Supporting information for "Enhancement of ionic liquid-aided fractionation of birchwood. Part 1: Autohydrolysis pretreatment"

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1. Materials

1-Ethyl-3-methylimidazolium acetate ([emim]OAc, 98% pure) was purchased from Iolitec GmbH, Germany. Birch sawdust (*Betula pendula*) was acquired from Metsän-tutkimuslaitos, Finland, and dried at 105 °C overnight, wherein a constant weight was achieved at 6 h. Loss on drying was 44.6±0.4% (in triplicate). The oven-dry sawdust was ground in a Wiley mill with a 0.6 mm (30 mesh) sieve, combined with mill holdup, and then sieved with a 125 µm sieve in a Retsch AS300 vibratory sieve shaker until the change of mass was <1% over 10 min. This procedure, while yielding only half the mass, reduces the recirculation of the wood meal in the Wiley mill. Extractives were removed with acetone in a Soxhlet extractor over three days, with the addition of 10% water on the last day.

Particle size distribution of the commercial sawdust was determined by sieving (Table S4). Compositions for severities P500 and P1500 with commercial sawdust are shown in Tables S5–6.

The prehydrolysis-kraft pulp used for comparison was dried to constant weight at 105 ºC before analysis.

2. Vertical kneader

The vertical kneader had a bucket-type reactor (3 l) with a heavy anchor blade reaching the full width and height of the reactor (diameter 12 cm). The separation of the blade and the reactor wall was 0.5–1 mm, providing high shear; the torque exerted was 100–160 mN/m. The vacuum was maintained at 10 mbar. For heating, the reactor was equipped with a single external jacket, where silicone oil, heated in a thermostat, was circulated. The temperature in the thermostat was adjusted such that the temperature in the reactor, measured by a separate internal thermometer, was maintained at 100 °C. Kneading was monitored with the shaft torque, a rough measure of viscosity.

3. Filtration unit

The press filter was designed by Seppo Jääskeläinen and constructed by the workshop of Aalto University. A 10-ton hydraulic press presses a piston into a cylinder equipped with a thermostat. The mesh stack has a diameter of 115 mm and consists of a twilled Dutch weave mesh (GKD SolidWEAVE #14377000, nominal 1 µm, 5-6 µm absolute (98%) hole size, Gebr. Kufferath AG, Germany), supported by a GKD Ymax2 mesh. The mesh stack is clamped between the cylinder and the backplate, which has a holed disk (2 mm holes in a hexagonal lattice) and a machined funnel surface with supports

for the disk for filtrate collection. The collecting flask is topped with a rubber cone, which provides a simple moisture barrier.

4. Washing and analysis of precipitate 1

The washing of precipitate 1 was monitored for the conductivity of the wash water (Figure S1), which exactly followed an exponential decrease that finally reached a value of 13.7 µS/cm (corresponds to 21.6 mg/l [emim]OAc, vs. a known concentration of [emim]OAc).

As the product (precipitate 1) contained 96% water, it collapsed into a black, insoluble and very tough mass if oven-dried, which causes overestimation of Klason lignin content. Direct addition of sulfuric acid overdiluted it, resulting in incomplete hydrolysis. Freeze drying overnight was thus necessary; it gave a fluffy aerogel with the same dry matter content as oven drying.

5. Metathesis

Metathesis was done by addition of 50% $HNTf_2$ in 1 ml steps, changing to 0.1 ml near the endpoint. Conductivity has an excellent fit to a 2nd degree curve until the endpoint, where an immediate increase is observed and the pH is a good fit to the Henderson-Hasselbalch equation.

6. Analysis of metathesis liquids

To analyze the metathesis liquid phases, the first step was omitted: concentrated sulfuric acid was added to bring the concentration to 4% (0.20 g 95-97% H_2SO_4 to 4.60 g liquid sample), and dilute acid hydrolysis done as in the solid samples. A blank sample was made from the metathesis reaction and subjected to the same hydrolysis; its absorbance

was subtracted from ASL spectra. ASL content was quantified with ε(280 nm) = 20.6 l/g-cm; wavelengths 205 and 240 nm were not suitable due to interference from the IL.

