Supporting information

On the effect of hot-water pretreatment in sulfur-free pulping of aspen and wheat straw

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17 pages, 9 figures, 4 tables

File S1. Supporting methods:

S1.1. Extraction procedure for removal of extractives

Extractives were removed from wheat straw by extracting twice with 70 % EtOH for 1 h and once with 1:1 $CHCl_3$:MeOH for 1 h at room temperature. The extracts were removed by filtration. The fine straw powder was then dried to constant weight in oven at 50 ºC for several days and stored in room temperature.

Extractives were removed from the aspen powder by extracting with acetone overnight. The extracts were removed by filtration and the aspen powder was dried to constant weight in oven at 50 ºC for two days and stored in room temperature.

S1.2. General procedure of the carbohydrate composition analysis

Briefly, 5-15 mg of the freeze-dried solid samples were depolymerized with acid methanolysis using 2 ml of 2 M HCl in anhydrous methanol. Calibration solutions containing 0.1 mg/ml of analyzed monomeric sugars except 4-O-Methyl glucuronic acid were used in analyses. 4-O-Methyl glucuronic acid was determined as glucuronic acid and glucuronic acids response factor was used to calculate the amount of 4-O-Me-glucuronic acid. Samples were kept in oven at 105 °C for 5 h. Samples and calibration solutions were treated similarly after acid methanolysis. Samples were cooled down and neutralized with 200 µl of pyridine. Internal standards were added on solutions, 1 ml of 0.1 mg/ml sorbitol and resorcinol to calibration solutions and 4 ml of same standards to samples. One ml of solutions was transferred to test tubes. Samples were first dried at 60 °C under nitrogen hood and further in a vacuum oven at 40 °C for 15 minutes. Samples were silylated after drying with 100 µl of pyridine, 150 µl of hexamethyl disilazane (HMDS) and trimethyl chlorosilane (TMCS).

S1.3. Lignin content analysis

Acetyl bromide soluble lignin concentration was calculated with the following equation based on the absorbance reading A. In the formula V is the volume of the diluted sample (100 ml), ε is the plant specific extinction coefficient (17.54 and 17.898 g^{-1} L cm^{-1} for wheat straw and aspen, respectively)[i], m is the weighed mass of the sample (4–6 mg) and L is the path length of the UV beam (1 cm).

$$\text{Lignin w/w content (\%)} = 100\% * V A / (\varepsilon m L) \qquad (1)$$

A solution of 25 % (w/w) acetyl bromide in acetic acid was prepared by adding 5 ml of 99 % acetyl bromide to 23.8 ml of glacial acetic acid. Lignin contents from lignocellulosic samples were determined by accurately weighing 4-6 mg of biomass to 8 ml glass vial using an analytical balance (± 0.1 mg). Then 5 ml of 25 % (w/w) acetyl bromide in acetic acid was added into the vials which were then capped with plastic caps equipped with Teflon liners. Vials were then placed into an oven at 50 ºC for 2 h to digest while gently shaking the vials every 15 minutes. After the digestion the solutions were flushed into 100 ml volumetric flasks which contained 10 ml of 2M NaOH and 25 ml of glacial acetic acid. Volumetric flasks were then further filled to 100 ml with glacial acetic acid. UV absorptions were read

immediately at 280 nm by using Varian Cary 50 Conc UV-VIS spectrometer. Blank sample was used as a baseline spectrum. Untreated sample of wheat straw or aspen was used as reference in every run.

S1.4. Lignin and hemicellulose NMR Characterization

^{13}C experiments were performed by collecting Bruker standard pulse sequence with 16000 scans using 1s pulse delay (d1). This resulted in the total experimental time of 10 h. HSQC spectra were collected with Bruker standard pulse sequence (hsqcetgp) with following conditions: 12.5 ppm spectral width in F2 (^{1}H) dimension with 1024 data points and 215 ppm spectral width in F1 (^{13}C) dimension with 128 data points, using 1.44347 s pulse delay and 16 scans. This resulted in the total experimental time of 53 min.

S1.5. Molecular weight distribution analysis

Lignin analysis: THF was used as an eluent with a flow rate of 0.8 mL min^{-1} at 30 °C. Both RI and UV-detector (254 nm) were used for monitoring. Calibration was performed using 8 polystyrene standards (Polymer Standards Service, Warwick, USA) with molecular weights between 474 and 76 000 g mol^{-1}. All the samples were acetylated by acetic anhydride in pyridine to make them soluble in THF prior to the GPC analyses. The sample concentration was 1 mg mL^{-1} and all samples were filtered through a 0.20 µm syringe filter (Acrodisc Pall GBH, Waters, USA) prior to injection with 10 µL injection volume.

Carbohydrate analysis: The eluent was 0.5 % LiCl in DMA (*N*,*N*-dimethylacetamide) with the flow rate of 1 ml min^{-1}. A ten-point pullulan standard set was used for the calibration with the original molar masses between 180 and 708 000 g mol^{-1} but according to Berggren *et al.*[ii] and Potthast *et al.*[iii] pullulan standards need to be corrected due to the different hydrodynamic volume (1-6 vs. 1-4 linkages between AGUs). Thus, in the calculations the cellulose corresponding values for pullulan standards between 700 and 445600 g mol^{-1} were used. The standards were dissolved to the eluent. All the samples and standards were filtered with Acrodisc 0.45 µm syringe filters (Pall GBH, USA) prior analysis. Sample concentration was 1 mg ml^{-1} and injection volume 50 µl.

S1.6. Ash content analysis

Ash contents in Wheat straw samples were determined by accurately weighing 2-5 mg of biomass to pre-dried pre-weighed ceramic TGA vessels. The vessels were placed in a muffler oven. The muffler oven was directly heated to 600 ºC and left to heat for 30 min at 600 ºC. The vessels were removed from the oven when the temperature inside the oven was decreased below 250 ºC. The vessels were weighed again to determine to mass of the residual matter. In present study, the Mettler Toledo TGA/SDTA851[e] balance was used to obtain the most accurate results when using such a small sample size.

S1.7. Pyrolysis-GC-MS analysis

Both samples were dried carefully, and homogenized prior analysis, and the pyrolysis was performed under the same conditions. Analytical scale Py–GC/MS equipment Pyrolab2000 (Pyrolab, Sweden) was adopted. The samples were analyzed as such using a platinum foil pulse pyrolyzer and 580 °C isothermal pyrolysis temperature. The system was directly connected to Bruker Scion SQ 456-GC/MS equipped with Agilent DB-5MS UI (5 %-phenyl)-

methylpolysiloxane, 30 m × 0.250 mm × 0.25 µm lm) capillary column. The injector temperature was 250 °C, ion source 250 °C with electron ionization of 70 eV, the MS scan range m/z 40–400 and helium as carrier gas at the flow rate of 1 mL/ min using a split ration of 1:2. From the base line corrected GC/ MS total ion count (TIC) chromatograms, the ratio of aromatic vs. carbohydrate based fragments identifed by selected reference compounds with their retention times and mass spectra, and by comparison with National Institute of Standards and Technology (NIST) library and with literature, as area, was compared to the total area (relative peak areas). The fragments used in calculations were measured between retention time of 3.04–20 min, and altogether 68 peaks for wheat straw and 50 peaks for aspen peaks were included in quantification.

Table S1. List of used solvent systems in the microwave treatments

The numerals of the entries are referred in upcoming data.

Wheat #	Aspen #	Solvent system
1	**10**	88 % HCOOH
2	**11**	HWE[a] + 88 % HCOOH
3	**12**	80 % [TEAH][HSO_4]
4	**13**	HWE[a] + 80 % [TEAH][HSO_4]
5	**14**	6:4 EtOH : 0.25 M Na_2CO_3
6	**15**	HWE[a] + 6:4 EtOH : 0.25 M Na_2CO_3
7	**16**	6:4 EtOH : 0.25 M NaOH
8	**17**	0.1 M HCl + 0.1 M NaOH
9	**18**	1 M NaOH

Table S2. GPC data of cellulose rich (C) fractions

Table S2. Weight average degree of polymerization (DPw) of wheat and aspen samples and their calculated polydispersity indexes (PDI). Cellulose denotes integration, and the corresponding value obtained from the GPC graph, at cellulose molecular weight area (16-22 min). "Whole" denotes the integrated values from the whole signal area.

