Electronic Supplementary Information (ESI)

Revisiting Organosolv strategies for sustainable extraction of valuable lignin: the CoffeeCat process

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Detailed analytical methods

Chemical composition

Ashes and water contents were determined by placing 300 mg of native biomass in crucibles in an oven at respectively 575°C for 4 h and 105°C for 24 h. They were then weighed until constant weight (less than ± 0.3 mg change in the weight upon one hour of re-heating the crucible). 250 mg of biomass were mixed with 3 mL of 72% H_2SO_4 at 30°C for 1 h. Deionized water (84 mL) was subsequently added to the samples, which were then autoclaved at 121°C for 1 h. The autoclaved samples were quickly cooled and filtered. The solid residue, called acid-insoluble (Klason) lignin (AIL) was washed with deionized water until the pH of washing water was neutral, then dried in an oven at 105°C until constant weight. Dried AIL was subjected to gravimetric analysis. AIL was determined following *Equation S1*.

Equation S1:

$$\text{AIL (\% wt.)} = \frac{\text{Dried AIL}}{\text{Initial sample weight}} \text{x100}$$

The liquid fraction was separated in two parts. The first one was diluted 50 times in ultrapure water and filtered (0.22 µm Syringe PTFE filter) prior to sugar content quantification by High Performance Anion Exchange Chromatography (HPAEC-PAD) (Dionex ICS 5000+ DC, Thermo Fisher Scientific, Massachusetts, United States) using an analytical CarboPac PA-1 or PA-20 (150 mm × 3 mm) column equipped with a guard column and kept at 25°C. Elution was carried out at 0.5 $mL.min^{-1}$ with a gradient method using sodium hydroxide as eluent, as described previously[1]. Quantification was based on calibration curves using standard sugar solutions (0 to 25 $\mu g.mL^{-1}$). The retention times of glucose and xylose were 10.22 and 10.92 min, respectively. The second part was used for acid-soluble lignin (ASL) quantification by measuring the absorbance at a wavelength of 205 nm using a UV spectrophotometer (Shimadzu 1800, Japan). This sample was diluted to bring the absorbance into the range of 0.7–1.0 with 2.5% H_2SO_4 (used as blank). AIL was determined following Equation S2.

Equation S2:

$$\text{ASL (\% wt.)} = \frac{\text{Abs}_{205\ nm} \text{x D x V}}{\varepsilon \text{ x } m_{\text{biomasse ini}} \text{x1000}} \text{x100}$$

Where D is the dilution factor, V the total volume (87 mL), and ε is the absorption coefficient of acid-soluble lignin (ε = 110 $L.g^{-1}.cm^{-1}$)[2].

Total lignin is the combined contents of AIL and ASL.

Ethanol extractives were also measured by using 5 g of biomass, a Soxhlet extractor and condenser, 200 mL of ethanol for a duration of 16 h (4 siphoning cycles/h).

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FTIR analyses

Alkali lignin, raw Mxg and extracted lignin fractions were characterized by infrared spectrometry using a FTIR-8400S (Shimadzu, France) equipped with a universal ATR sampling accessory with germanium crystal. The solid samples were analyzed without further preparation. Spectra were recorded from 600 to 4000 cm^{-1}, with a resolution of 4 cm^{-1} for 64 scans. Raw data were collected, then processed by subtracting the reference spectra from air. The results presented have undergone a vector normalization and a baseline correction.

This analysis is often performed to compare the functional groups of the obtained lignins and to determine the chemical changes occurring during extraction. Bands were assigned based on previously published literature data[3–5].