7. Sugar analysis

Sugar analysis of the Klason hydrolysis liquors was accomplished by HPAEC-PAD. For detection of ASL and carbohydrates, the sample was diluted 1:50. ASL was detected with a Shimadzu UV-2550 spectrophotometer using ASL specific absorbance of ε(205 nm) = 110 l/g-cm, using a path length of 1 cm. Acetyl content was determined by quantification of acetic acid with HPLC from the Klason hydrolysis liquors, using a Dionex UltiMate 3000 HPLC system with a Acclaim Organic Acid 5 µm/120 Å column. Elution was with acetonitrile-water 30/70 then 70/30 and detection with UV at 280 nm.

8. Analysis of furans

Furans were also determined by HPLC (elution with 2.5 mM methanesulfonic acid in water, with a ramp to 45% acetonitrile, detection by UV at 210 nm), and converted to anhydrosugar equivalent by the factor 0.778 for hydroxymethylfurfural and 0.727 for furfural (molecular weight ratios of the C_5 or C_6 furan and the corresponding C_5 or C_6 anhydrosugar).

Furans are formed in the Klason hydrolysis. With unprocessed wood, furan yield was 0.10% in residual solution, but was essentially absent in wood insolubles (0.0005%) and washwaters (0.0004%). A small yield (1.37%) was liberated from autohydrolyzed wood on Klason hydrolysis, and furans were also found (0.70%) in the Klason hydrolysate of precipitate 1. Thus, of the 3.53% carbohydrate unaccounted for, 2.28% is explained by furans. Furans were included in the xylan content. Accordingly, in order to normalize to

100%, the polysaccharide (cellulose, xylan, etc.) contents were divided by a factor of (100% – F/P), where F is the furan (anhydrosugar equivalent) content and P is noncarbohydrate content (P = 100% – lignin – acetyl).

9. CP/MAS ^{13}C NMR

CP/MAS ^{13}C NMR analysis was with a Bruker Avance DPX300 spectrometer operating at a resonance frequency of 75.46 MHz for ^{13}C. The samples, wetted with water (1:1), were packed into a 7 mm zirconium oxide rotor. Experiments were carried out with a Bruker 7 mm MAS probe at ambient temperature (26 °C). The MAS spinning rate was 4 kHz, the ^{1}H decoupling field was 50 kHz, the contact time was 1 ms and the delay between repetitions was 3 s; 32768 transients were accumulated for each spectrum. The FIDs were multiplied with an exponential window function (LB=40 Hz) prior to Fourier transformation. Chemical shifts were referenced to the methylene signal in external adamantane (δ=29.5 ppm). Cellulose II was identified with the peaks of 88 and 89 ppm. Lignin peaks identified were 56 (methoxyl), 63, 75, and 84 (γ-, α-, and β-C, respectively in β-O-4), 102 (syringyl aromatic CH), 136 (aromatic C–C) and 153 ppm (aromatic C–O).

10. Karl Fischer titration

Karl Fischer titration was used for water content determination. For gelatinous material, if directly injected, the titration solution reacts only with the surface of an injected drop. Thus, the sample was pressed between two glass slides, which were separated and quickly dropped whole into the titration cell. This forms a thin film that releases moisture quantitatively. The method is not applicable to nonhomogeneous samples. When kneading and filtering unprocessed birch in [emim]OAc, the system was not fully

sealed, and thus water was absorbed from the atmosphere into the wood solution. The water content was 7.92% in the filtrate, and filtrate wood content was corrected accordingly in the balances.

11. Solid content

Solid content was determined by residual mass on freeze drying or oven drying (105 °C) overnight. Dry matter weighing error is 0.0001 g or for precipitates 1 and 2, 0.07–0.15% as relative error, which is negligible in this context.

12. Error estimates

For the gravimetric determination yields, precision of weighing is estimated as ±0.02 g, but given that most fractions are weighed wet, weighing error is very small based on wet fraction weighing and dry matter content. The total sum of weighing errors amounts to 0.14% / ow and 0.07% / ow for unprocessed and autohydrolyzed birch experiments, respectively, which is also negligible. For component analysis, difference between duplicates in precipitate 1 was 0.5% for Klason lignin and 0.2% for ASL. The mild hydrolysis of metathesis liquids produces an error of 0.02–0.05 AU for ASL, which corresponds to a 2–4% lignin content difference. In HPAEC, mutual agreement of carbohydrate integrals was high with duplicate injections (0.1 area-% with glucose and 0.02 area-% with other sugars), and cellulose content variation between duplicate hydrolysis runs was also small, 0.1–0.3% although 1.3% with washwater solute.