#	DPw wheat cellulose	PDI wheat cellulose	DPw wheat whole	PDI wheat whole	#	DPw aspen cellulose	PDI aspen cellulose	DPw aspen whole	PDI aspen whole
1	3548	2.9	2933	9.5	**10**	2124	3.7	1885	6.3
2	3140	3.0	2678	8.1	**11**	1213	2.8	1059	4.6
3	1849	3.5	1545	9.3	**12**	872	3.6	799	6.0
4	856	3.3	805	5.0	**13**	484	2.3	440	3.3
5	5305	1.5	2151	8.6	**14**	4126	1.9	1744	8.6
6	4227	1.9	2807	11.9	**15**	1666	2.8	1499	6.1
7	5326	1.5	2185	8.2	**16**	3925	2.0	1775	9.0
8	3317	2.6	2277	18.8	**17**	1482	3.2	1129	8.0
9	4346	1.8	3149	6.4	**18**	3591	2.0	2308	7.6

Figure S1. GPC graphs of cellulose rich (C) fractions

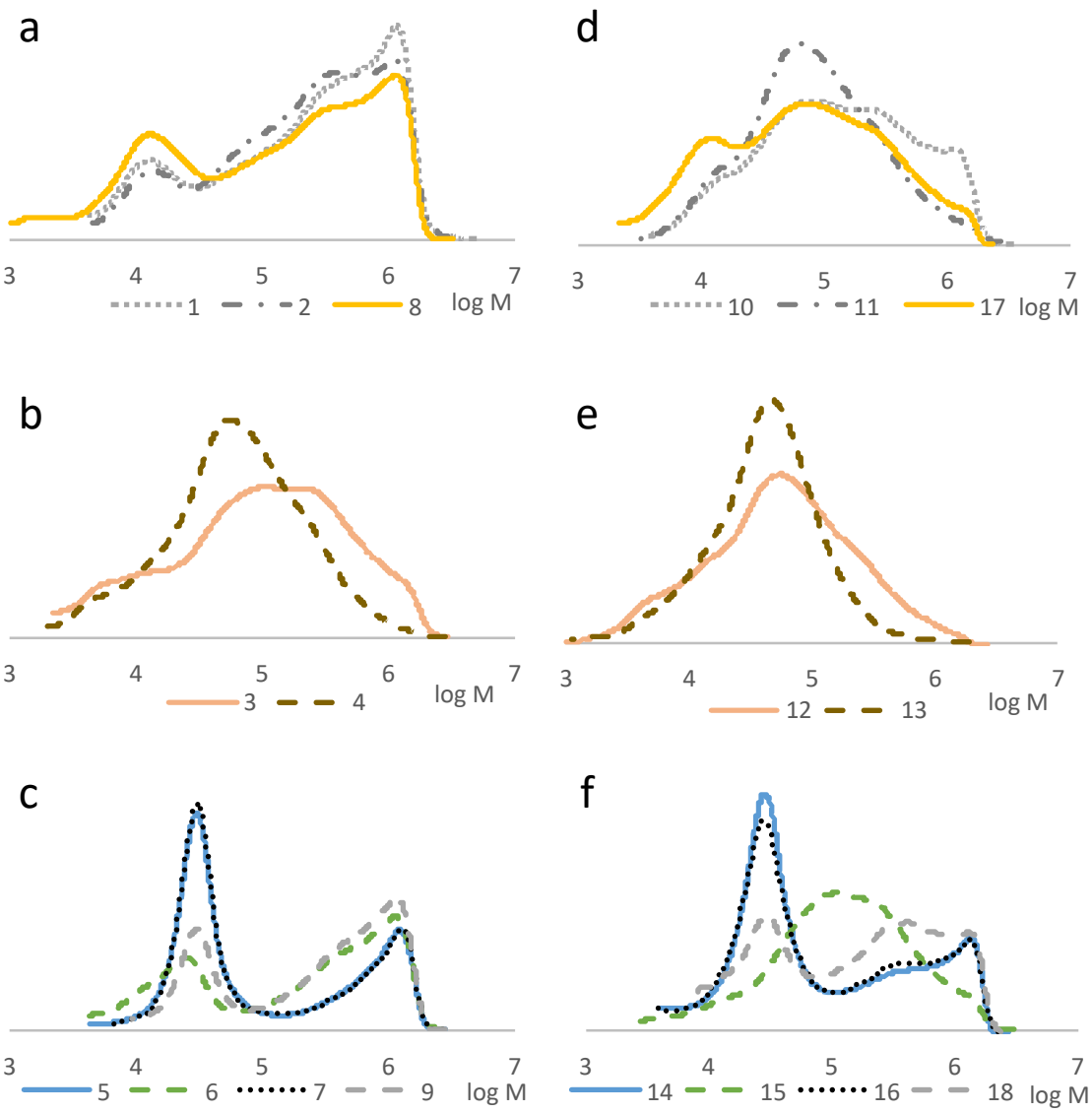


Figure S1. GPC graphs of wheat straw (**a**, **b** and **c**) and aspen (**d**, **e** and **f**) cellulose rich fractions from different extraction experiments. Graphs **a** and **d** describe the samples treated in acidic conditions, **b** and **e** in ionic liquid-water mixtures and **c** and **f** were done in alkaline conditions.

Table S3. GPC data of hemicellulose rich fractions (A)

Table S2. Weight average degree of polymerization (DPw) of wheat and aspen HWE samples and their calculated polydispersity indexes (PDI). $DP_w=M_w/AXU$ (Anhydroxylose Unit = 132 g mol^{-1})

Fraction	M_w	DP_W	PDI
Aspen HWE	1530	12	1.4
Wheat straw HWE	7950	60	4.0

Figure S2. GPC graphs of hemicellulose rich (A) fractions

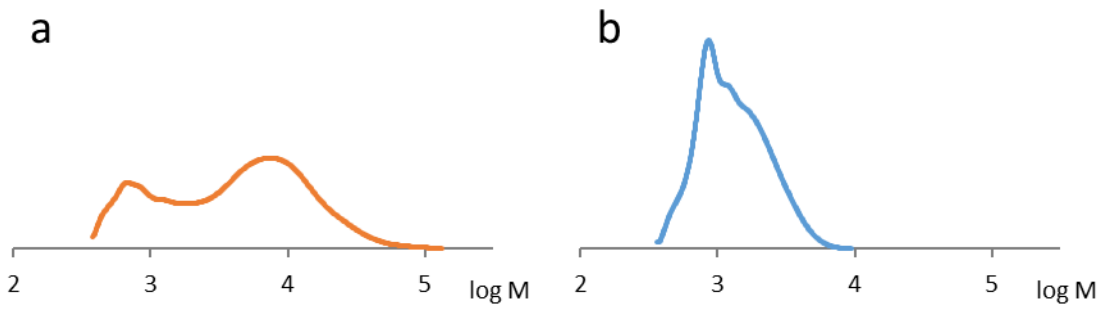


Figure S2. a) Wheat and b) aspen hemicellulose GPC results as graphs

Figure S3. Wheat straw lignin (fraction D) HSQC NMR Spectra

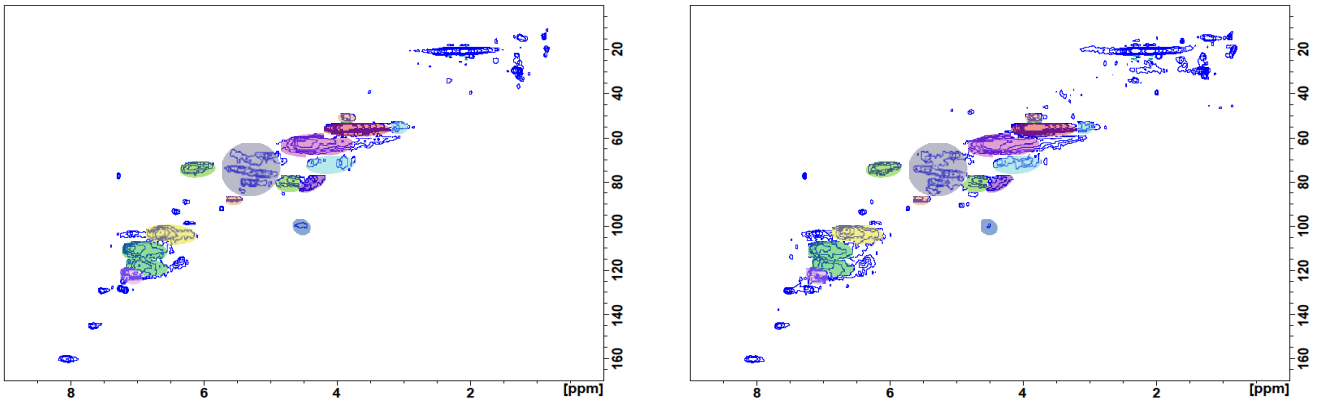


Figure S3.1. Formic acid (**1**) (Left)
Figure S3.2. Hot water extraction + Formic acid (**2**) (Right)