NMR analyses

The extracted lignins were analysed by NMR after solubilization in dimethyl sulfoxide-D6 (10% w/v) using 1D and 2D NMR techniques. The experiments were performed on a spectrometer (Bruker Avance III 500 MHz spectrometer) equipped with a 5 mm probe operating at 125.7452 MHz (13C channel) and 500.0800 MHz (1H channel) at 298 K. The ^{1}H, ^{13}C and ^{1}H-^{13}C HSQC were performed under standard conditions (using the pulse program hsqcetgpsisp2.3). The spectral widths were 6500 and 24000 Hz for the ^{1}H and ^{13}C dimensions, respectively. The central solvent peak was used as an internal reference (δC 39.51 ppm; δH 2.50 ppm). HSQC cross-signals were assigned based on the literature[6–12]. A semiquantitative analysis of the intensities of the HSQC cross-signal intensities was performed[9]. In the side chain region (δC 50-90 ppm and δH 2.5-6.0 ppm) of HSQC spectra of lignin, the relative abundance of interunit linkages was estimated from the correlation's intensities of β-5/β-β/β-O-4 units and expressed as a percentage of interunit linkages (β-5/β-β/β-O-4 interunit linkages/100 units). In the aromatic region (δC 100-135 ppm and δH 5.0-8.5 ppm) of HSQC spectra of lignin, the $C_{2,6}$–$H_{2,6}$ correlations from the sum of S and S' units, the sum of C_2–H_2, C_5–H_5 and C_6–H_6 correlations from G units and the sum of $C_{2,6}$–$H_{2,6}$ and $C_{3,5}$–$H_{3,5}$ correlations from H units were used to estimate the S/G/H ratio[13]. NMR spectra were processed using Bruker's Topspin 4.1.1 windows processing software.

SEC-MALLS analyses

SEC experiments were performed with a HPLC pump (LC10AD, Shimadzu) coupled to an autosampler (Autosampler VE 2001, Malvern Panalytical) and a multi-detectors system recording UV, light scattering (RALS: right angle light scattering and LALS: left angle light scattering) and refractive index signals (Viscotek TDA305, Malvern Panalytical). A SEC column (Viscotek T6000M, 10 µm particle size, 8 mm ID x 300 mm, Viscotek Malvern Panalytical) was used and equipped with a post-column nylon filter (0.22 µm). The column was equilibrated with THF. The flow rate was 0.3 $mL.min^{-1}$, the temperature was 30°C and the injection volume was 10 µL. Data were processed with the Omnisec software (v5.12, Malvern Panalytical). The calibration procedure and cross validation were performed with polystyrene standards (Viscotek PolyCal standards, Malvern Panalytical). The refractometer was used as the concentration detector and the refractive index increments values (dn/dc) used to determine the molecular weight were 0.185 and 0.145 $mL.g^{-1}$ for polystyrene and lignin, respectively[14]. Lignin samples were solubilized in THF at 25 $g.L^{-1}$ and filtered through a 0.22 µm Nylon-filter just before injection.

Lignin phenol content

The phenol content of the extracted lignin samples was determined. It was measured according to the Folin-Ciocalteu's method using gallic acid as reference[15,16]. A control stock solution of gallic acid was prepared (1 $g.L^{-1}$ in DMSO) to obtain a calibration curve from 0.2 $g.L^{-1}$ to 1 $g.L^{-1}$ from absorbance measurements at 760 nm using a UV spectrophotometer (Shimadzu 1800, Japan). For the analysis, 0.5 mL of lignin solution (2 $g.L^{-1}$), 2.5 mL of Folin-Ciocalteu's reagent and 5 mL of Na_2CO_3 (200 $g.L^{-1}$) were added to 50 mL flask and covered with distilled water. The mixtures were stirred for 30 min in the dark at room temperature. The absorbance was then measured at 750 nm. The recorded values for lignins were compared to the gallic acid standard curve (Abs 760 nm = 0.0011 [Gallic acid] $g.L^{-1}$, $r^2 = 0.998$) to determine the phenolic content expressed in mg GAE (gallic acid equivalent).g $lignin^{-1}$. All analyses were performed in duplicate.

Physico-chemical properties of the solvents and the acids

Table S1. Comparison of the physico-chemical properties of the solvents.

