Supramolecular interaction-driven delignification of lignocellulose

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Materials and methods
Materials
Refined Norway spruce chips (thermomechanical pulp) used as a raw material and kraft lignin used as a lignin reference were kindly provided by UMP and Metsä, respectively. Ethanolytic lignin was provided by Vertoro. Thymol (99-101%), menthol (≥98.0%), phenol (≥99%), eugenol (99%), ethyl acetate (≥99.5%), p-cymene (99%), methanesulfonic acid (MSA, ≥99.0), p-toluenesulfonic acid monohydrate (≥98%), sodium methanesulfonate (98%), and trifluoromethanesulfonic acid (98%) were obtained from Merck. Ethanol (≥96%), 2-isopropyl-5-methylanisole (≥96.0%), 4-hydroxybenzenesulfonic acid (≥85.0%), and hexane (≥95%) were obtained from Avantor.

Delignification of softwood using supramolecular interaction-driven chemistry
MSA (24.25 g) and thymol (75.75 g) at a molar ratio of 1:2 were weighed in a beaker (total weight of 100 g) and placed in an oil bath at the desired temperature (40, 50, 60 °C). When a clear liquid was formed, 10 g of air-dried wood was added and the mixture was stirred for the designated time (0.5, 1, 2.5, or 5 min) followed by the addition of 200 ml ethanol to quench the reaction. The ethanolic suspension containing dissolved lignin and solid fraction was filtered, and solid cake was washed with ethanol until the color of the reaction mixture was removed. The solid fraction was oven-dried at 60 °C for approximately 12 h and stored at room temperature.

Recovery of the spent chemicals and lignin
MSA was first neutralized by the addition of equimolar NaOH dissolved in water (20 wt. %). Precipitated sodium methanesulfonate was filtered and washed with ethanol. The purity of the salt was characterized by ^{1}H nuclear magnetic resonance (NMR) spectroscopy.
Ethanol in the washing liquor was evaporated using a rotary evaporator. Thymol and two lignin fractions were separated with column chromatography using a mixture of ethyl acetate and hexane as an eluent. The purity of thymol was characterized by ^{1}H NMR.

Reference delignification of softwood
To investigate the effect of hydrogen bond donors on delignification, reference delignifications were performed by replacing thymol with p-cymene, 2-isopropyl-5-methylanisole, or menthol with two equivalents per mole of MSA. In the case of p-cymene and 2-isopropyl-5-methylanisole, an uneven mixture was formed with MSA, and therefore, they were not used. A clear liquid was

formed between menthol and MSA, and delignification was performed in a similar manner to that used with thymol at 60 °C for 5 min.

MSA is fully miscible with water, and two reference delignification experiments were conducted by replacing thymol with water. First, MSA was mixed with water at a molar ratio of 1:2 (water content of 18.02 wt. %), and delignification was performed at 60 °C for 5 min. Second, an MSA-water mixture with a water content of 75.75 wt. % was used to represent the mass ratio between MSA and thymol at a molar ratio of 1:2. Delignification was performed at 60 °C for 5 min.

Three selected novel delignification systems described in the literature were also studied for delignification of softwood. A deep eutectic solvent between choline chloride and lactic acid was used at a molar ratio of 1:2. Two compounds were first mixed and heated at 120 °C until a clear liquid was formed. Air-dried wood was added at a liquid:solid ratio of 1:10. The reaction was allowed to proceed for 180 min, and delignification was stopped by the addition of ethanol. The mixture was then filtered and washed with a high excess of ethanol.

Delignification with 80% aqueous p-toluenesulfonic acid was performed according to a previous publication at 80 °C for 20 min. Delignification with aqueous 4-hydroxybenzenesulfonic acid was performed at 60 °C for 5 and 20 min.

Production of nanocellulose from delignified cellulose fraction
After delignification of softwood at 60 °C for 5 min, ethanol-washed wood was washed with water to exchange solvent from ethanol to water. Nondried samples were then diluted with water to 0.5 wt. %, and samples were sonicated with an ultrasound probe (Heilscher UP 400 s power discharge 0.5 s and pause 0.5 s, 100% amplitude and 7 mm probe tip diameter) for 30 min in an ice water bath. Nanocellulose film was produced by vacuum filtration on a membrane (Durapore DVPP 0.65 µm, Merck Millipore Ltd., Ireland) at a negative pressure of ~800 mbar.

Characterization
The lignin, carbohydrate, and extractive contents of the original and delignified softwood were determined using the National Renewable Energy Laboratory method.(1) Chemical characterizations of lignin fractions were performed using a Bruker Vertex 80v diffuse reflectance infrared spectrometer and a Bruker Ascend 400 MHz NMR spectrometer. The quantitative determination of the molecular weight of the products was performed using Agilent 8890 gas chromatography equipped with an HP-5MS Ultra Inert column from Agilent (model: USR577054H). The thermal properties of thymol, menthol, lignin, and their mixtures were characterized using differential scanning calorimetry (DSC) (Mettler Toledo DSC 821e). The morphology of the nanocellulose and light transmittance of the nanocellulose film were investigated using transmission electron microscopy (TEM, JEOL JEM- 2200FS, Japan) and UV − vis spectrophotometry (Shimadzu, Japan), respectively.

Enzymatic hydrolysis
Orignal and MSA-thymol delignified wood were tested for enzymatic digestibility by Celignis Analytical, Limerick, Ireland (analysis package P121: https://www.celignis.com/package.php?value=67). During the hydrolysis, the target pH for the was 4.8 to 5.0 and sodium azide at final concentration of 0.025% was used as anti-bacterial agent and citrate buffer at final concentration of 0.05M as buffering agent. Cellulase, enzyme blend (Sigma-Aldrich) was used to perform the reaction at 50 °C for 72h and samples were taken every 12h for the time course study. The enzyme is used at the dosage of 0.3g/g glucan. The solids loading was aimed at 1%, however the actual weights used has resulted in solids loading between 1.2 and 1.4 percentage. The final obtained sugars concentration is corrected for evaporation loss and normalised for solid loading of 1%.

Production of nanocellulose from delignified cellulose fraction

After delignification of softwood at 60 °C for 5 min, ethanol-washed wood was washed with water to exchange solvent from ethanol to water. Nondried samples were then diluted with water to 0.5 wt. %, and samples were sonicated with an ultrasound probe (Heilscher UP 400 s power discharge 0.5 s and pause 0.5 s, 100% amplitude and 7 mm probe tip diameter) for 30 min in an ice water bath. Nanocellulose film was produced by vacuum filtration on a membrane (Durapore DVPP 0.65 μm, Merck Millipore Ltd., Ireland) at a negative pressure of ~800 mbar.

Supplementary Text

Study of the acid counterpart

We observed that initial liquid formed between sulfuric acid and thymol slowly solidified at room temperature, which could be attributed to the sulfonation/sulfation of phenolic thymol.[2] Meanwhile, together with MSA, thymol formed clear liquid at molar ratio of 1:2, and no solidification was observed when heated at temperature of 100°C. When cooled, MSA-thymol mixture remained liquid even at -24°C when left undisturbed. However, when manually mixed, rapid crystallization was observed, indicating strong supercooling property. At temperature of 40 °C, mixture was liquid even when mixed. In addition to the MSA, trifluoromethanesulfonic acid also formed liquid with thymol. However, the use of trifluoromethanesulfonic acid with thymol resulted in complete dissolution of wood within 5 min at 60°C (no precipitation occurred by the addition of ethanol). Compared to MSA, trifluoromethanesulfonic acid is a significantly stronger acid (pKa of -14.7 vs. −1.9) and is strong enough to cause complete degradation of wood components even in short reaction time.

Characterization of isolated lignin fractions

As a powder, both lignin fractions exhibited strong reddish color, however, when samples are dissolved in ethyl acetate and cast on the glass Petri dish, both fractions are transparent and mildly colored (Figure S5a). LF1 showed more yellowish color compared to LF2, which appears as red. In DMSO, both fractions showed strong absorption peak at wavelength of 285 nm (Figure S5b), which is typical UV absorption maximum for lignin. The LF1 exhibited slightly higher absorption at visible light region as well as small shoulder around 265 nm, indicating that two fractions have different characteristics. When the UV and visible light absorption properties of two lignin fractions are compared to oligomeric lignin, notable differences can be observed at visible light region. The light absorption of oligomeric lignin begins already at the halfway of the visible light spectrum, whereas in case of LF1 and LF2, to absorption begins around wavelength of 450 nm. The maximum absorption of oligomeric lignin at UV region is higher compared to two lignin fractions obtained by thymol-MSA method, however, the LF1 and LF2 showed notable higher maximum UV absorption compared to the absorption at low end of visible light spectrum (400 nm) (24, 47, and 8 for LF1, LF2, and oligomeric lignin, respectively). Therefore, two lignin fractions exhibit high potential as UV-absorbing component (especially against UVB radiation), for example in cosmetics and polymers, without sacrificing the visible light transparency, as was demonstrated by addition of LF2 to the poly(propylene carbonate) film (Figure S5c).