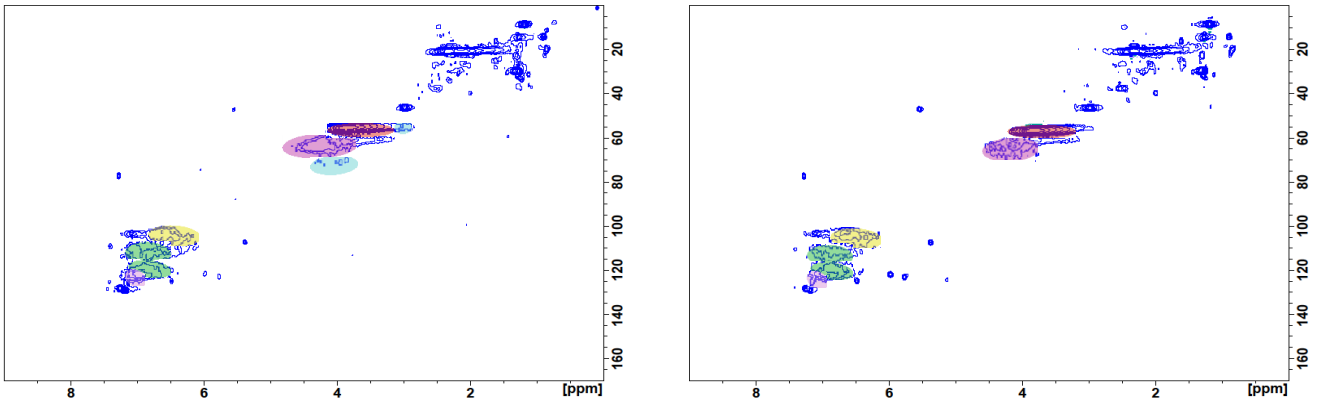


Figure S3.3. [TEAH][HSO_4] (**3**) (Left)
Figure S3.4. Hot water extraction + [TEAH][HSO_4] (**4**) (Right)

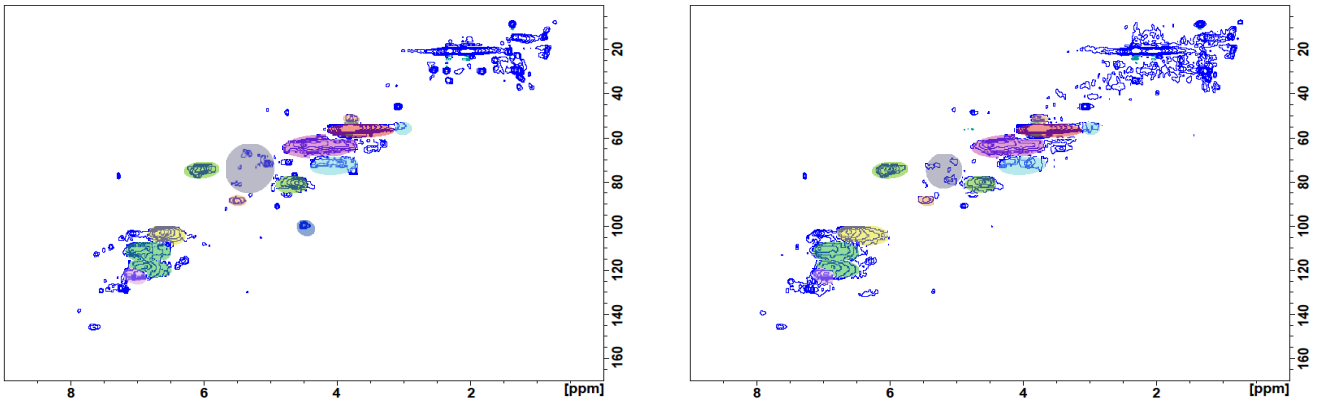
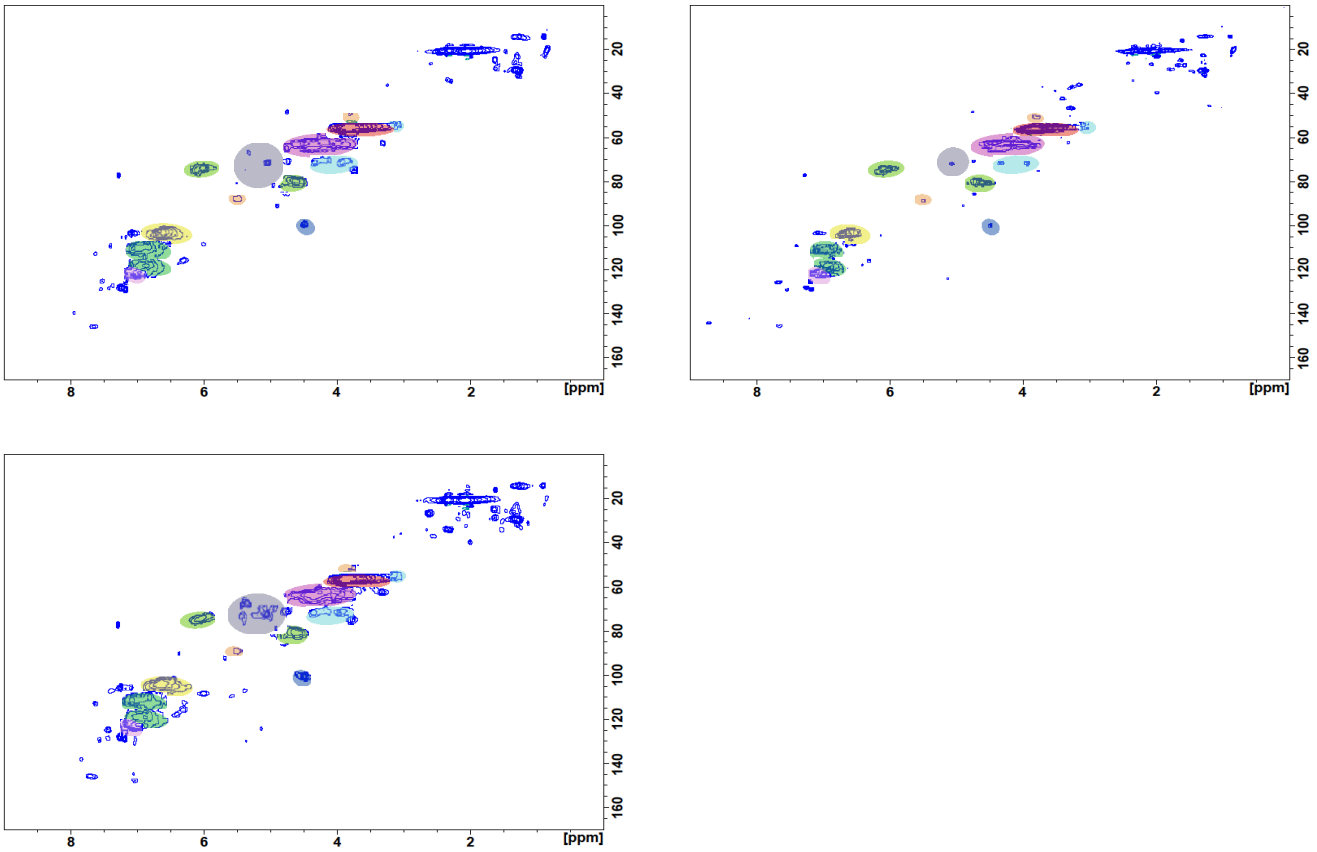
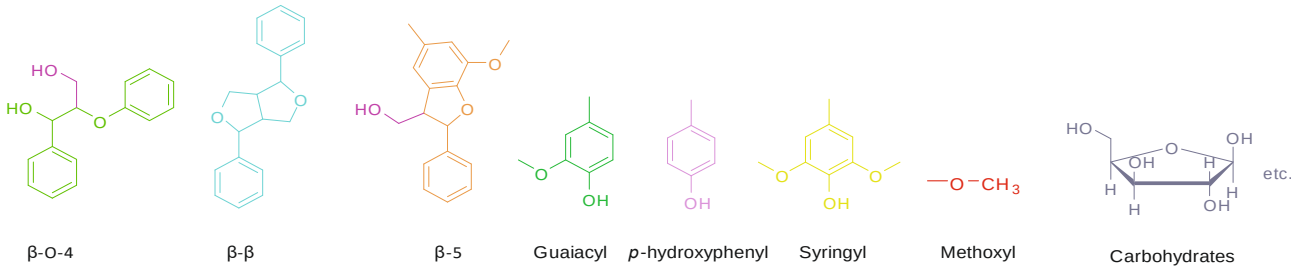


Figure S3.5. EtOH/0.25 M Na_2CO_3 (**5**) (Left)
Figure S3.6. Hot water extraction + EtOH/0.25 M Na_2CO_3 (**6**) (Right)





β-O-4 β-β β-5 Guaiacyl *p*-hydroxyphenyl Syringyl Methoxyl Carbohydrates

Figure S3.7. EtOH/NaOH (**7**) (Top left)
Figure S3.8. Dilute HCl + dilute NaOH (**8**) (Top right)
Figure S3.9. 1M NaOH (**9**) (Bottom left)