Product	2-Methyltetrahydrofuran[17,18]	2-Methyltetrahydrofuran-3-one[17]
Molecular formula	$C_5H_{10}O$	$C_5H_8O_2$
Molar mass ($g.mol^{-1}$)	86.134	100.117
Density ($g.mL^{-1}$)	0.854	1.034
Boiling point (°C)	80.2	138
Melting point (°C)	-136	N.D.
Solvent/water mixture	Biphasic system	Monophasic system
logP (o/w)	0.85	-0.65

Table S2. Comparison of the physico-chemical properties of the acids.

Product	Oxalic acid[19,20]	L-Aspartic acid[21,22]	L-Glutamic acid[22,23]
Molecular formula	$C_2H_2O_4$	$C_4H_7NO_4$	$C_5H_9NO_4$
Molar mass ($g.mol^{-1}$)	90.03	133.1	147.13
Density ($g.mL^{-1}$)	1.9	1.7	1.5
Boiling point (°C)	365.1	324	267.2
Melting point (°C)	189.5	270	205
Thermal decomposition (°C)	127-157	185-280	185-280
Solubility in water 25°C ($g.L^{-1}$)	220	4.5	8.6
Acidity (pKa)	$pKa_1 = 1.23$ $pKa_2 = 4.30$	$pKa_1 = 1.92$ $pKa_2 = 3.87$ $pKa_3 = 9.87$	$pKa_1 = 2.19$ $pKa_2 = 4.25$ $pKa_3 = 9.67$
logP (o/w)	-0.81	-3.89	-3.69

Preliminary experiments

Preliminary experiments were performed to rationally select biomass loading and acids concentration before scaling up. Freeze-dried *Mxg* (1, 5 or 15% w/v) and 0.1 M or 0.2 M acid (oxalic (ox), aspartic (asp) or glutamic (glut) acid) were added to a 12 mL MeTHF-3-one/water mixture (volume ratio of 3:1), then incubated at 140°C, 350 rpm in 20 mL glass tubes with a Carousel 12 Plus Reaction Station™ (Radleys, United Kingdom) coupled to a Haake K35 thermoregulated bath (Thermo Fisher Scientific, Heysham) at 10°C to avoid evaporation. The process was stopped after 6 h and finally cooled in an ice bath for 20 min. The liquid and solid phases were separated by vacuum filtration. The solid phase was discarded. The solvent was removed from the liquid fraction by evaporation with a SpeedVac Concentrator (SPD121P, Thermo Savant, Waltham, MA, USA). The obtained residue was washed with water and then centrifuged. This step was repeated at least 3 times until the water was colorless. Finally, the resulting solid was freeze-dried and weighted. The enriched-lignin fraction yields (%) were calculated based on the weight on the latter fraction, using Equation S3.
Equation S3:

$$\text{Enriched-lignin fraction yield } (\%) = \frac{\text{Lignin extracted in pretreatment solvent (g)}}{\text{initial lignin in raw } \textit{Mxg} \text{ (g)}}$$