13. Molar mass distribution of holocelluloses

Acid chlorite delignification conditions are as follows: in 40 ml water, a sample of 1 g was delignified with 0.8 g $NaClO_2$ and 0.16 g AcOH at 75 °C for 2 h; the chemicals

were added in two portions. The suspension was cooled in an ice bath and centrifuged to separate the holocellulose, which was extensively washed (10 x water and 2 x acetone). The conditions for the birch meal were more severe: 1.5 g $NaClO_2$ and 0.3 ml AcOH were added in three equal portions over 3 h.

EDA pretreatment of birch holocellulose was done with the following method: a sample of 0.5 g was incubated in 5 ml EDA for 1 day at room temperature and then washed with 5 ml EDA and 10 ml DMAc. EDA-soluble material was precipitated with 1-propanol. The combined holocellulose was solvent-exchanged in 5 ml DMAc for 1 day at room temperature.

Molar mass distributions were measured with a Dionex Ultimate 3000 gel permeation liquid chromatography system with four PLgel MIXED-A columns and a refractive index detector, with LiCl/DMAc as the eluent. Calibration was achieved by comparison with pullulan standards between 343–1,600,000 Da, with retention times corrected with the cellulose/pullulan apparent molar mass ratio derived from Berggren *et al*. (R. Berggren, F. Berthold, E. Sjöholm and M. Lindström, *J. Appl. Polym. Sci.*, 2003, **88**, 1170-1179). Deconvolution of the peaks was done in OriginPro 8.5.0 SR1 by fitting three Gaussians to the logarithmic chromatogram. The moments of the distributions (M_n and M_w from ΣN_i, $\Sigma N_i M_i$ and $\Sigma N_i M_i^2$) were then calculated from the fitted curves.

For GPC, deconvolution was done using OriginPro 8.5.0 SR1. The first peak was considered hemicellulose and the other peaks cellulose. For convenience, an extra peak at log M_w 4.7 was fitted to data from autohydrolyzed wood and precipitate 1, in order to account for the irregular shape of the main peak. This was considered as one peak in analysis, since we suspect it to be a peak fronting artifact.

The commercial prehydrolysis-kraft pulp used for comparison was analyzed by Michael Hummel and Heini Lehtonen. The properties are M_w = 291 kg/mol, M_n = 62 kg/mol and PDI = 3.5, 18% alkali resistance (R18) 98.0% and kappa number 0.69 ml/g. The glucan, mannan and xylan contents (94.9%, 0.0% and 2.5%, respectively) were determined according to NREL/TP-510-42618. The kappa number (0.69 ml/g) was determined according to SCAN-C 1:00 (Kappa Number, SCAN-C 1:00, Scandinavian Pulp, Paper and Board Testing Committee, 2000). 1 kappa number corresponds to 0.15% lignin, thus the residual lignin content is ca. 0.1%.

14. Yield and composition data from the fractionations

See Tables S2–3.

15. Dissolved wood lignin

Dissolved wood lignin was prepared according to Fasching *et al.* (M. Fasching, P. Schroeder, R. P. Wollboldt, H. K. Weber and H. Sixta, Holzforschung, 2008, 62, 15). Ball-milled birch was dissolved in DMSO/NMI (dimethyl sulfoxide/*N*-methylimidazole), carbohydrates removed by precipitation with 9:1 dioxane-water, the supernatant evaporated, the solid redissolved in 75% acetic acid and precipitated in water. The total lignin content was 91.79% of which 85.66% Klason lignin and 6.13% ASL.

