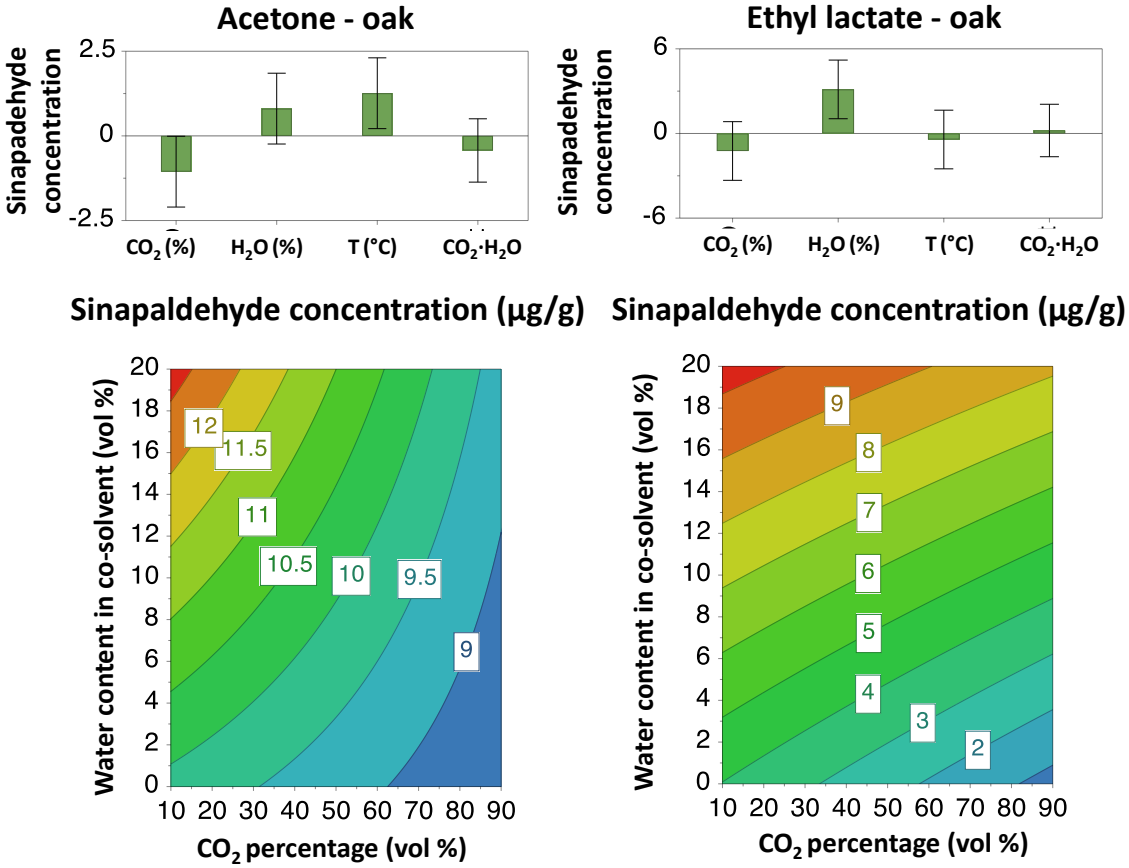


*Figure S2 - Coefficient plots from the interaction model with a full factorial design showing the influence of the investigated factors and two-factor interactions on the sinapaldehyde concentration and contour plots showing the influence of the $CO_2$ percentage and water content in the co-solvent on the sinapaldehyde concentration of oak wood using acetone and ethyl lactate as co-solvent. $CO_2$ (%): $CO_2$ percentage; $H_2O$ (%): water content in co-solvent. Temperature was 60 °C and pressure 350 bar.*

**Figure S3**. Base peak ion chromatogram of the lignin extracted from oak wood obtained with UHPSFC/ESI-HRMS.

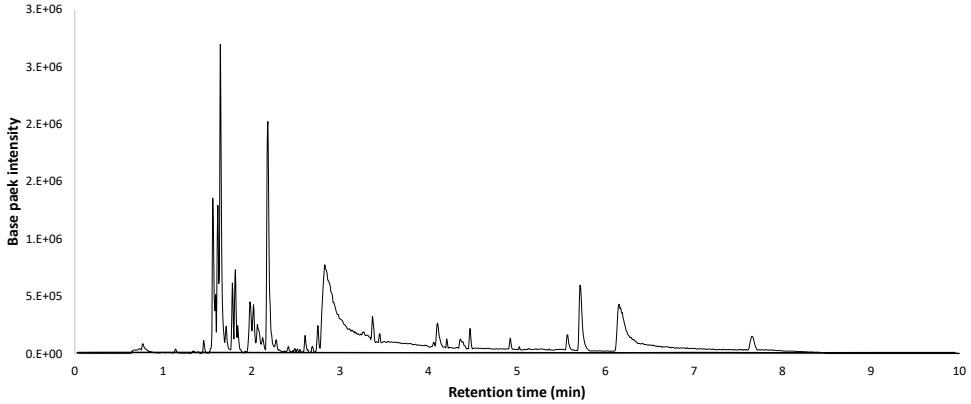

**Figure S4.** Loading plot of the KMD-PCA-QDA classification model for lignin dimers showing the latent variables (LVs) 1 and 3. #C = number of carbon atoms, #H = number of hydrogen atoms, #O = number of oxygen atoms.