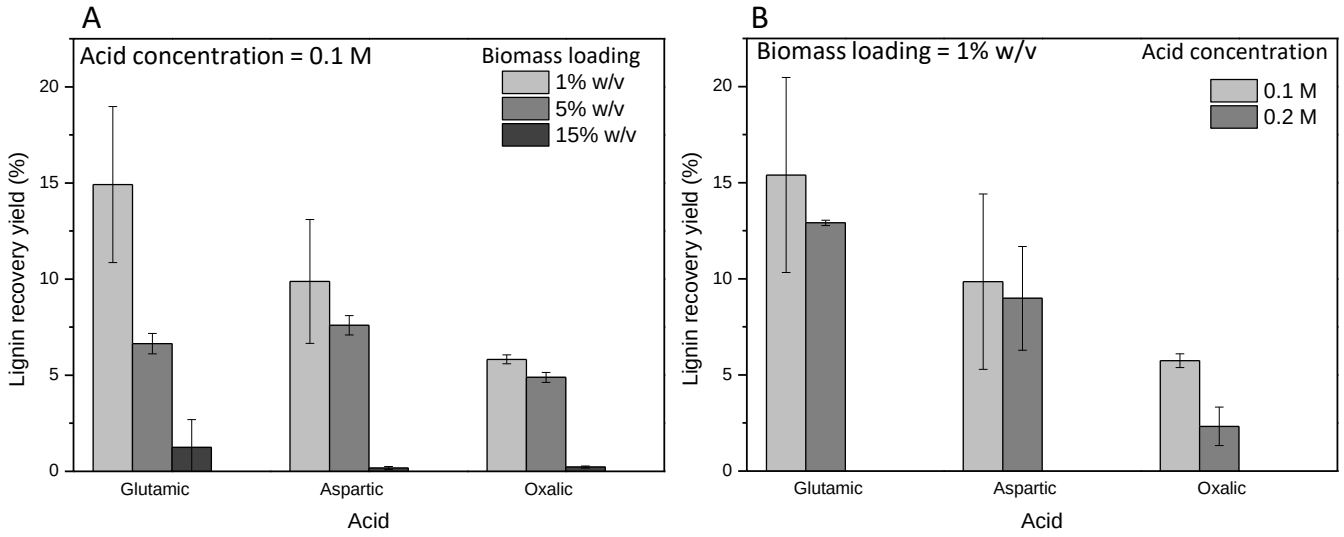

Figure S1. Preliminary experiments to confirm biomass loading and acid concentration for the pretreatment: (A) Enriched-lignin fraction yields obtained at 140°C for 6h at different biomass loading and (B) Enriched-lignin fraction yields obtained at 140°C for 6h at different acid concentration.

Lignin solubility

Determination of lignin solubility in MeTHF-3-one:water mixtures was performed by gravimetric analyses. 10 to 150 mg of Alkali lignin were introduced into 1 mL of the solvents' mixture to reach 1 to 15% w/v loading. The dispersions were stirred for 24 h at 50 °C. After centrifugation (13,000 g, 15 min, MiniSpin®, Eppendorf Austria GmbH), the supernatants were removed. The resulting amounts of lignin were weighed (using a precision analytical balance, Sartorius Practum 224–1S, Germany) and used for non-solubilized lignin percentage determination. All measurements were performed in triplicate and the solubility values were expressed as mean values with a standard deviation (±) in %.

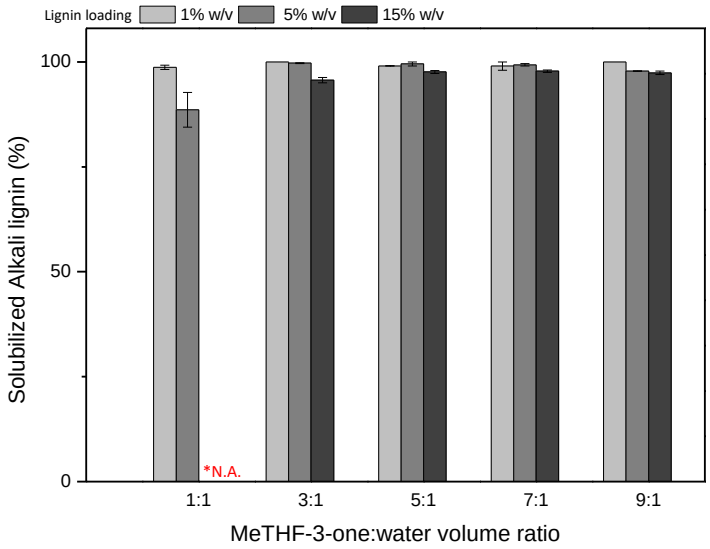

Figure S2. Alkali lignin solubility in MeTHF-3-one:water mixtures after 24h at 50°C. *N.A. means not analysed due to too high viscosity.

