Mild Organosolv Lignin for Enhancing UV Protection in Sunscreen

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1. Abstract

Value-added applications for lignin are of immediate interest given its immense untapped potential as a renewable alternative to unsustainable petroleum-based platform chemicals. The evaluation of lignin as a bio-based sun blocker pushes it into high-end utilisation but this is limited by its darkening due to typically harsh industrial extraction conditions. In this work, a mild organosolv extraction procedure (40 - 60˚C for 16 h) is used to obtain relatively lighter lignin samples that are analysed by extraction efficiency, UV-Vis spectrophotometry, 2D HSQC NMR spectrometry and GPC. The most promising samples are blended with pure commercial cream and evaluated based on colour and reduction of solar UV transmittance. The ethyl acetate extraction had the highest extraction efficiency (21.5%) and one of the lightest coloured samples. The most optimal cream blend is determined to be the propyl acetate/propanol extracted lignin that increased the Solar Protection Factor of the 1 wt% blend to 15.4, confirming that extraction conditions can be tuned to obtain lignin more suitable for sunscreen incorporation.

2. Introduction

Long-term exposure to ultraviolet radiation can have detrimental effects on the eyes, skin and general health. Frequent sunscreen use is vital for protection against a variety of skin diseases that are a potential consequence of unprotected exposure to the sun. Based on the wavelength, UV radiation is classified into three main categories; UV-A, UV-B and UV-C. UV-C rays cannot pass through the Earth's ozone layer. Notably, UV-B (290-320 nm) is the most harmful and UV-A (320-400 nm) is the most penetrating with significant effects on the epidermis layer of the skin[5].

Two categories of sunscreen currently dominate the market: physical and chemical. The physical variety works using the reflection mechanism and contains mainly zinc oxide and titanium dioxide. There are no health drawbacks with this type but there is commonly discomfort associated with its use. Chemical sunscreens eliminate this problem but their incorporation of synthetic chemicals can have harmful effects on skin tissue over time[4]. Thus, it is of interest to explore naturally sourced alternatives that may function as UV-blocking agents in sunscreen.

The valorization of biomass sources has grown in importance due to the depletion of fossil-based fuels over the years. As one of the most abundant biobased polymers, lignin has attracted much attention in the chemical industry to move forward in a sustainable, environmentally friendly direction while reducing dependence on petroleum-based products. Despite there being a magnitude of about 10^8 tons of lignin produced naturally and more than 50 million tons of industrial lignin produced per annum, lignin has never been used as an ingredient in pharmaceuticals or cosmetics due to health concerns[4]. Recent studies demonstrated that lignin is not cytotoxic[1], allowing for potential high-end utilization. A relatively unexplored application is the inclusion of lignin in sunscreens due to the UV-blocking properties of the aromatic rings present in its structure[2].
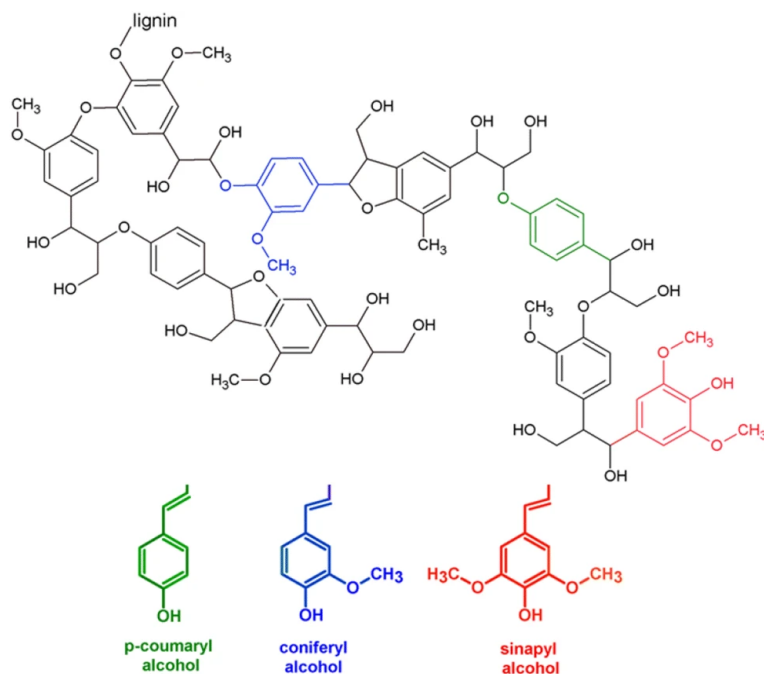
Figure 1: Representative fragment of lignin structure with its three main monolignols (reproduced from Ref 12)