FTIR spectra of both lignin fractions showed typical peaks of lignin (Figure S6). The most notable difference between the two lignin was the presence of C=O stretching of conjugated carbonyls at wavenumber of 1651 cm^{-1} in the spectrum of LF1. The peak of conjugated carbonyl groups is only slightly visible in the spectrum of LF2, which could explain the different light absorption properties of two lignin samples. The conjugated carbonyls are identified as one of the main light absorption functional moieties in lignin, and therefore LF1, which exhibited higher quantity of conjugated carbonyls, showed elevated absorption at both UV and visible light region.

The common features of ^{1}H NMR spectra of both lignin fractions are regions of phenolic (8.5–9.2 ppm), aromatic (6–7.5 ppm), methoxy (3.5–4 ppm), and aliphatic (0.5–3.1 ppm) protons (Figure

S7). The strong signal is observed around 1.1 ppm, which could originate from CH_3-protons of isopropyl group of thymol, indicating that both lignin fractions are modified with thymol during the fractionation process (similar to phenolation of lignin(3)). The broadening of the aliphatic signals in both lignin samples compared to pure thymol indicates that chemical environment of these groups is altered due to the addition of thymol to lignin. Furthermore, although the small peaks of aldehyde around 10–10.5 ppm can be seen in spectrum of LF2, in case of LF1, these peaks are more notable in case of LF1, which is in agreement with FTIR data.

The thymolation of lignin is also evident in the Heteronuclear single quantum coherence spectra of both lignin samples (Figure S8). Compared to HSQC spectra of pure thymol, the absence of peak (δ_C/δ_H 126.00/6.96) related to the proton at meta-position compared to hydroxyl group, indicates that this position might be the main reaction side between thymol and lignin. However, as described by the phenolation of lignin(4), formation of several different type of addition product can be expected and reaction of lignin with different position of aromatic ring of thymol cannot be excluded.

Typical peaks of methoxy group of lignin can be seen in both samples, however, the absence of characteristic β-O-4′ substructure (δ_C/δ_H 59.5/3.5(5)) indicates that this bond is either cleaved or chemically modified during the delignification. The cleavage of the β-O-4′ is backed by the low molecular weight of both lignin samples.

Nanocellulose from delignified cellulose fraction

Ultrasonication of cellulose in water at consistency of 0.5 wt.% resulted in formation of highly gel-like dispersion (Figure S9a and c). Nanocellulose showed elongated structure with diameter of few nanometer as can be seen in TEM image presented in (Figure S9b). The film obtained by vacuum filtration exhibited very high transparency at visible light region (Figure S9d).
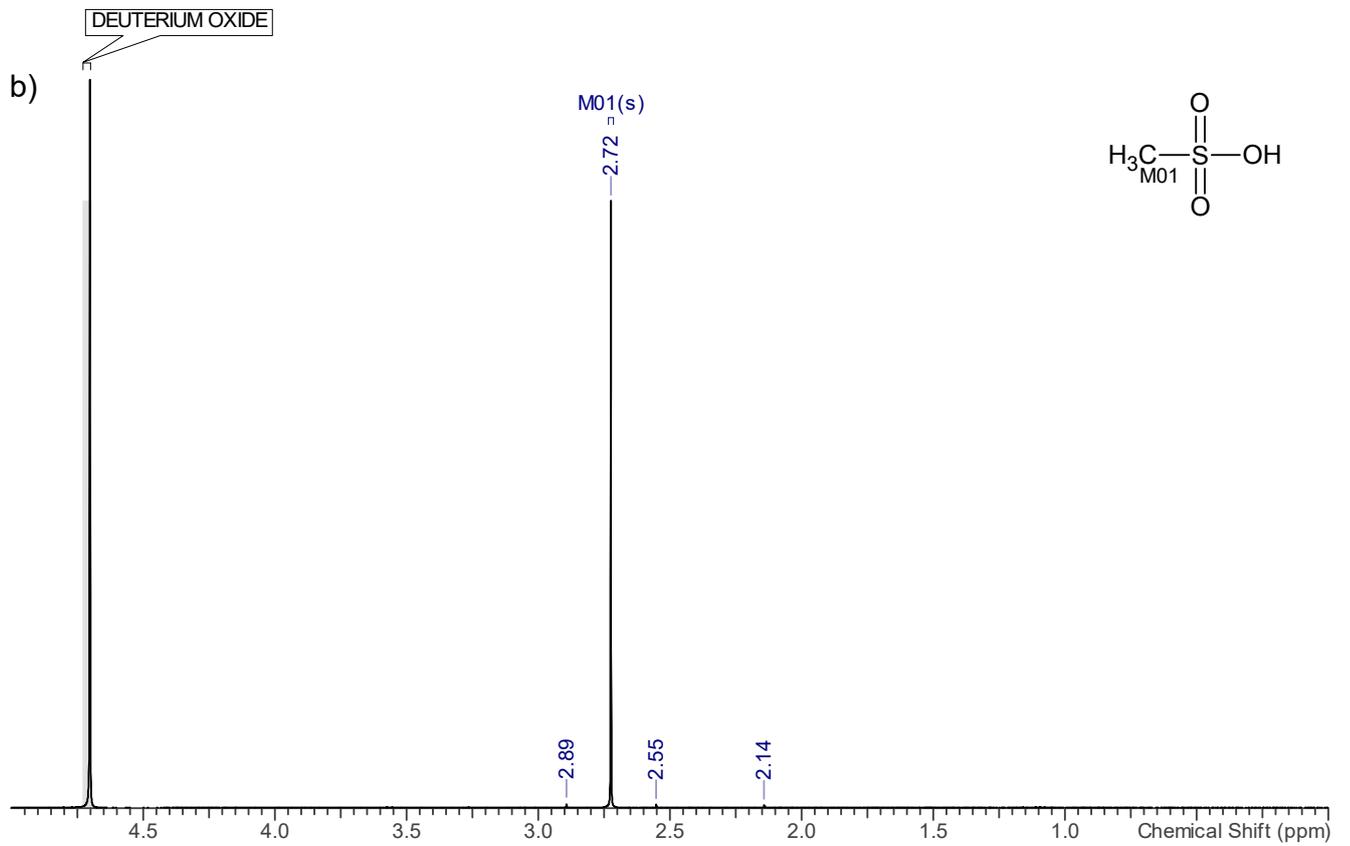
a)

M01(s)

2.74

DEUTERIUM OXIDE

2.90 2.56

H₃C—S—OH (methanesulfonic acid structure, M01)

4.5   4.0   3.5   3.0   2.5   2.0   1.5   1.0   Chemical Shift (ppm)

b)

DEUTERIUM OXIDE

M01(s)

2.72

2.89 2.55 2.14

H₃C—S—OH (methanesulfonic acid structure, M01)