FTIR spectra of the Enriched-lignin fractions and raw *Mxg*

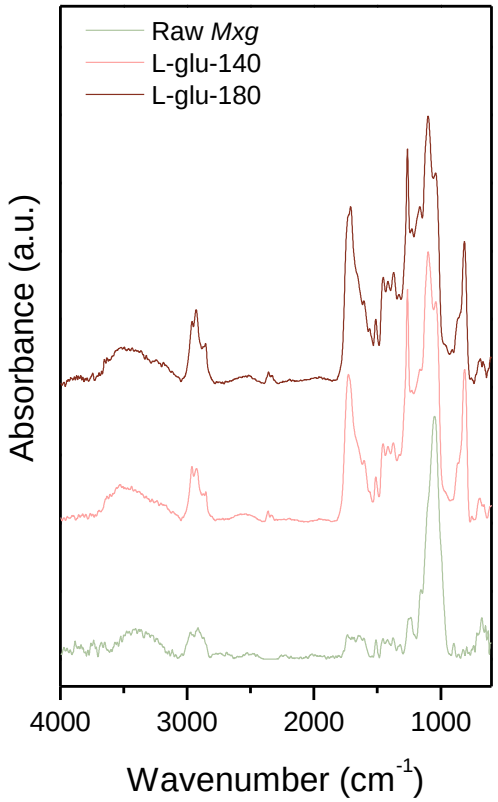

Figure S3. FTIR spectra of raw *Mxg*, **L-glu-140** and **L-glu-180**, in the range 600 to 4000 cm^{-1}.

NMR characterization

The extracted lignins were analysed by NMR after solubilization in dimethyl sulfoxide-D6 (10% w/v) using 1D and 2D NMR techniques. The experiments were performed on a spectrometer (Bruker Avance III 500 MHz spectrometer) equipped with a 5 mm probe operating at 125.7452 MHz (13C channel) and 500.0800 MHz (1H channel) at 298 K. The ^{1}H, ^{13}C and ^{1}H-^{13}C HSQC were performed under standard conditions (using the pulse program hsqcetgpsisp2.3). The spectral widths were 6500 and 24000 Hz for the ^{1}H and ^{13}C dimensions, respectively. The central solvent peak was used as an internal reference (δC 39.51 ppm; δH 2.50 ppm). NMR spectra were processed using Bruker's Topspin 4.1.1 windows processing software. HSQC cross-signals were assigned based on the literature[6–9,13]. A semiquantitative analysis of the intensities of the HSQC cross-signal intensities was performed as previously done in literature standards[9].

In the aromatic region (δC 100-150 ppm and δH 6.2-7.8 ppm) of HSQC spectra of lignin, the $C_{2,6}$–$H_{2,6}$ correlations from the S and S' units, the C_2–H_2, C_5–H_5 and C_6–H_6 correlations from G units and the $C_{2,6}$–$H_{2,6}$ correlations from H units were used to estimate the S/G/H ratio[9]. The following regions were used for the determination of the aromatic ratios:
$S_{2/6}$: [(6.60-7.02)(102.0-105.5)]
$S'_{2/6}$: [(7.31-7.38)(103.6-105.3)]
G_2: [(6.89-7.19)(109.2-112.3)]
G_5: [(6.66-7.24)(112.8-117.0)]
G_6: [(6.71-7.00)(117.6-120.3)]
$H_{2/6}$: not detected

The H/G/S ratios were calculated, as previously done in literature standards[9], with the following equations:
Equation S4: Total aromatic =(((S2/6 + S'2/6)/ 2)) + ((G2 + G5 + G6 – H2/6) / 3) + (H2/6 / 2)
Equation S5: Ratio S = (((S2/6 + S'2/6) / 2)) : total aromatic x 100%
Equation S6: Ratio G = ((G2 + G5 + G6 – H2/6) / 3) : total aromatic x 100%
Equation S7: Ratio H = (H2/6 / 2) : total aromatic x 100%

p$CA_{2/6}$: [(7.41-7.72)(128.6-131.5)]
pCA_8: [(6.24-6.36)(112.7-114.5)]
pCA_7: [(7.45-7.65)(143.8-145.9)]