16. GPC of lignins

Although Ac-DWL dissolved in THF, P2 was not fully soluble in THF when acetylated with acetic anhydride/pyridine for NMR analysis. Hence, P2 was further acetylated after pre-dissolution into LiCl/DMAc. P2-Ac (21 mg) was added to DMAc (500 μl),

followed by LiCl (30 mg). The sample was heated at 80 °C for 2 h. Pyridine (200 μl) was added. The homogenized mixture was allowed to cool to room temperature. Acetyl chloride (150 μl) was then added, and the homogenized mixture was allowed to stir overnight. The reaction was quenched with 10 ml water for 10 min. The lignin was removed by centrifugation, washed with 10 ml water, and then dried in a vacuum oven to a brown solid (21 mg), which was fully soluble in THF.

For GPC of lignin, samples were dissolved in the eluent in a concentration of 1 mg/ml, the solution filtered with a syringe filter (PTFE, 0.45 μm pore size) and injected into the GPC. The GPC system (Agilent, Santa Clara, CA, USA) used includes a degasser, pump, auto-sampler, column oven (Agilent 1100 series), diode array UV detector (Agilent 1050 series), and refractive index (RI) detector (Agilent 1200 series). The mobile phase was THF (HPLC grade, without stabilizer) with a flow rate of 0.5 ml/min. The columns used were 300 mm × 7.8 mm i.d., Styragel HR-5E and Styragel HR-1, connected in series with a 30 mm × 4.6 mm i.d. guard column of the same material (Waters, Milford, MA, USA). The system was calibrated with polystyrene standards (500, 890, 1000, 4000, 9000, 42 300, 177 000, 434 000, 1 270 000 Da) using UV detection at 280 nm. The molar masses of the samples were calculated using the UV detection wavelength at 280 nm and RI detection. Agilent Chemstation (rev. A. 10.02) with Agilent GPC add-on (rev. A 02.02.) was used to calculate the molar mass distributions.

Table S1. Compositions of the acid chlorite-delignified holocelluloses with PHK pulp for comparison

	Birch	Autohydr.	Precip. 1	PHK
Klason lignin	5.18	0.00	0.00	–
ASL	6.67	3.42	1.07	–
Lignin	11.85	3.42	1.07	–
Cellulose	53.53	83.42	93.24	94.9
Glucomannan	3.22	1.78	1.30	0
Xylan	29.96	11.37	4.38	2.5
Anhydrorhamnose	0.51	0.00	0.00	–
Anhydroarabinose	0.34	0.02	0.00	–
Anhydrogalactose	0.59	0.00	0.00	–
Total hemicellulose	34.61	13.17	5.69	2.5
Total carbohydrate	88.15	96.57	98.93	97.4

Table S2. Composition of each fraction in the unprocessed birch experiment. Solid and liquid refer to solid and water-soluble fractions from the metathesis reaction.

	Birch meal	Precipitate 1	Precipitate 2	Insolubles (solid)	Insoluble (liquid)	Residual (solid)	Residual (liquid)	Washwater (solid)	Washwater (liquid)
Dry yield (% wood)	100.00	73.08	2.28	13.45	1.86	4.88	3.39	5.66	0.14
Klason lignin	18.24	16.60	60.08	11.62	0.00	31.52	0.00	0.00	0.00
ASL	3.09	4.40	7.86	6.31	61.08	9.19	82.02	4.61	46.71
Lignin	21.33	20.99	67.94	17.93	61.08	40.71	82.02	4.61	46.71
Acetyl	4.31	0.45	0.50						
Furans					0.03		3.52		0.30
Cellulose	47.78	55.89	17.55	53.94	9.34	11.16	3.41	5.38	23.84
Glucomannan	2.46	2.16	0.85	3.41	0.00	1.57	0.00	0.00	0.00
Xylan	23.04	19.86	12.16	24.12	17.59	38.10	9.76	90.02	24.02
Anhydrorhamnose	0.28	0.00	0.03	0.00	2.57	0.86	1.08	0.00	1.13
Anhydroarabinose	0.30	0.28	0.45	0.00	0.00	2.96	0.00	0.00	0.00
Anhydrogalactose	0.49	0.37	0.54	0.60	9.42	4.63	3.73	0.00	4.29
Total hemicellulose	26.58	22.67	14.01	28.13	29.59	48.13	14.57	90.02	29.44
Total carbohydrate	74.35	78.56	31.56	82.07	38.92	59.29	17.98	95.39	53.29

Table S3. Composition of each fraction in the autohydrolyzed birch experiment.