4.5   4.0   3.5   3.0   2.5   2.0   1.5   1.0   Chemical Shift (ppm)
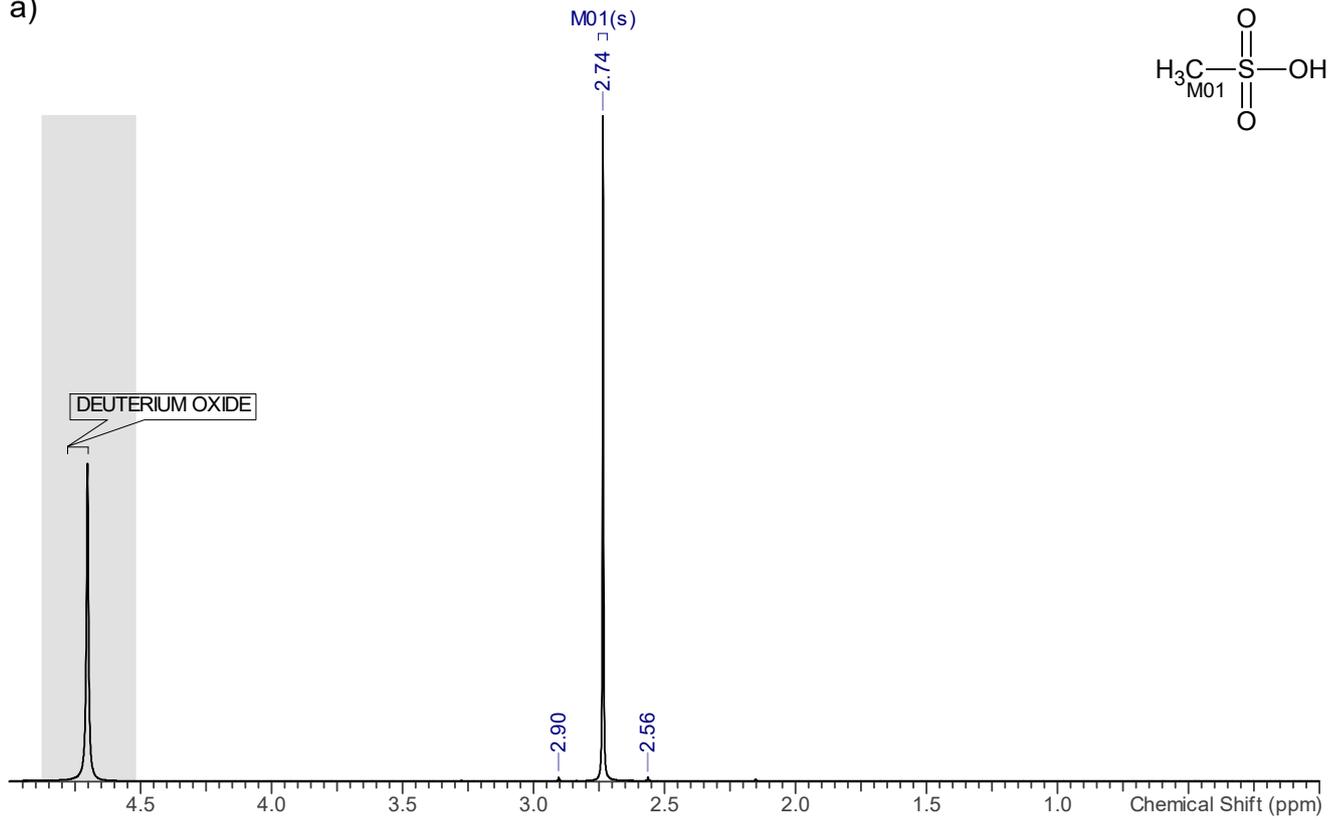
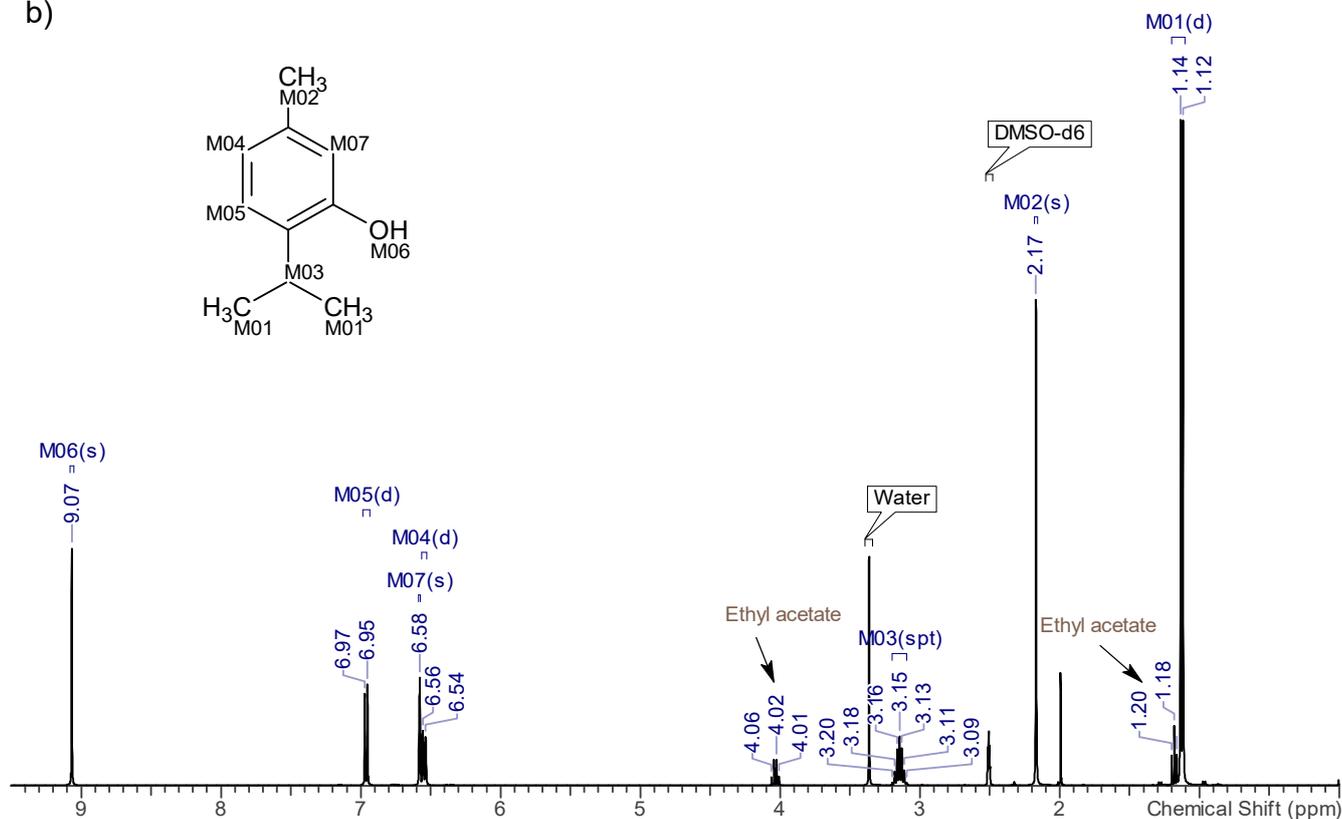**Fig. S1**. ^{1}H NMR spectra of a) commercial sodium methanesulfonate and b) sodium methanesulfonate precipitated from washing liquor with NaOH demonstrating high purity.
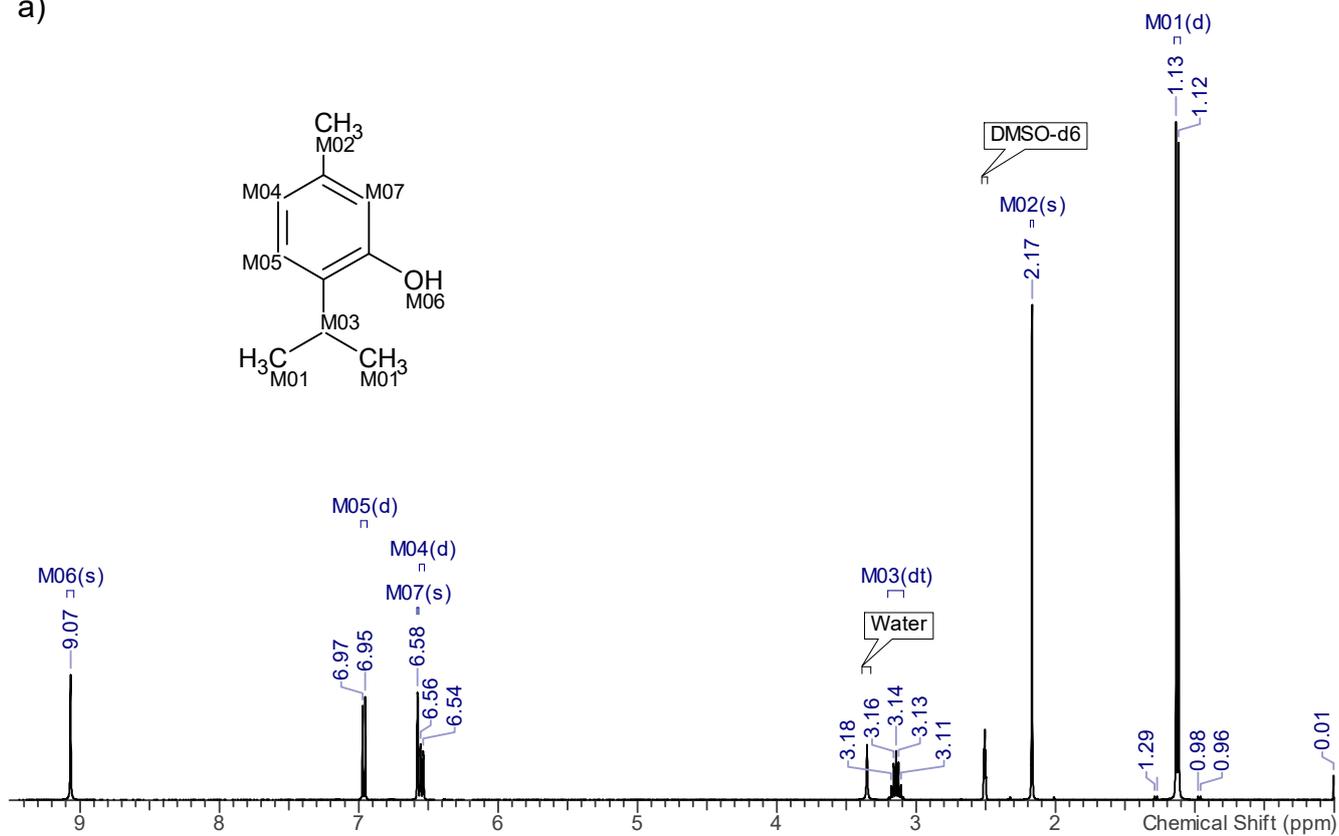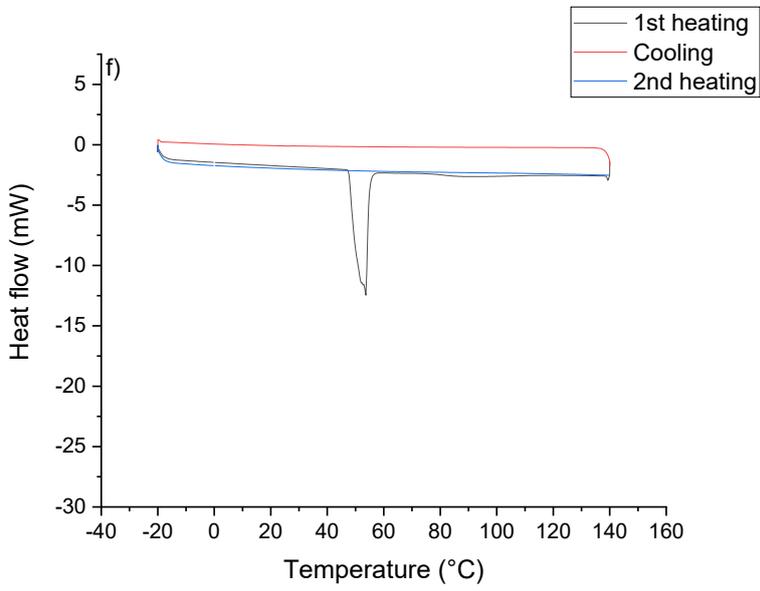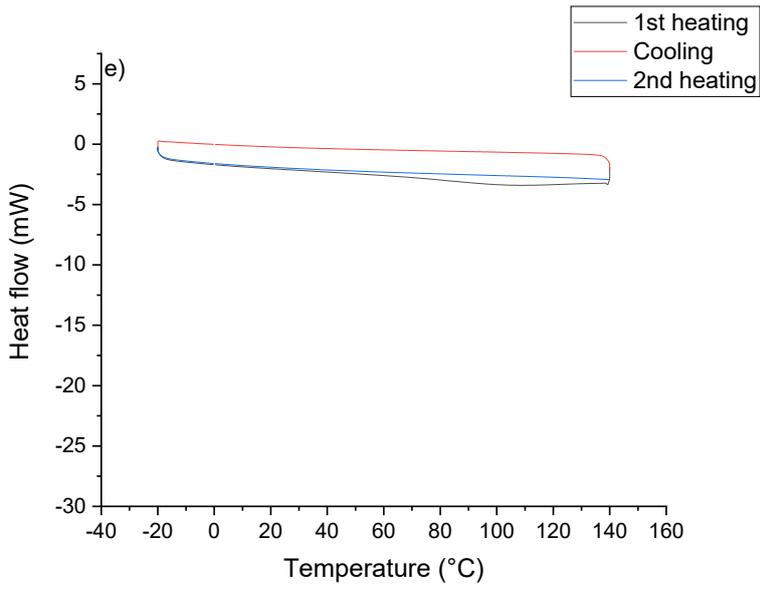
Fig. S2. ^{1}H NMR spectra of a) commercial thymol and b) thymol isolated from washing liquor by column chromatography with eluent (ethyl acetate) as the main impurity.