Figure S4. Wheat straw lignin (fraction D) ^{13}C NMR-spectra

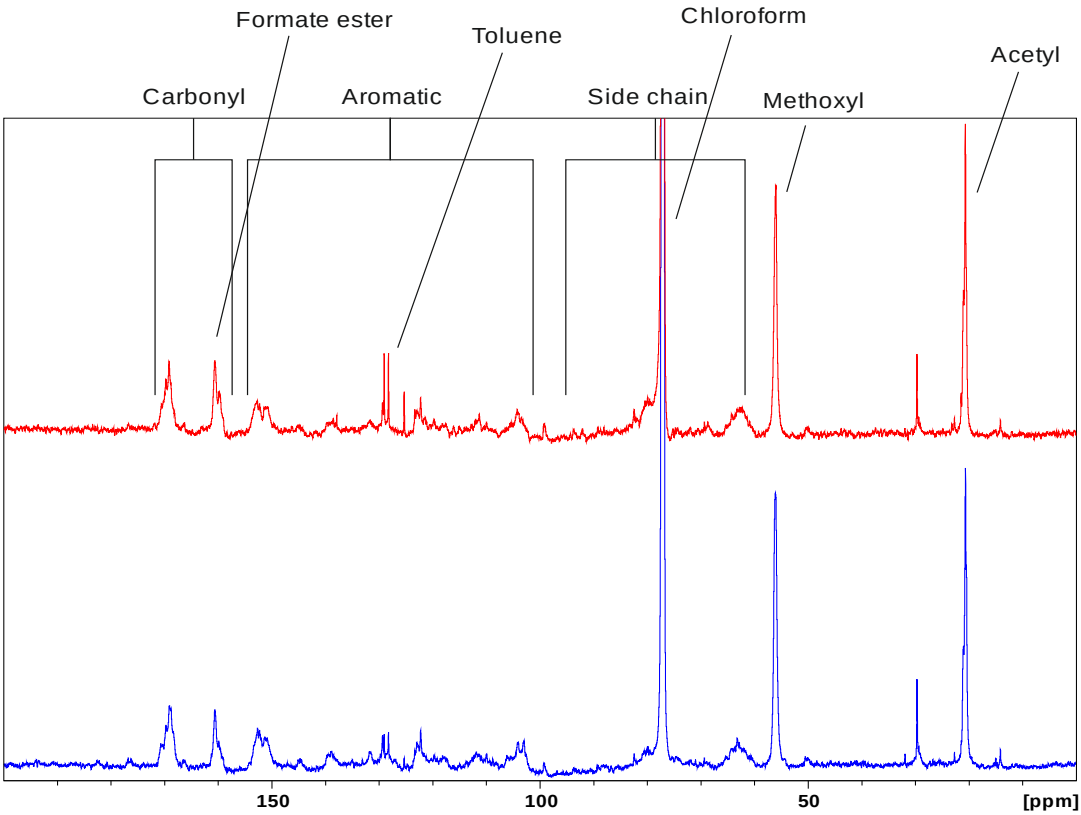


Figure S4.1. The ^{13}C-NMR of Formic acid (**1**) (Top spectrum) and Hot water extraction + Formic acid (**2**) (Bottom spectrum)

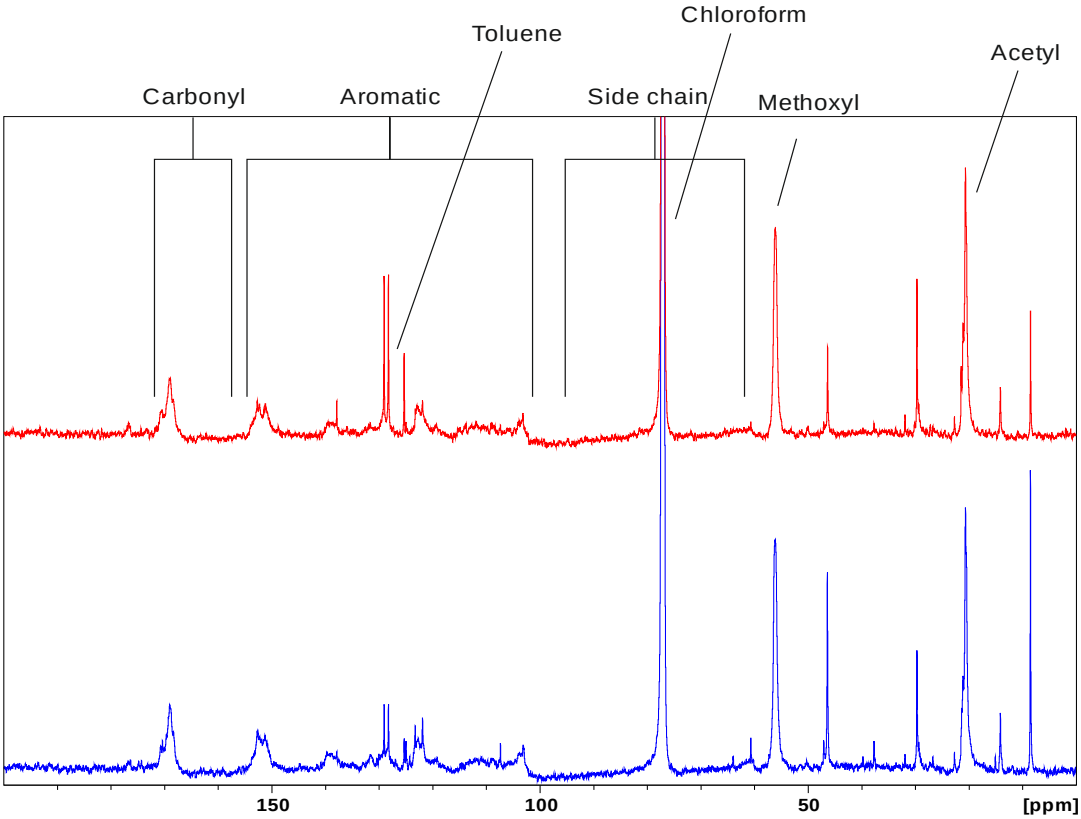


Figure S4.2. The^{13}C-NMR of [TEAH][HSO_4] (**3**) (Top spectrum) and Hot water extraction + [TEAH][HSO_4] (**4**) (Bottom spectrum)

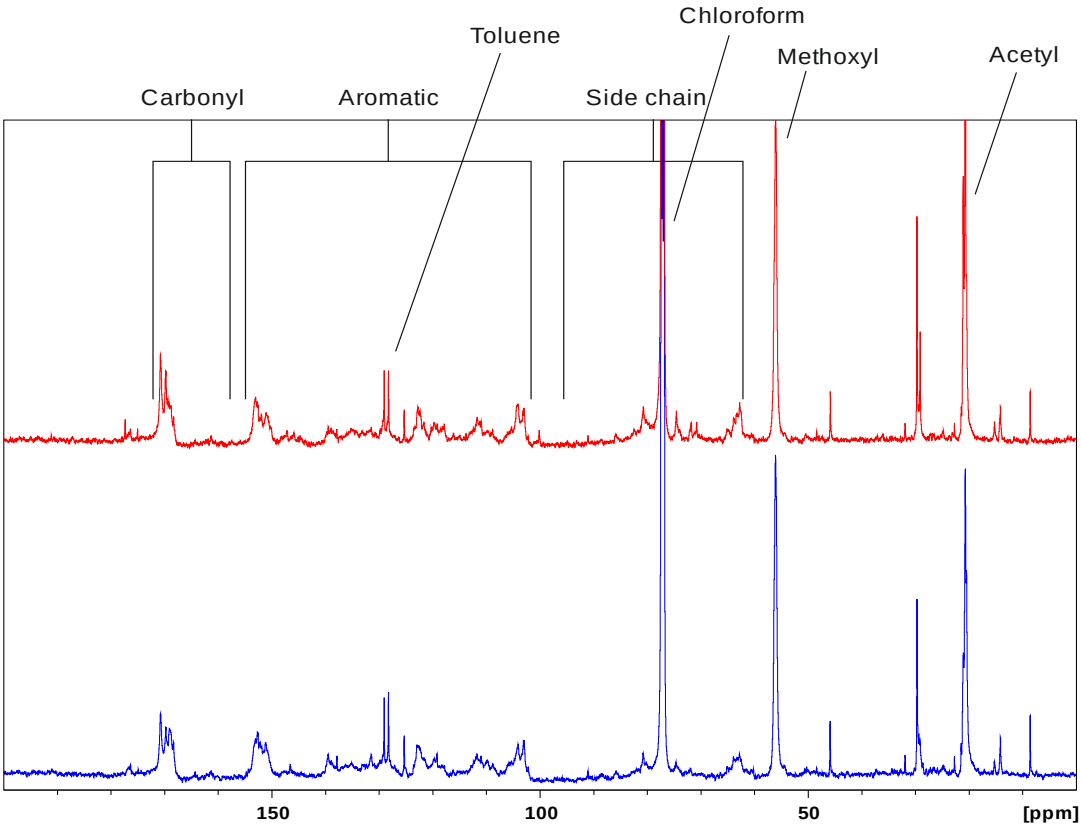


Figure S4.3. ^{13}C-NMR of EtOH/0.25 M Na_2CO_3 (**5**) (Top spectrum) and Hot water extraction + EtOH/0.25 M Na_2CO_3 (**6**) (Bottom spectrum)