In the side chain region (δC 50-90 ppm and δH 2.8-5.6 ppm) of HSQC spectra of lignin, the relative abundance of interunit linkages was estimated from the correlation's intensities of β-5/β-β/β-O-4 units and expressed as a percentage of interunit linkages (β-5/β-β/β-O-4 interunit linkages/100 aromatic units). The obtained values for the linking motifs are divided by a factor 1.3 as the HSQC measurements overestimate these values[9]. The following regions were used for the determination of the linking motifs:
β-O-4_α [(4.70-5.08)(70.9-73.1)]
β-O-4_β [(4.24-4.50)(83.3-85.1)]
β'-O-4_β [(3.90-4.25)(85.5-87.5)]
β-O-4_γ / β'-O-4_γ [(3.20-4.30)(58.8-61.4)]
β-5_α [(5.40-5.65)(86.6-88.0)]
β-5_β [(3.42-3.52)(53.0-54.1)]
β-β_α [(4.65-4.72)(84.5-86.0)]
β-β_β [(3.05-3.10)(53.2-54.4)]

The number of linking motifs were calculated with the following equations[9]:
Equation S8: %β-O-4 linkages = ((β-O-4_α + β'-O-4_α) / total aromatic x 100)/1.3
Equation S9: %β-5 linkages = (β-5_α / total aromatic x 100)/1.3
Equation S10: %β-β linkages = (β-β_α / total aromatic x 100)/1.3

SEC-MALLS characterization

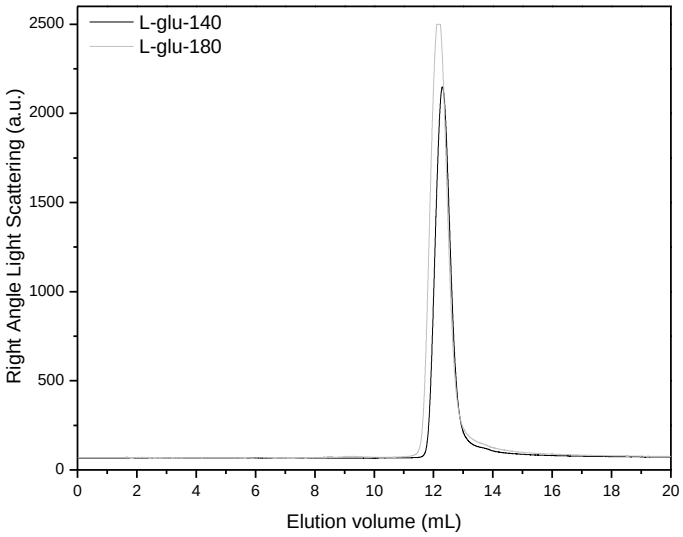

Figure S4. Size exclusion chromatogram with Right Angle Light Scattering detection of **L-glu-140** (black line) and **L-glu-180** (grey line).

FTIR spectra of the delignified fractions before and after [Emim][OAc] pretreatment and enzymatic hydrolyses