Fig S3. Mixture of oligomeric lignin and thymol at a mass ratio of 1:2 at room temperature, demonstrating the liquid appearance of the mixture.
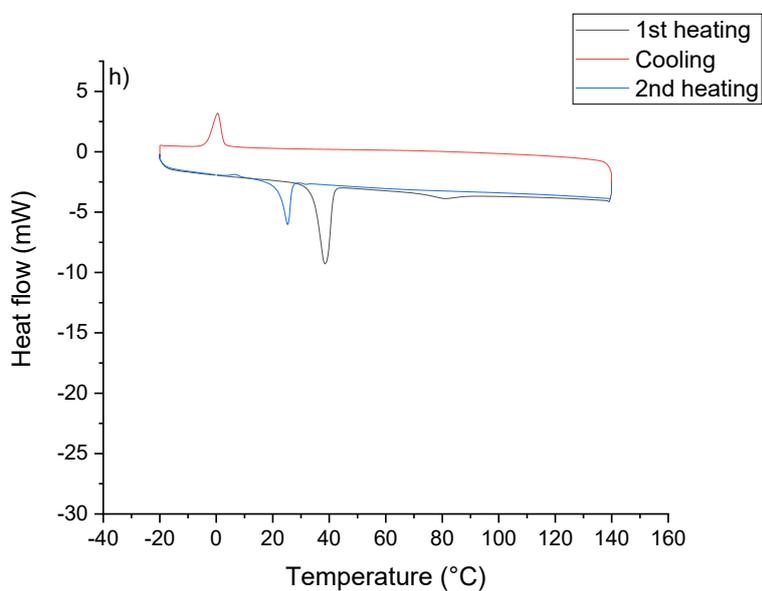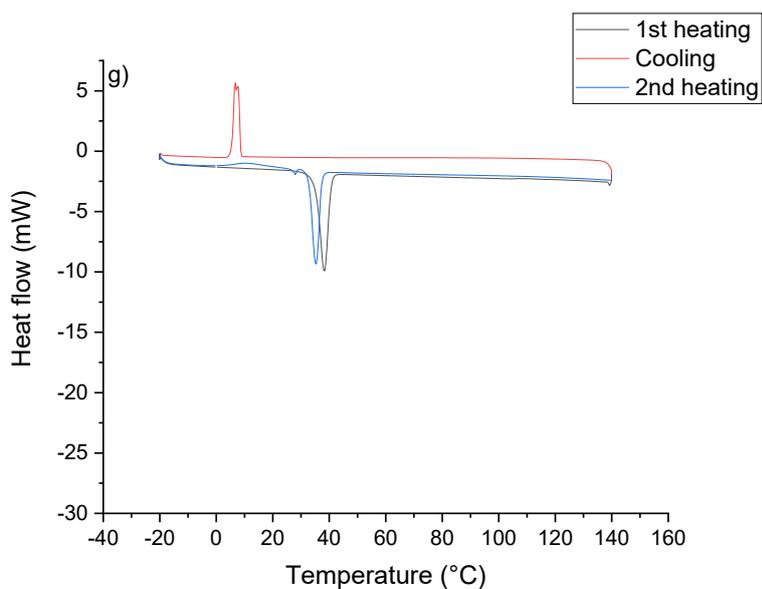
c)

d)

e)