Figure S5. Wheat straw and aspen lignin (fraction D) GPC data

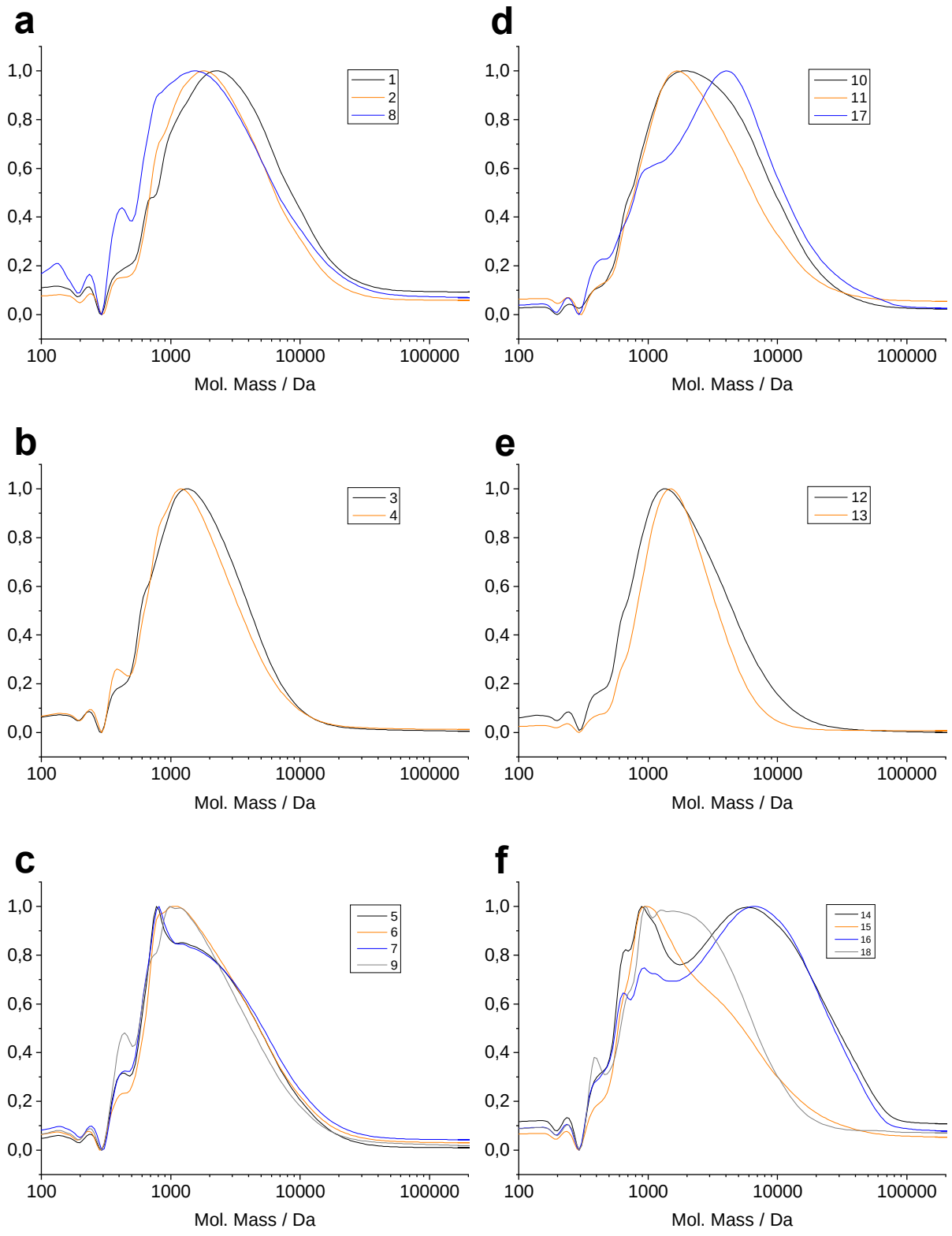


Figure S5. GPC graphs of wheat straw (a, b and c) and aspen (d, e and f) lignin rich fractions D from different extraction experiments measured at 254 nm. Graphs a and d describe the samples in acidic conditions, b and e in ionic liquid-water mixtures and c and f were done in alkaline conditions.

Figure S6. Aspen lignin (fraction D) HSQC NMR-spectra

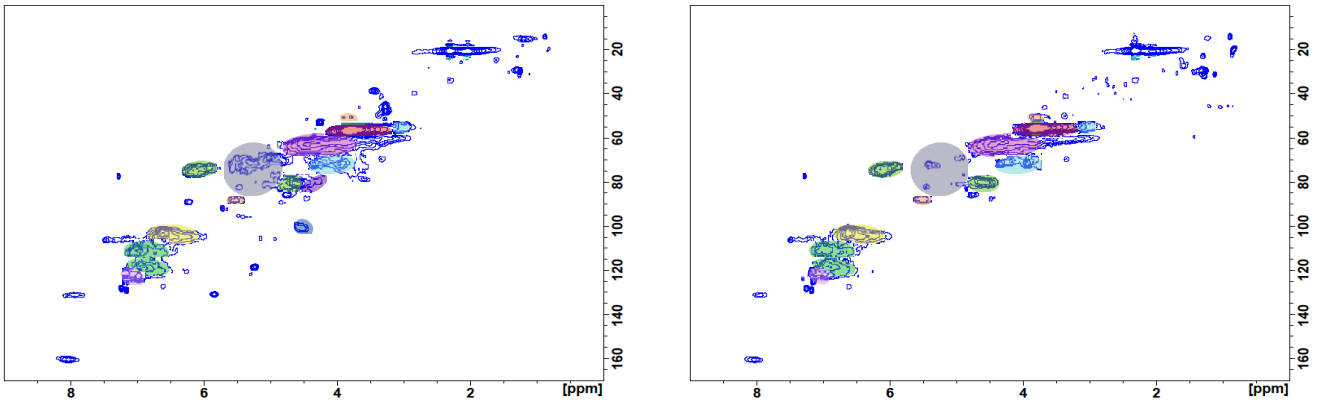


Figure S6.1. Formic acid (**10**) (Left)
Figure S6.2. Hot water extraction + Formic acid (**11**) (Right)

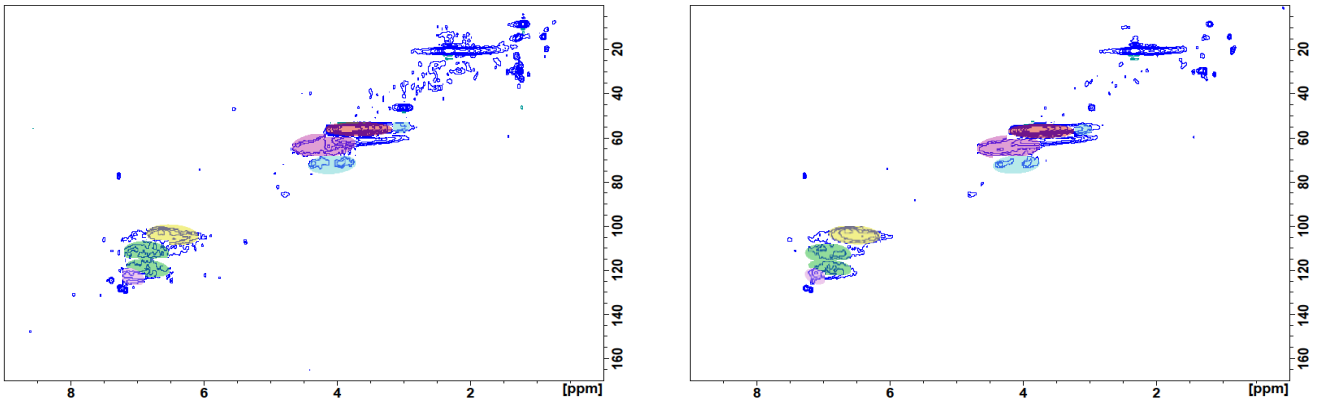


Figure S6.3. [TEAH][HSO_4] (**12**) (Left)
Figure S6.4. Hot water extraction + [TEAH][HSO_4] (**13**) (Right)