	Birch meal	AH-birch	Precipitate 1	Precipitate 2	Insolubles (solid)	Insoluble (liquid)	Residual (solid)	Residual (liquid)	Washwater (solid)	Washwater (liquid)
Dry yield (% wood)	100.00	75.02	52.78	3.87	1.71	0.04	6.36	3.71	1.63	0.34
Klason lignin	18.24	24.40	12.06	85.49	18.56	0.00	84.22	0.00	22.16	0.00
ASL	3.09	2.36	1.59	3.29	5.56	51.25	7.93	65.67	5.04	51.78
Lignin	21.33	26.76	13.65	88.79	24.12	49.48	92.15	63.04	27.20	49.54
Acetyl	4.31	0.69	0.49	0.47						
Furans		1.82	1.33	0.19	0.80	3.45	0.23	4.01	0.64	4.32
Cellulose	47.78	56.93	77.57	6.65	67.86	3.59	5.29	1.76	43.49	2.05
Glucomannan	2.46	1.52	1.07	0.23	0.92	3.21	0.00	2.63	0.00	6.01
Xylan	23.04	13.84	7.22	3.87	7.10	43.72	2.53	31.92	29.31	42.05
Anhydrorhamnose	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Anhydroarabinose	0.30	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Anhydrogalactose	0.49	0.19	0.00	0.00	0.00	0.00	0.03	0.64	0.00	0.35
Total hemicellulose	26.58	15.62	8.29	4.09	8.02	46.93	2.56	35.20	29.31	48.41
Total carbohydrate	74.35	72.29	85.87	10.75	75.88	50.52	7.82	36.32	72.80	50.11

Table S4. Particle size distribution of the commercial sawdust obtained by analytical sieving.

Sieve (mm)	Fraction (%)
<0.05	0.02
0.05–0.1	1.3
0.1–0.2	1.7
0.2–0.5	9.3
0.5–1.0	26.2
1.0–2.0	52.0
2.0–4.0	6.4
4.0–8.0	2.2
>8.0	1.1

Table S5. Compositions of fractions (w-%) for P500 and average particle size 1.63 mm

	Birch	AH	Insoluble	P1	P2
Klason lignin	19.7	23.6	23.4	12.5	61.8
ASL	4.4	2.3	7.0	2.5	8.3
Lignin	24.0	25.9	30.3	14.9	70.1
Cellulose	45.0	60.4	51.4	73.6	23.9
Glucomannan	2.3	1.5	0.0	1.2	0.0
Xylan	26.3	12.2	18.3	10.3	6.0
Rhamnose	0.5	0.0	0.0	0.0	0.0
Arabinose	1.1	0.0	0.0	0.0	0.0
Galactose	0.8	0.0	0.0	0.0	0.0
Hemicellulose	31.0	13.7	18.3	11.5	6.0
Carbohydrate	76.0	74.1	69.7	85.1	29.9

Table S6. Composition of fractions (w-%) for P1500 and average particle size 1.63 mm

	Birch	AH	Insoluble	P1	P2
Klason lignin	19.9	29.1	41.1	13.1	84.6
ASL	4.3	2.1	5.9	2.8	7.3
Lignin	24.1	31.2	47.0	15.9	91.9
Cellulose	45.9	61.4	41.1	78.0	3.8
Glucomannan	2.1	0.4	0.0	0.0	0.0
Xylan	26.4	6.9	11.9	6.1	4.3
Rhamnose	0.4	0.0	0.0	0.0	0.0
Arabinose	0.3	0.0	0.0	0.0	0.0
Galactose	0.8	0.0	0.0	0.0	0.0
Hemicellulose	30.0	7.4	11.9	6.1	4.3
Carbohydrate	75.9	68.8	53.0	84.1	8.1

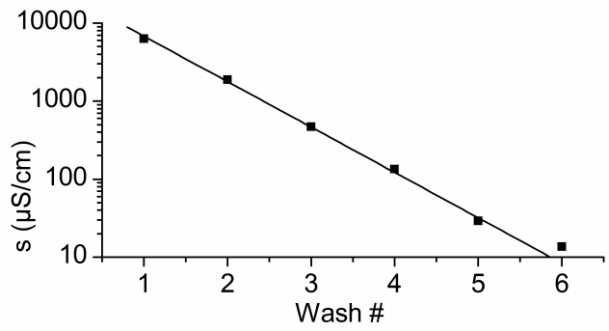


Figure S1. Washing of precipitate 1 in the unprocessed birch experiment, monitored by conductivity.