f)
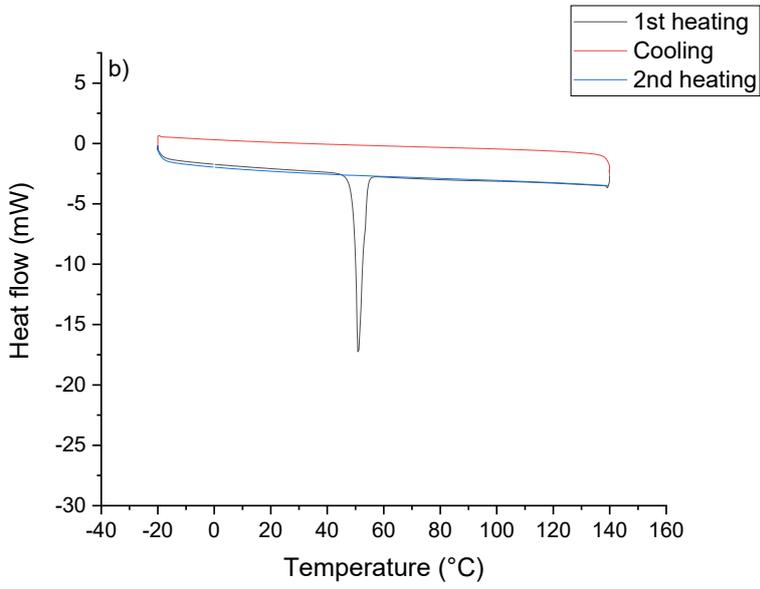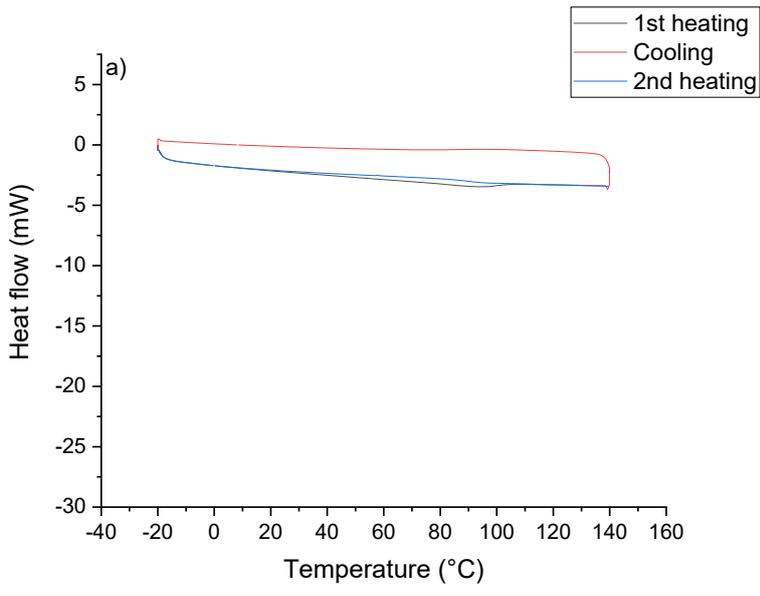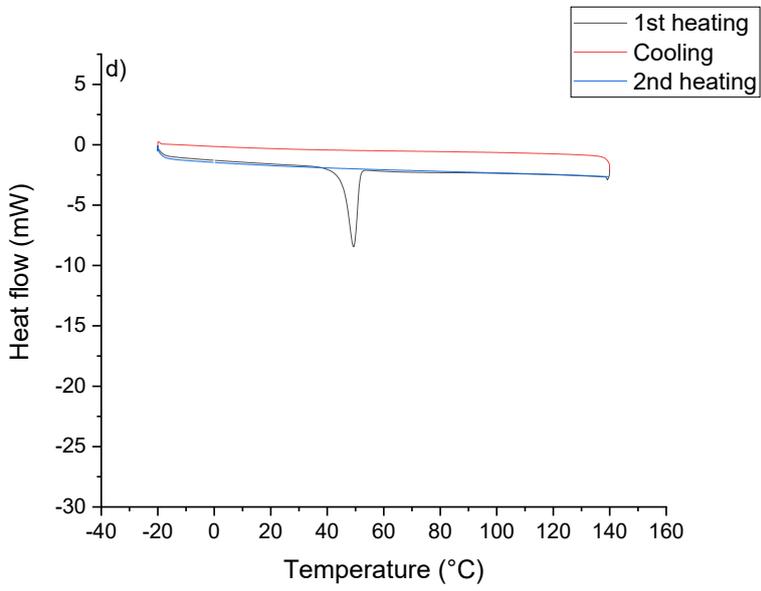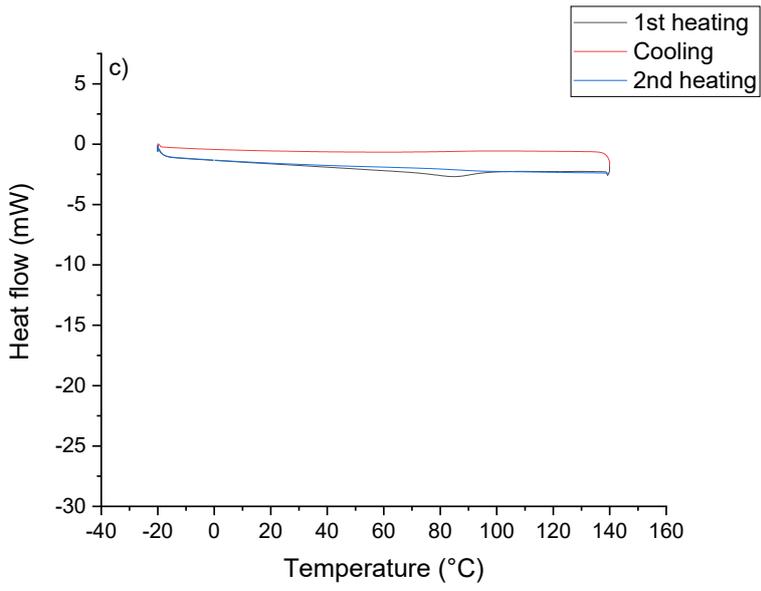
Fig. S4. Differential scanning calorimetry curves of a) isolated lignin (LF2), b) LF2-thymol, c) oligomeric lignin, d) oligomeric lignin-thymol, e) kraft lignin, f) kraft lignin-thymol, g) menthol, and h) oligomeric lignin-menthol. The mass ratio between lignin and thymol/menthol was 1:2.
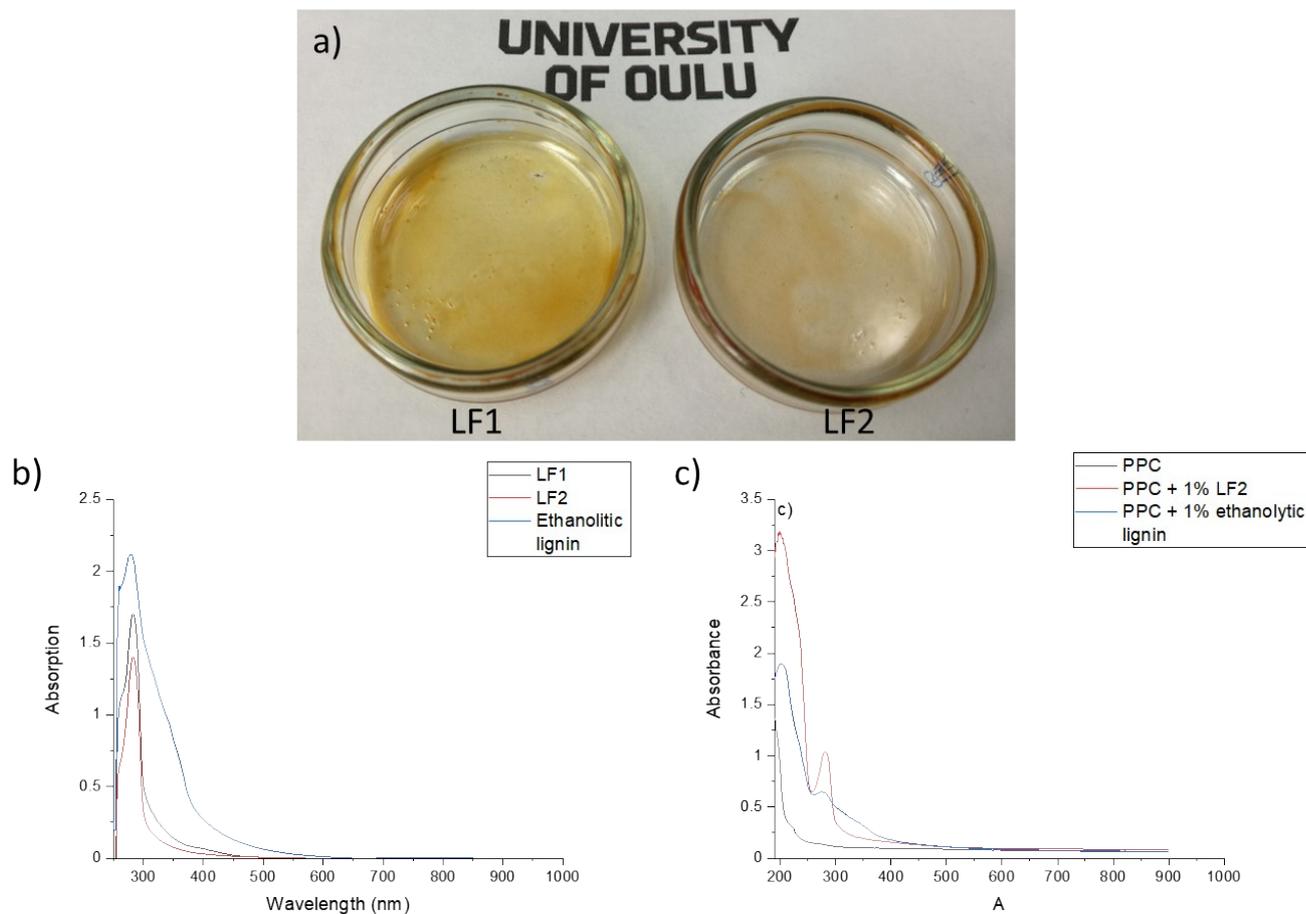
Fig. S5. a) visual appearance of two lignin fractions cast on the glass from ethyl acetate, b) UV Vis spectra