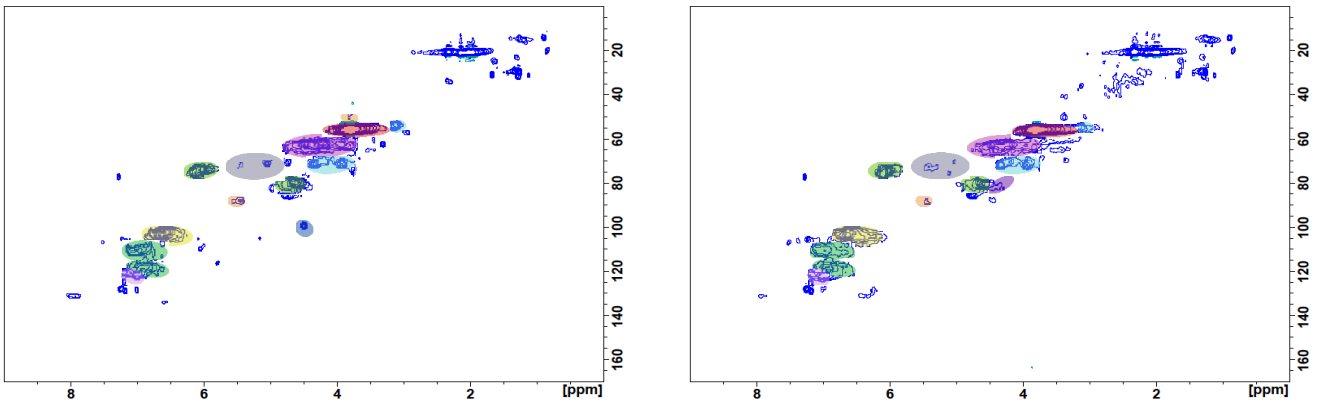
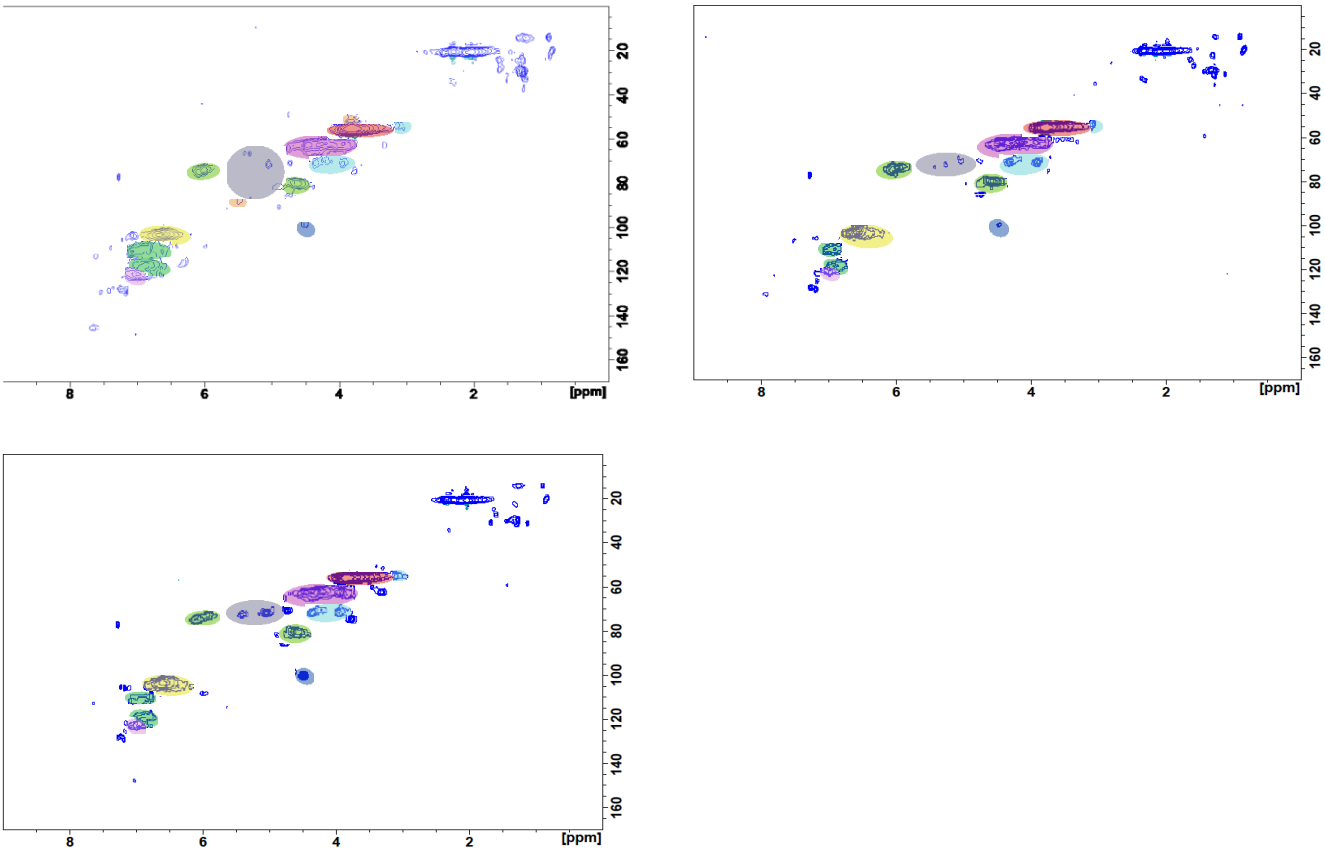


Figure S6.5. EtOH/0.25 M Na_2CO_3 (**14**) (Left)
Figure S6.6. Hot water extraction + EtOH/0.25 M Na_2CO_3 (**15**) (Right)



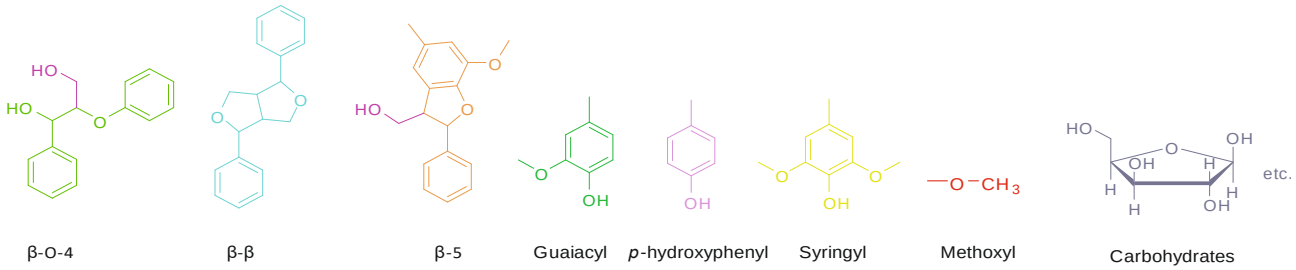


Figure S6.7. EtOH/NaOH (**16**) (Top left)
Figure S6.8. Dilute HCl + dilute NaOH (**17**) (Top right)
Figure S6.9. 1M NaOH (**18**) (Bottom left)

Figure S7. Aspen lignin (fraction D) ^{13}C NMR-spectra

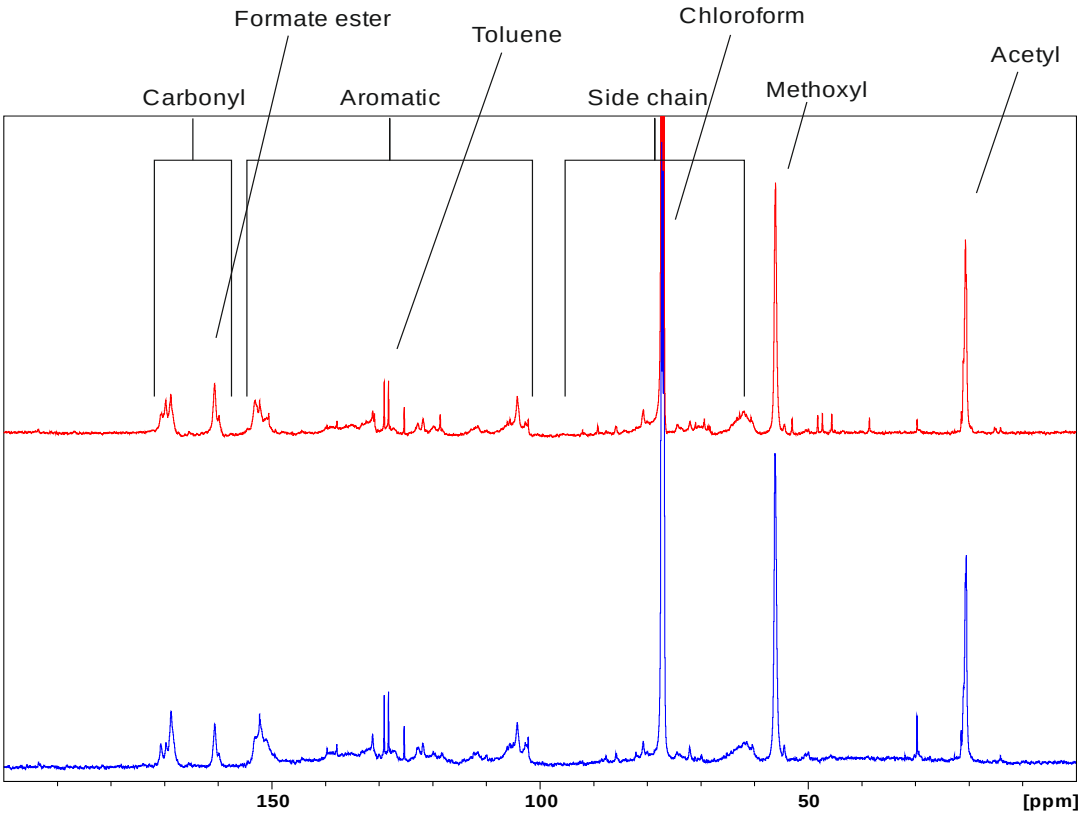


Figure S7.1. The^{13}C-NMR of Formic acid (**10**) (Top spectrum) and Hot water extraction + Formic acid (**11**) (Bottom spectrum)