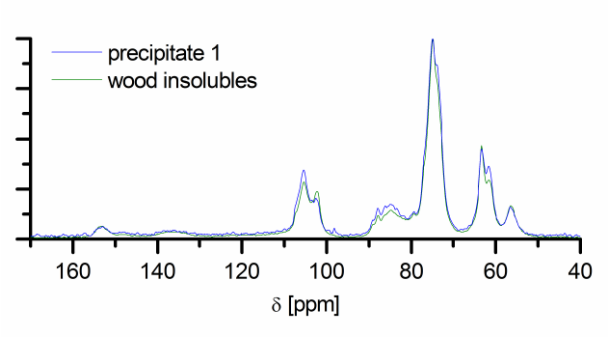


Figure S2. CP/MAS ^{13}C NMR spectra for precipitate 1 and wood insolubles.

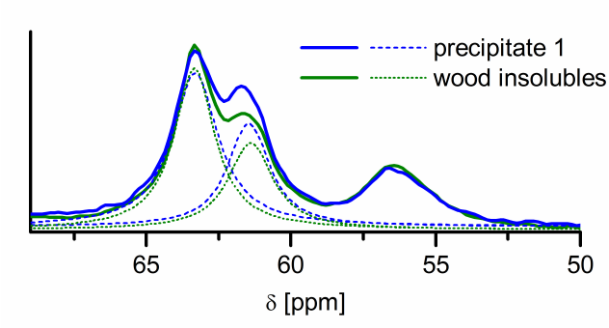
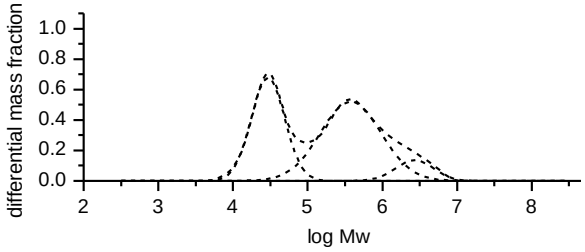
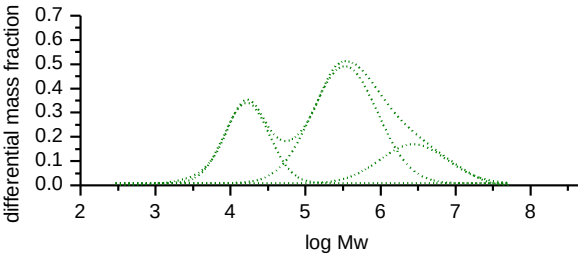


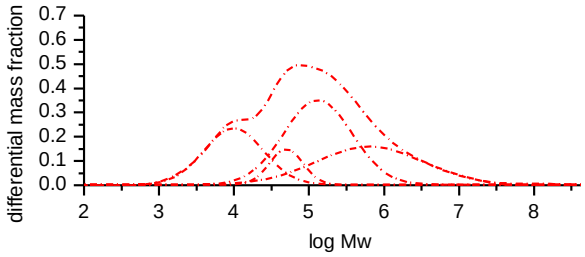
Figure S3. Deconvolution of the C6 peaks of regenerated cellulose II in unprocessed birch filter retentate and precipitate 1; the 63/61 ppm integral ratios are 1.69 and 1.72, respectively.



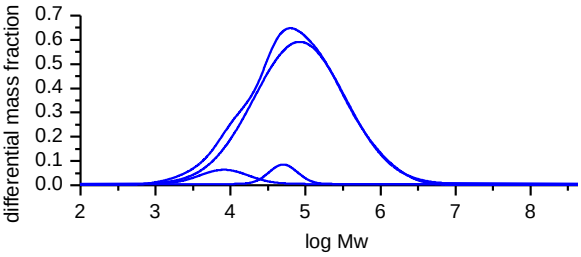
Birch



Kraft pulp



Autohydrolyzed birch



Precipitate 1

Figure S4. Deconvolution of individual GPC results into size fractions.