Lignin is the most abundant component of biomass and is composed of three major aromatic building blocks (Figure 1). These varieties of phenylpropane units lend to the aromaticity of lignin and subsequently its broad range UV-blocking abilities. This is significant because most natural UV-blockers offer only partial protection and are unable to block the full UV range. Lignin is also rich in other UV blocking functional groups such as ketone, phenolic and other chromophores. Phenolic groups in particular have a free radical scavenging ability, giving antioxidant properties to lignin in addition to the UV absorption that makes it suitable for applications that require UV blocking. Nevertheless, this potential is hindered by the dark colour of the industrial lignin leading to a sunscreen that would be difficult to market to the general public[4].

Two factors that greatly influence the colour of lignin are the micromorphology and extraction conditions. In terms of micromorphology, it was seen that lignin with finer particles and lower bulk density resulted in a higher colour performance[3]. Milder extraction procedures are also key to reducing darkening of lignin during recovery and this entails using relatively lower temperatures and neutral solvents, for example. Despite being almost colourless in wood, industrial or "technical" lignin such as Kraft lignin, alkali lignin or lignosulfonate typically has a dark brown color because various types of chromophores are introduced into the structure via the harsh lignin isolation procedure and pulping process. The extent of process modification depends on the process severity (i.e., pH, temperature, cooking duration and pulping concentration).[14-15] The use of milder conditions allow for retention of the native C-O bonds and β-ether units instead of forming the complex, condensed C−C bonded chemical structures that arise during harsh lignin fractionation which result in a much darker lignin. However, these milder conditions usually require a trade-off with yield/extraction efficiency as higher temperatures usually allow for more efficient

delignification. Thus, various combinations of extraction conditions can be employed in order to find a good compromise between colour and lignin yield.

Using alcohols and/or acetates as extraction solvents is of interest due an alkoxylation modification that results in the incorporation of the β'-O-4 linkage (Figure 2) which may allow the extraction of larger lignin fragments by protecting the structure from degradation and subsequent condensation that leads to darkening. It also increases the solubility of the lignin in the solvent by replacing the α-OH group with an aliphatic tail, which should theoretically improve the extraction efficiency.
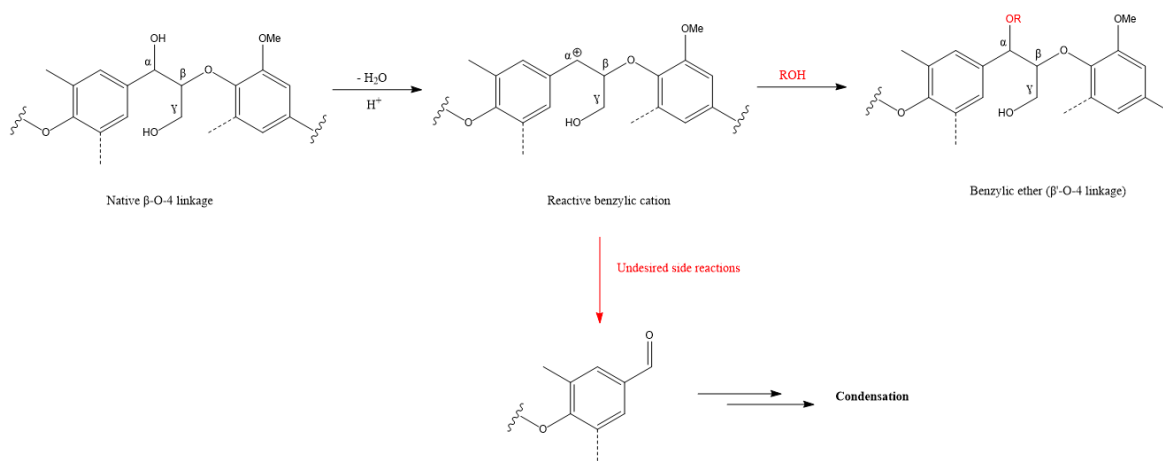