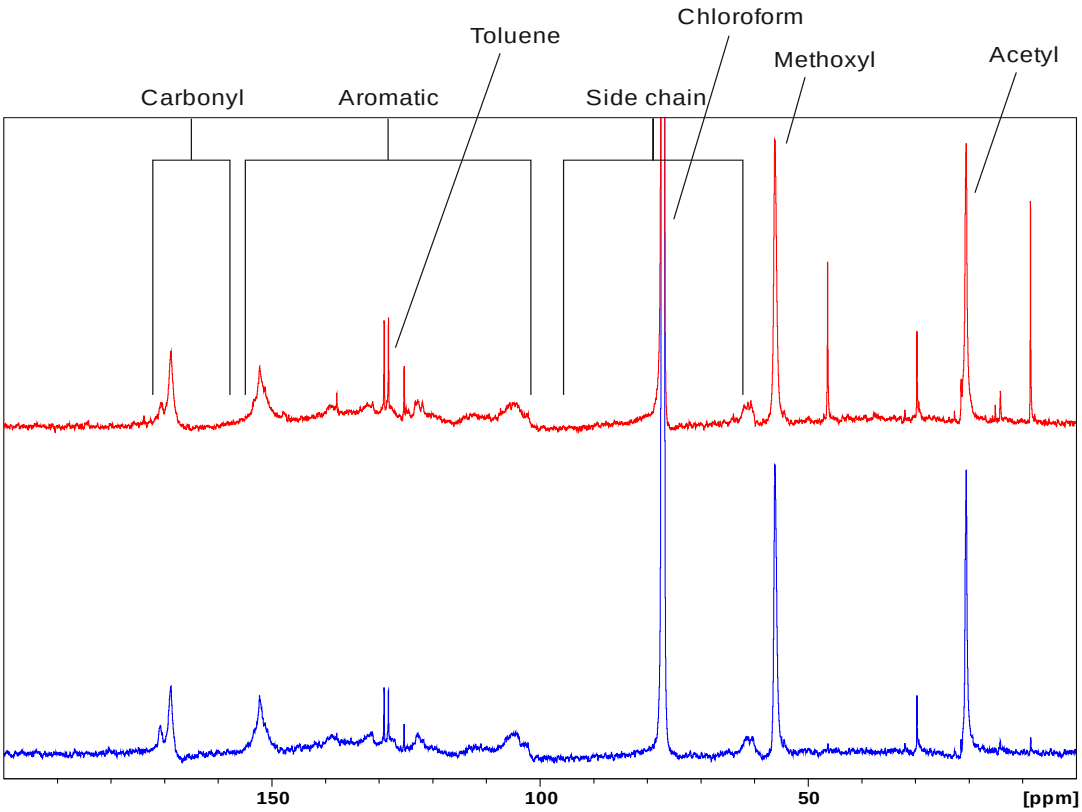
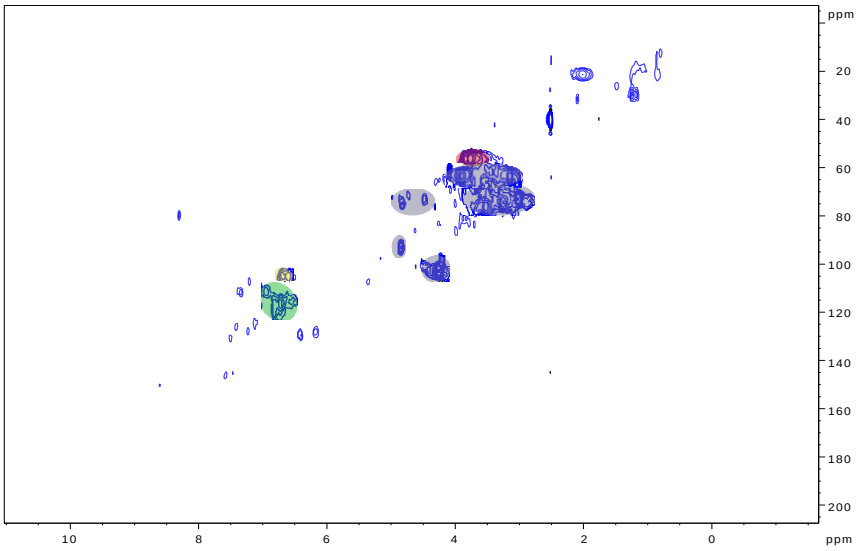


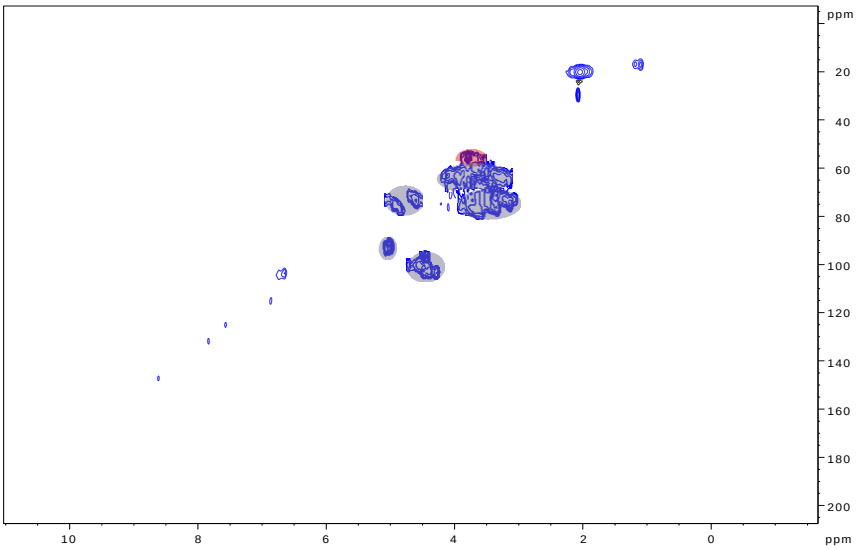
Figure S7.2. The^{13}C-NMR of [TEAH][HSO_4] (**12**) (Top spectrum) and Hot water extraction + [TEAH][HSO_4] (**13**) (Bottom spectrum)

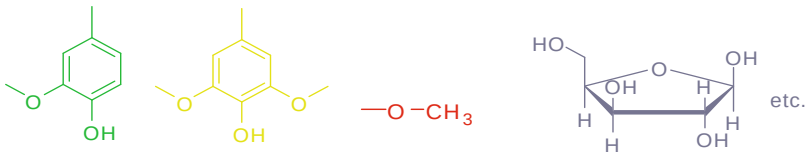
Figure S8. HSQC NMR of hemicellulose rich (A) fractions

S8.1



S8.2





Guaiacyl Syringyl Methoxyl Carbohydrates

Figure S8. HSQC spectra of HWE fractions (A) of S8.1) wheat straw in d6-DMSO and S8.2) aspen in d6-acetone.

Table S4. Carbohydrate composition analysis

Table S4.1 Carbohydrate composition of wheat straw and the isolated cellulose rich fraction C samples from different treatments (**1**-**9**). The samples have been depolymerized with acid methanolysis and silylated for GC-MS analysis.