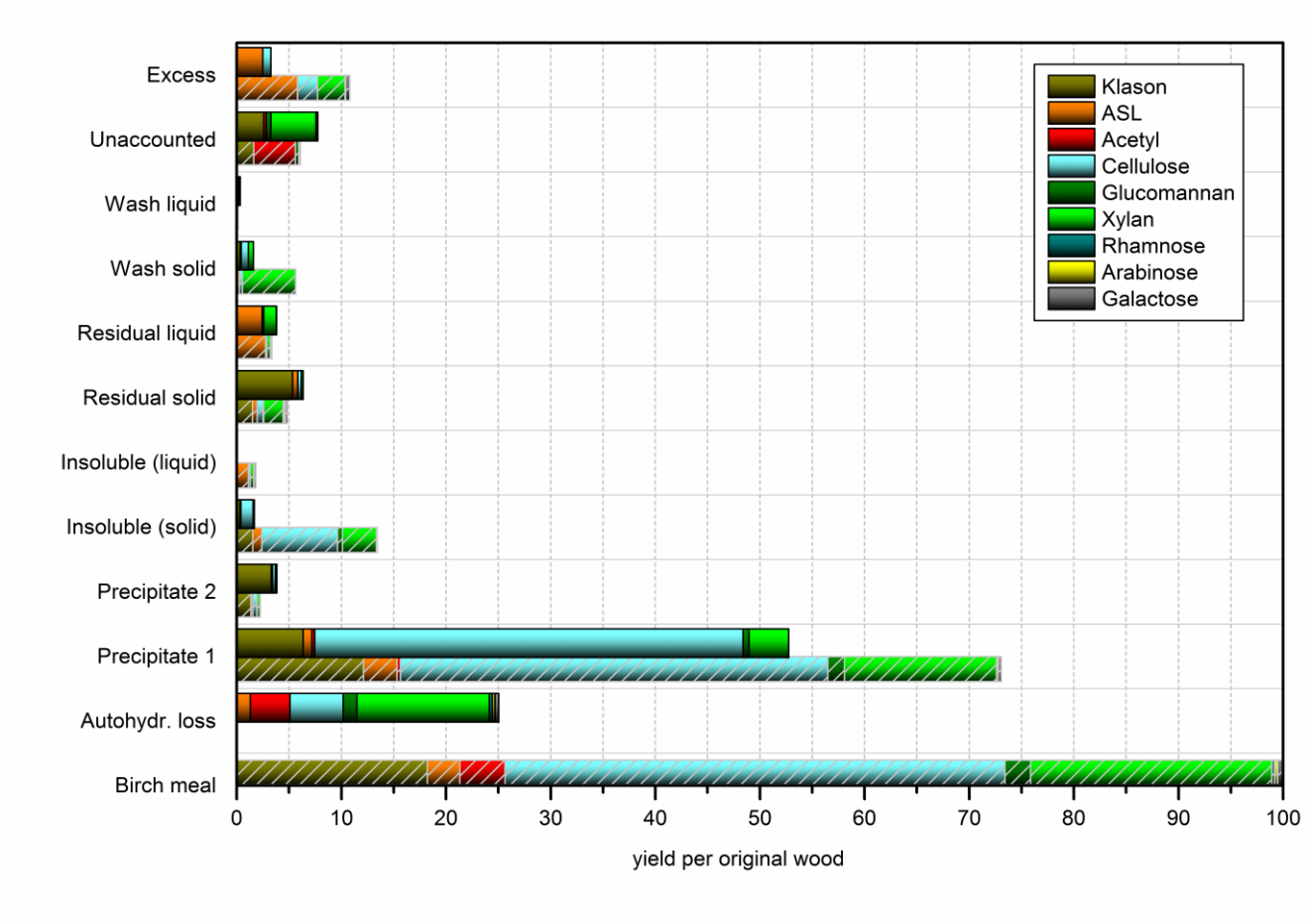


Figure S5. Yields per original wood for each fraction and component; striped: native birch, unpatterned: autohydrolysed birch as starting material