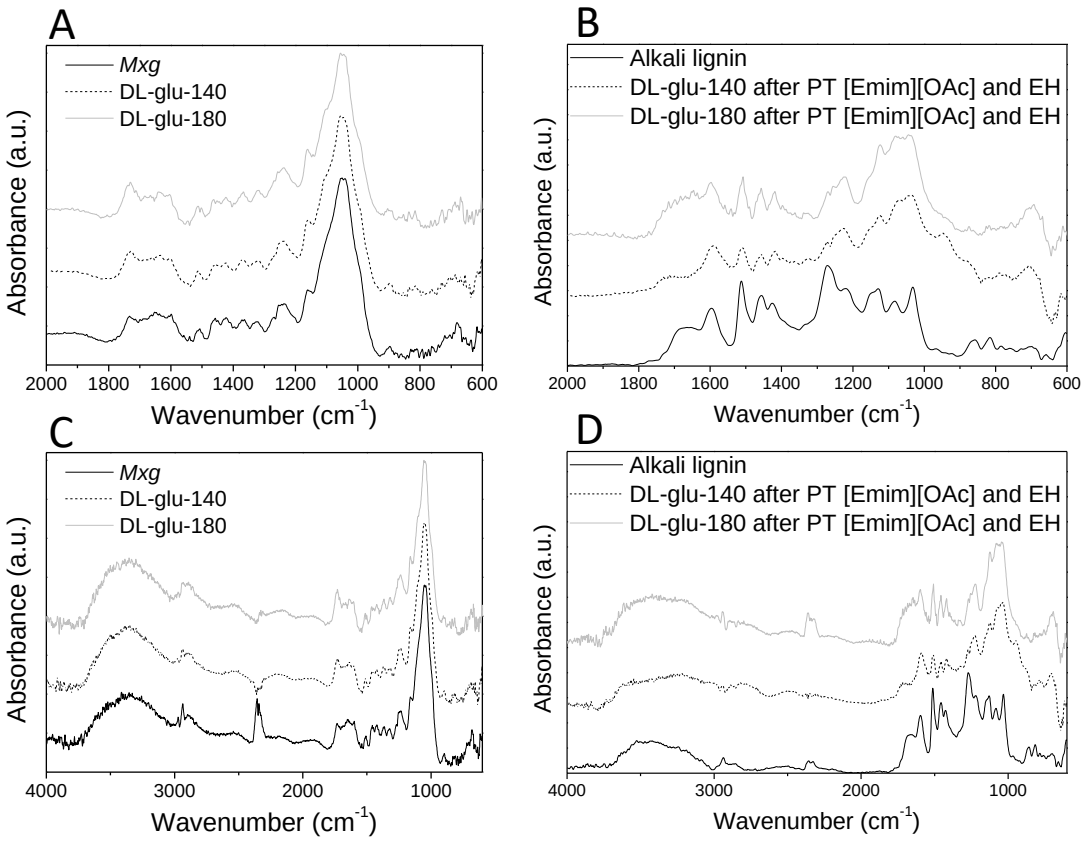

Figure S5. FTIR spectra (in the range 600 to 2000 cm^{-1}) of (A) raw *Mxg* and delignified biomass pulp after the CoffeeCat process at 140°C (**DL-glu-140**) and 180°C (**DL-glu-180**), (B) Alkali lignin, Lignin recovered from the solid fraction after [Emim][OAc] pretreatment (PT) and enzymatic hydrolyses (EH) of the delignified biomass pulp from the process at 140°C (**DL-glu-140 after [Emim][OAc]**) and at 180°C (**DL-glu-180 after [Emim][OAc]**). The complete spectra (600 to 4000 cm^{-1}) of (A) and (B) are presented in (C) and (D), respectively.

FTIR spectra of PLA, enriched-lignin fraction (L-glut-140) and PLA-lignin composite powder

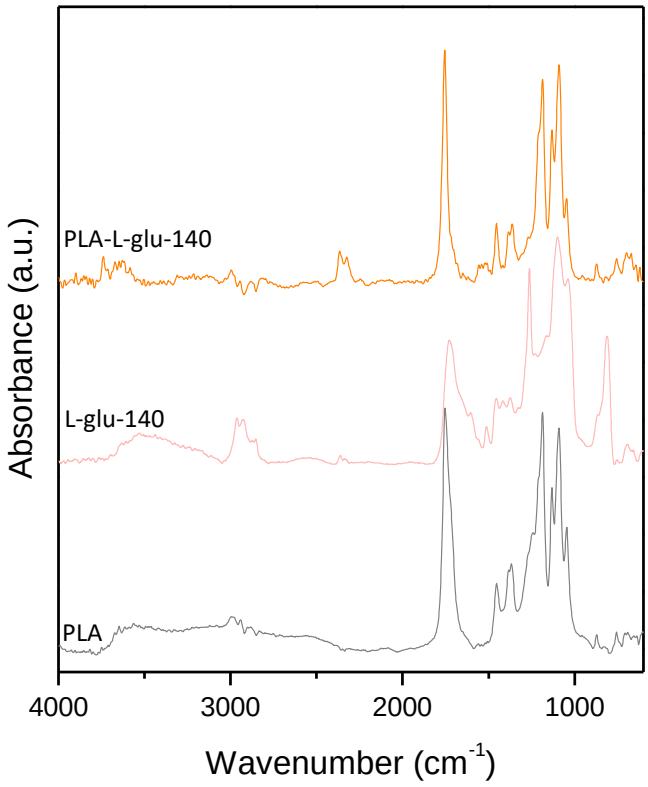

Figure S6. FTIR spectra of PLA, **L-glu-140** and **PLA-L-glu-140 powder**, in the range 600 to 4000 cm^{-1}.