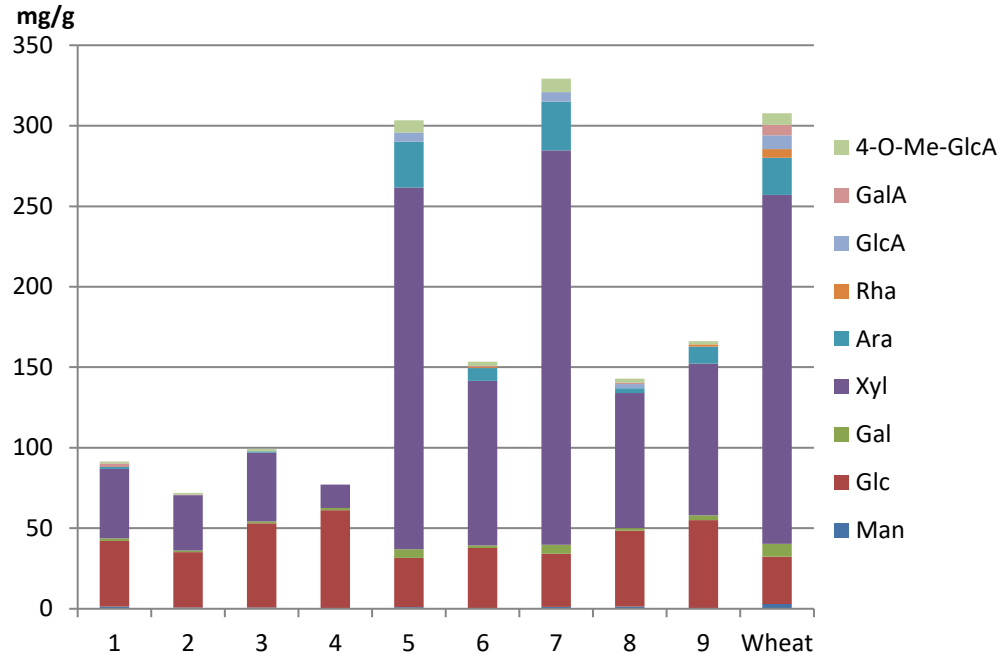


Table S4.2 Carbohydrate composition of aspen and the isolated cellulose rich fraction C samples from different treatments (**10**-**18**). The samples have been depolymerized with acid methanolysis and silylated for GC-MS analysis.

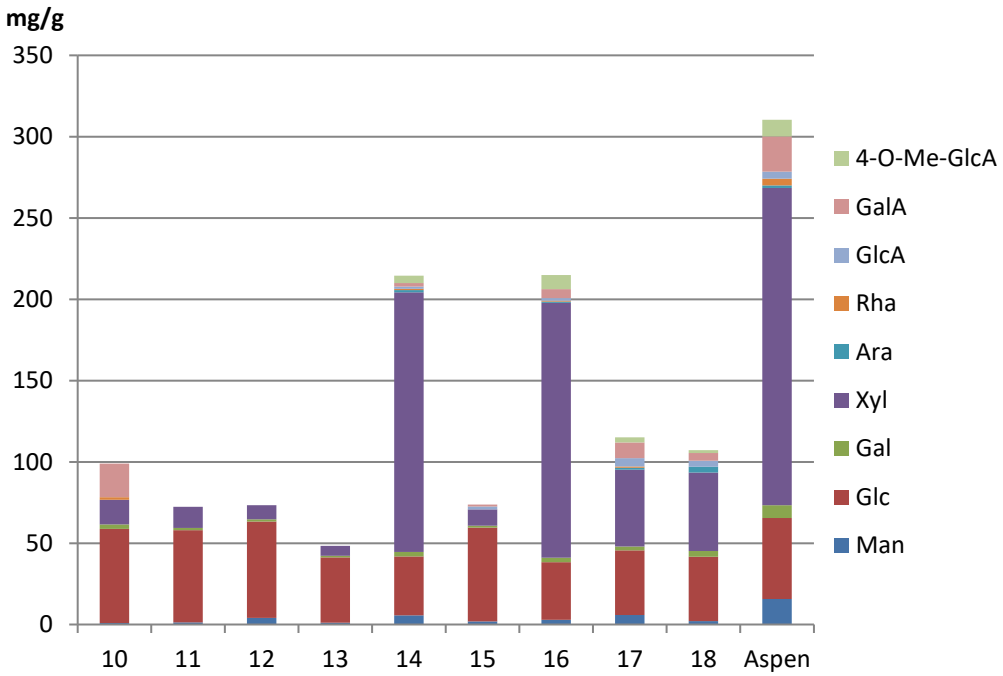


Figure S9: HSQC-NMR of Aspen in concentrated 1M NaOH-experiments

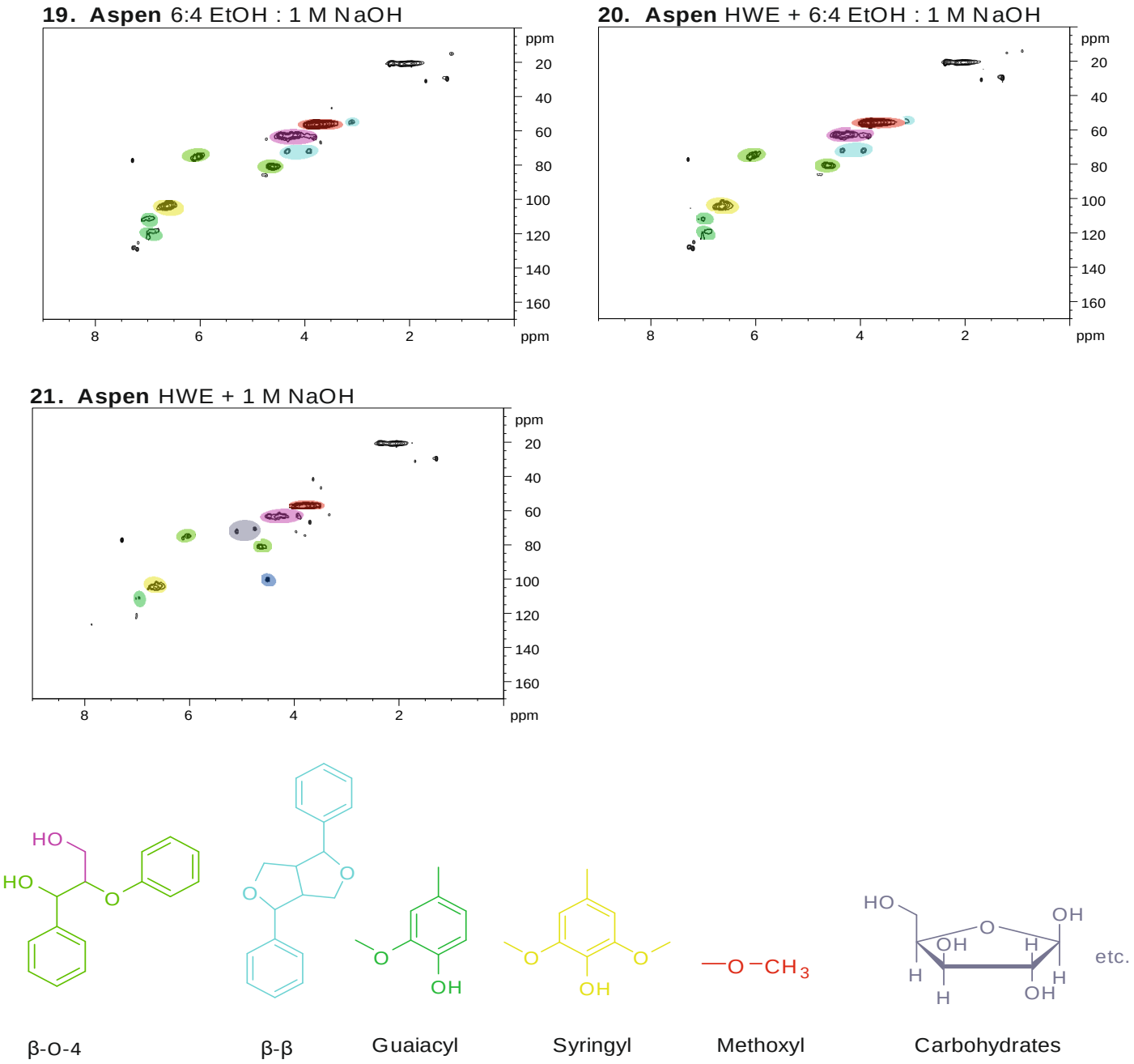


Figure S9.1 HSQC-spectra of EtOH/1 M NaOH (**19**) (Top left)
Figure S9.2. HWE+ EtOH/1 M NaOH (**20**) (Top right)
Figure S9.3. HWE+1M NaOH (**21**) (Bottom left)

References

[i] Fukushima, R. S. and Hatfield, R. D. Comparison of the acetyl bromide spectrophotometric method with other analytical lignin methods for determining lignin concentration in forage samples. *J. Agric. Food Chem.* **2004**, *52*, 3713-3720.
[ii] Berggren, R., Berthold, F., Sjöholm, E. and Lindström, M. Improved methods for evaluating the molar mass distributions of cellulose in kraft pulp. *J. Appl. Polym. Sci.* **2003**, *88*, 1170-1179.
[iii] Potthast, A., Radosta, S., Saake, B., Lebioda, S., Heinze, T., Henniges, U., Isogai, A., Koschella, A., Kosma, P., Rosenau, T., Schiehser, S., Sixta, H., Strlič, M., Strobin, G., Vorwerg, W. and Wetzel, H., Comparison testing of methods for gel permeation chromatography of cellulose: coming closer to a standard protocol. *Cellulose* **2015**, *22*, 1591-1613.