of two lignin fractions obtained from washing liquor by column chromatograph and ethanolitic lignin as comparison (0.01 wt.% in DMSO), and c) UV Vis spectra of poly(propylene carbonate) (PPC) films with 1 wt.% of LF2 or ethanolytic lignin presenting that lignin isolated with MSA-thymol exhibits excellent UV absorption properties with minimal absorption at visible light region.
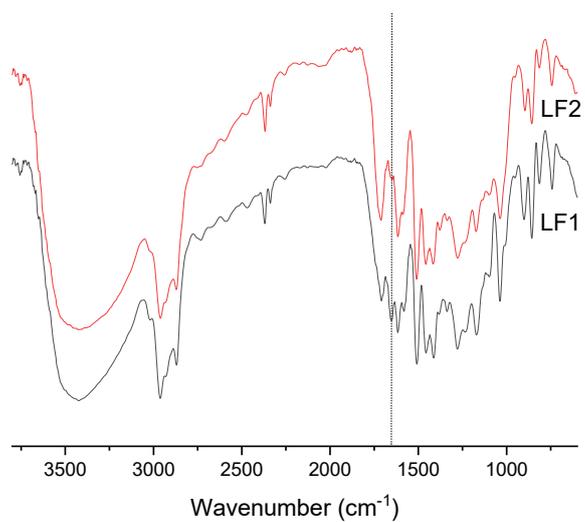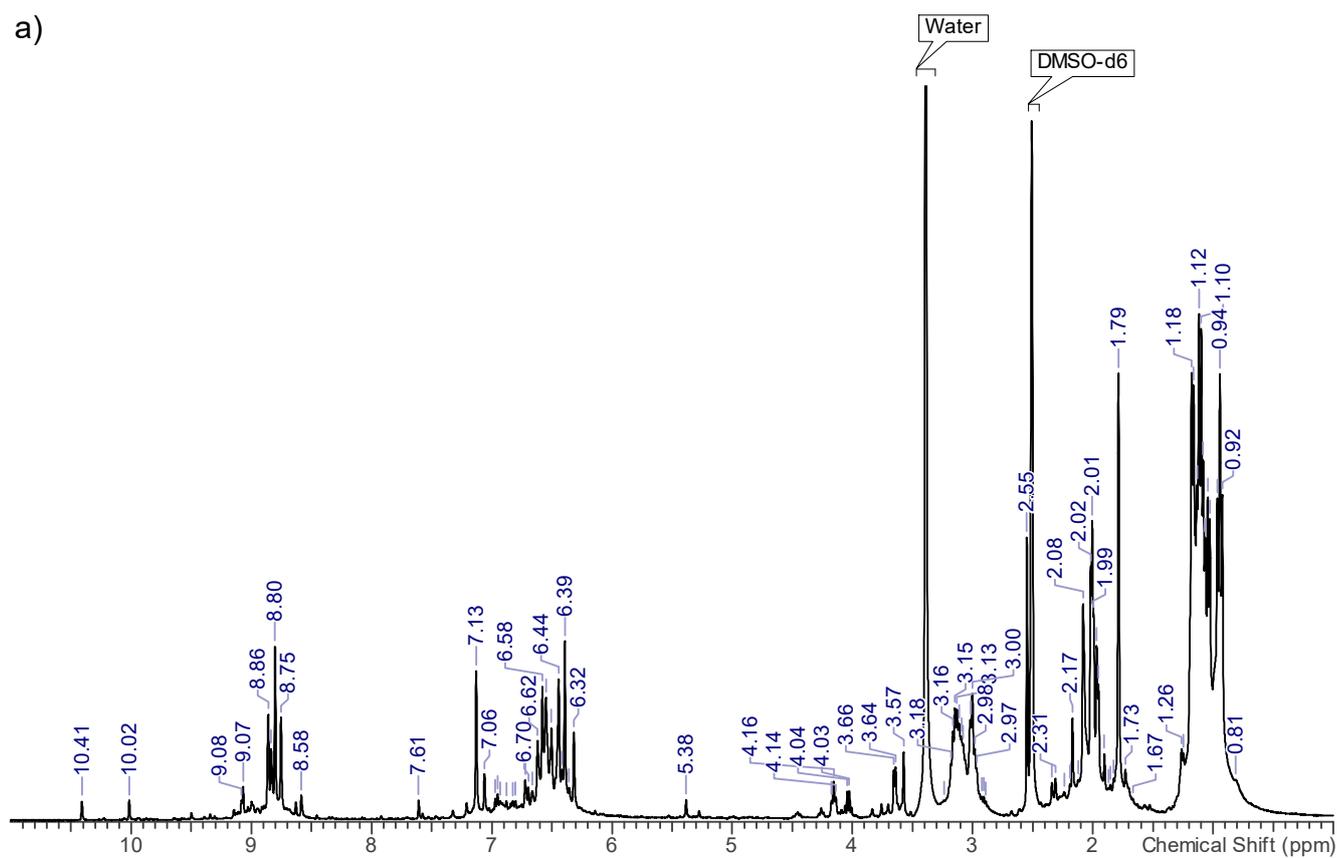
Fig. S6. FTIR spectra of two lignin fractions. Dashed line indicates C=O stretching of conjugated carbonyls at wavenumber of 1651 cm^{-1}.