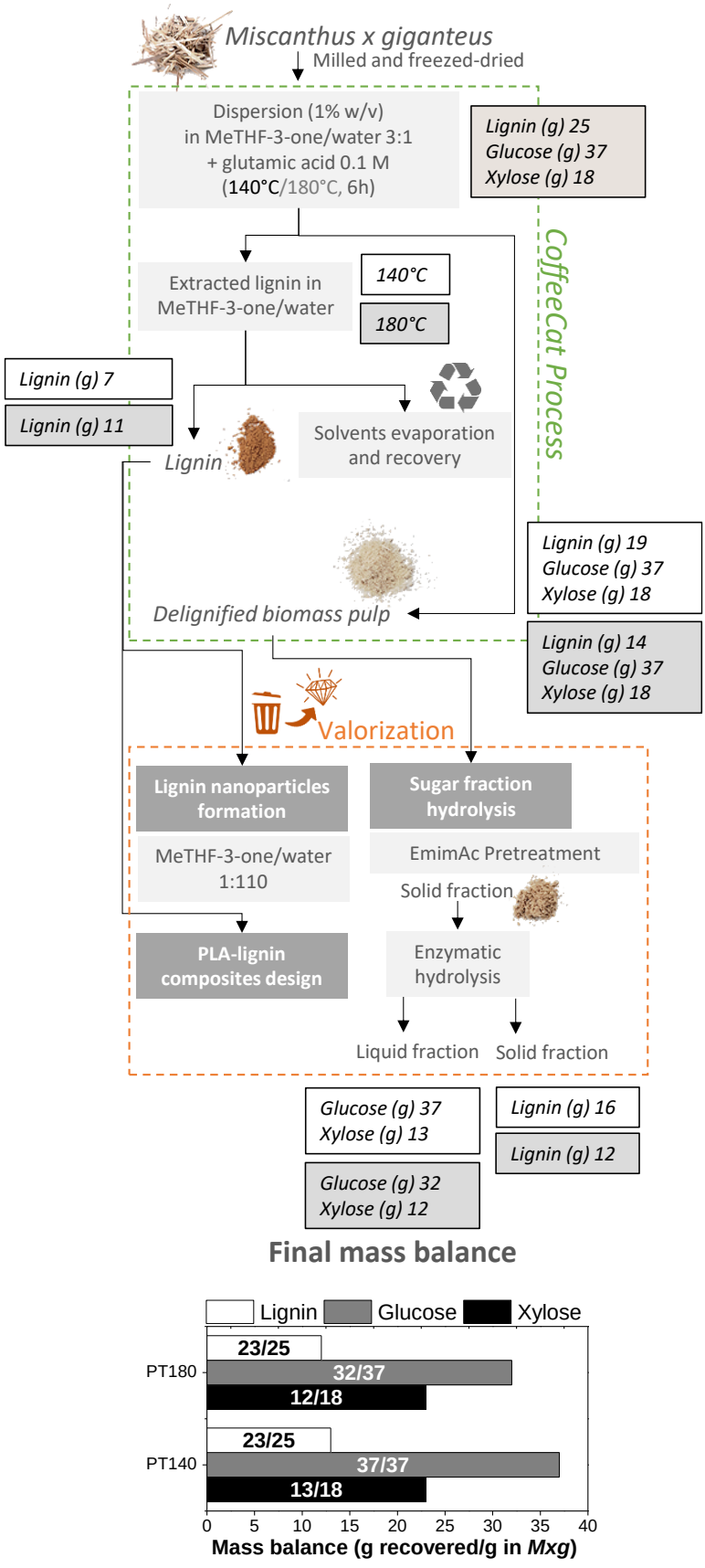

Figure S7. Overview and mass balance of the process.

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