a)

b)
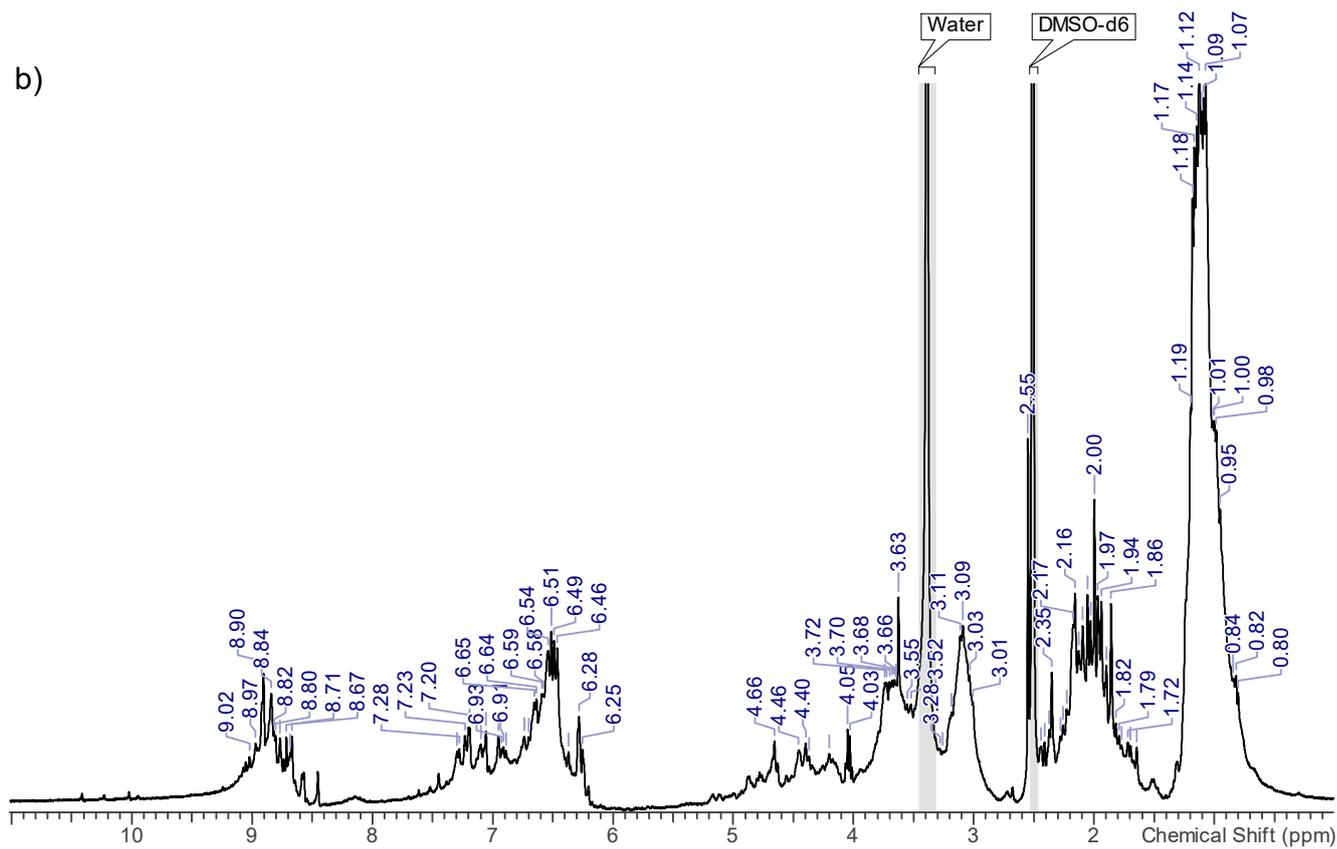
Fig. S7. ^{1}H NMR spectra of two lignin fractions: a) LF1 and b) LF2

a)

LF1

1.95, 15.25
2.00, 19.04
1.18, 14.65
1.08, 23.00
3.09, 26.40
2.16, 21.16
1.78, 19.09
3.64, 35.28
3.01, 40.27
2.07, 19.28
4.15, 42.46
2.50, 40.15
2.54, 40.77
3.65, 56.02
3.57, 56.33

LF2

1.78, 19.34
2.02, 19.67
1.74, 19.24
1.08, 23.03
3.08, 26.47
2.34, 19.67
2.44, 20.12
3.66, 55.89
2.50, 40.21
2.54, 41.02

Thymol

2.17, 21.02
1.13, 22.98
3.15, 26.32
DMSO-d6

b)

LF1

6.41, 116.58
6.54, 116.97
6.69, 117.93
7.06, 125.16
7.12, 125.16
6.50, 126.60
6.30, 128.05
6.61, 127.11

F2 Chemical Shift (ppm)

LF2

6.20, 116.45
6.26, 116.52
6.47, 116.87
6.34, 116.60
7.19, 126.25
7.24, 127.34

F2 Chemical Shift (ppm)

Thymol

6.58, 115.88
6.54, 120.03
6.96, 126.00

F2 Chemical Shift (ppm)

Fig. S8. HSQC spectra at a) aliphatic and b) aromatic regions of LF1, LF2, and thymol

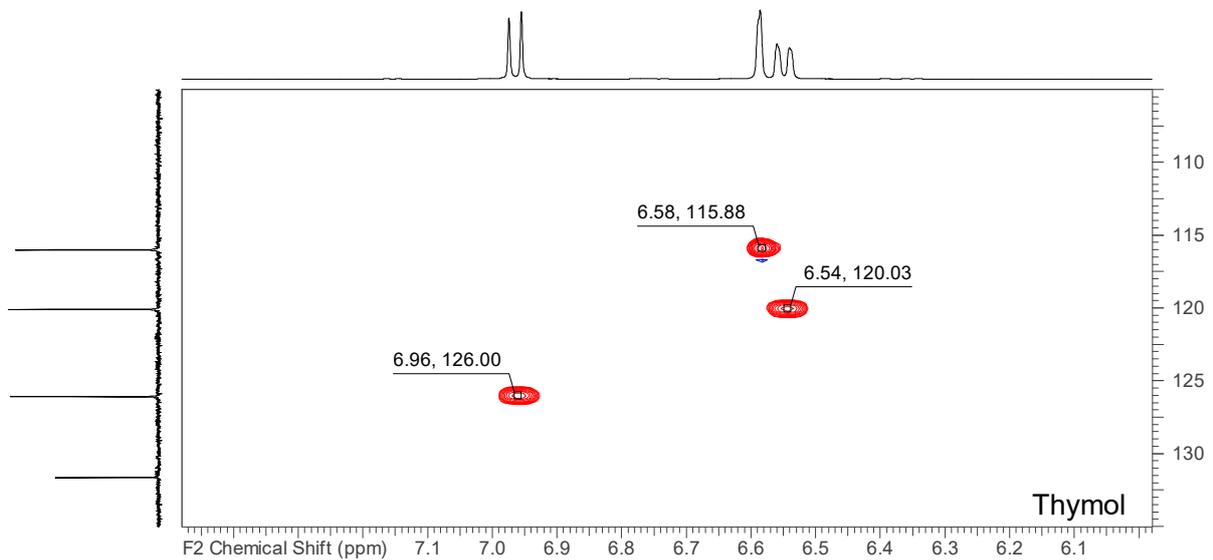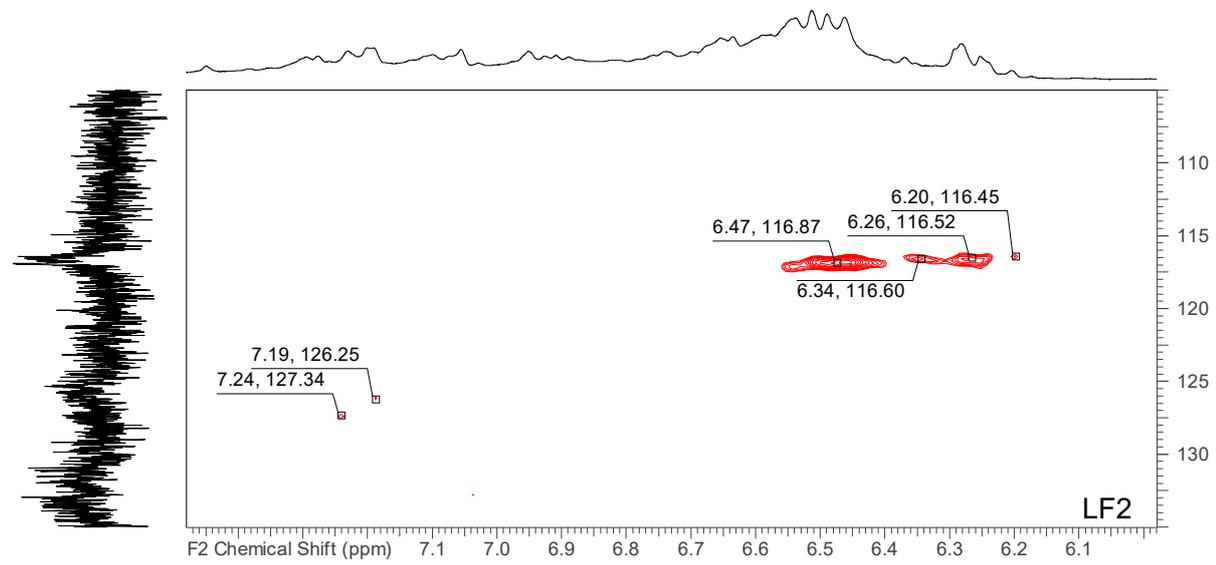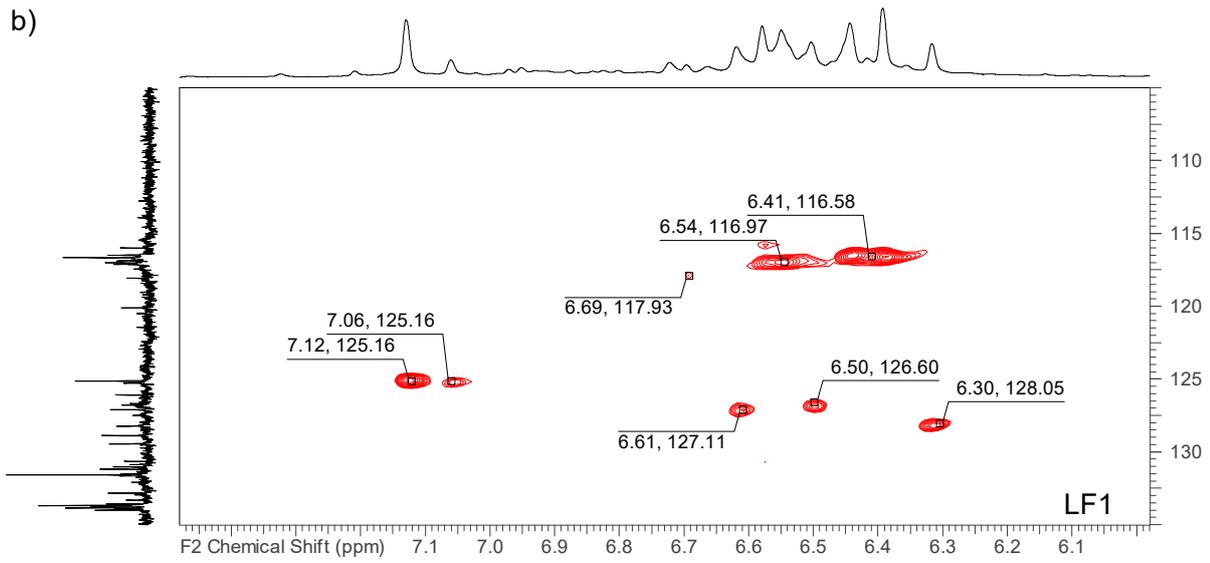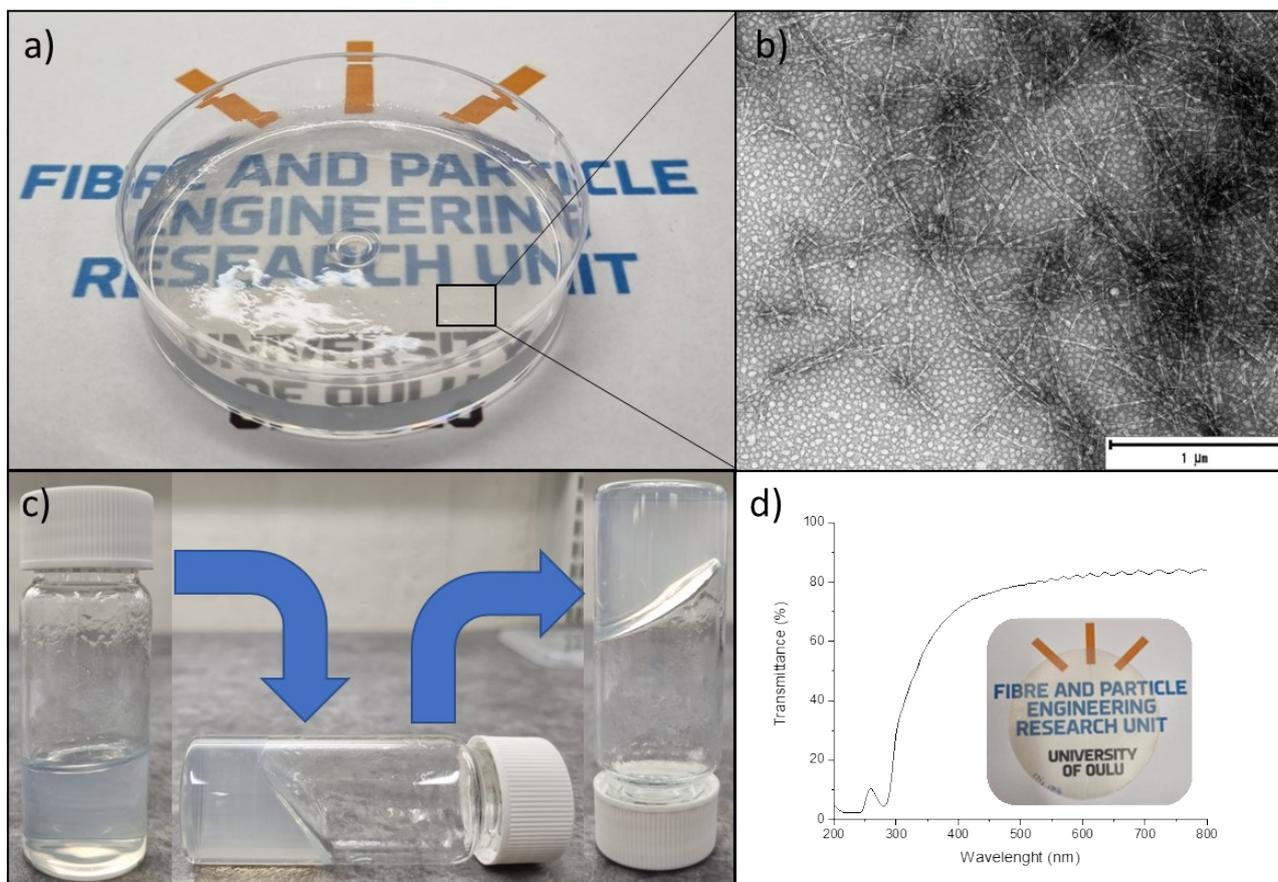**Fig. S9**. a) Nanocellulose solution (0.5 wt.% in water) on the Petri dish demonstrating high transparency and b) transmission electron microscope image of nanocellulose; c) gel-like appearance of nanocellulose solution demonstrated in glass bottle; d) UV-Vis spectrum of nanocellulose film

Table S1. The weight and number average molecular weight and polydispersity index of two lignin fractions.

Lignin	The weight average molecular weight	Number average molecular weight	Polydispersity index
LF1	790	580	1.4
LF2	1900	1090	1.7

Table S2. Comparison of the delignification experiment conducted in this study with delignification of softwood and hardwood reported in literature.

	Wood	Chemistry	Temperature (°C)	Time (min)	Recidual lignin	Lignin removal	Reference
Softwood	Spruce	MSA-Thymol	40	5	4.8		This work
		MSA-Thymol	50	2.5	4.6		This work
		MSA-Thymol	60	5	3.0		This work
		Choline chloride-lactic acid	120	180	6.8		This work
		p-toluenesulfonic acid	80	20	18		This work
		4-hydroxybenzenesulfonic acid	60	120	5.9		This work
		4-hydroxybenzenesulfonic acid	60	5	15		This work
	Radiata pine	p-toluenesulfonic acid	80	60		42.15	(6)
	Douglas fir	Choline chloride – lactic acid	145	540		58.2	(7)
	Hydrothermal treated pine	Choline chloride – lactic acid	130	180		26.4	(8)
	Spruce	Peracetic acid	85	45 (multiple times)	2.3		(9)
	Spruce	Peracetic acid	70	1500	1.28		(10)
	Radiata pine	Benzyltrimethylammonium chloride - formic acid	150	120	7.41	87.0	(11)
Hardwood	Poplar	p-TsOH	80	30		73.77	(6)
	Poplar	Choline chloride – lactic acid	145	360		78.5	(7)
	Hydrothermal treated poplar	Choline chloride – lactic acid	130	180		70.5	(8)
	Poplar	Choline chloride – glycerol - AlCl$_3$	110 (microwave)	10	2.25		(12)
	Eucalyptus	γ-valerolactone - water	180 (microwave)	120	7.1		(13)
	Aspen	γ-valerolactone – water - sulfuric acid	125	180	1.5		(14)

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