Figure 2[16]: Reaction scheme displaying alkoxylation modification for alcohol extractions (reproduced from Ref 16).

For acetate extractions, the first step is acetylation of the γ-OH group which releases a methyl alcohol that may in turn react with the acetylated lignin as above to form this β'-O-4 linkage.
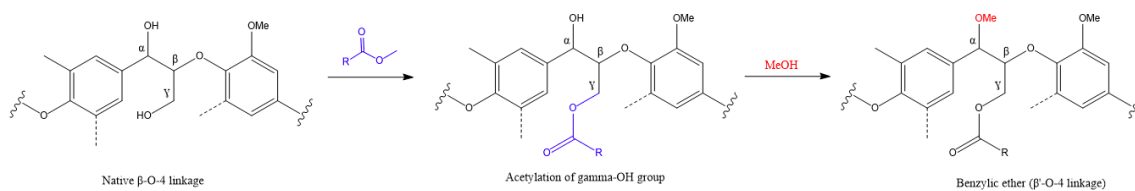

Figure 3: Reaction scheme displaying acetylation modification for acetate extractions.

The effect of these modifications on UV absorbance has yet to be explored in literature; hence, the purpose of this work is to examine whether there is a link between extraction conditions and potential sunscreen performance. Mild organosolv lignin extracted from walnut using different extraction conditions is compared in terms of colour, yield, and UV performance. Based on initial data, a select number of samples were evaluated on UV protection and colour upon addition to pure cream in an attempt to determine the most suitable combination for an effective lignin-based sunscreen.
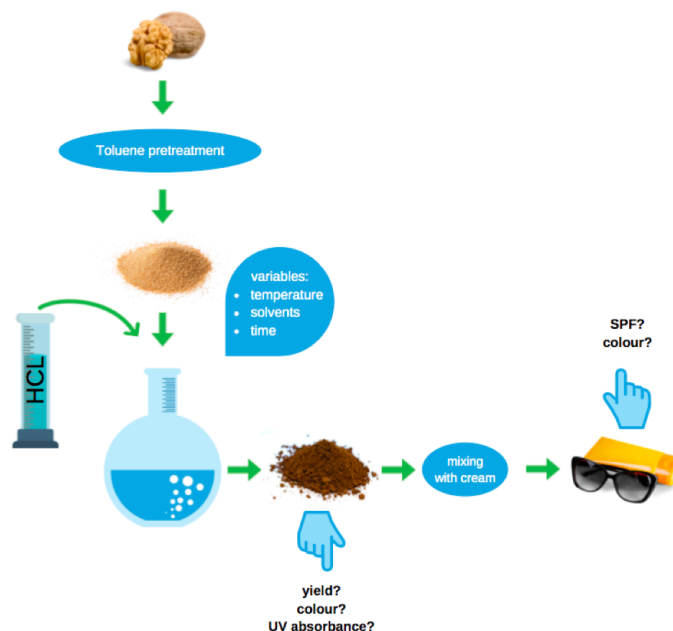
Figure 4: Schematic representation of performed experimental work including pretreatment, extraction, sunscreen blending, and points of analysis.

3. Experimental Methods[8][9]

3.1 Pretreatment of Feedstock for Fatty Acid Removal

Commercial walnut powder was obtained from Bramble Berry. 150 g of the walnut powder was added to a 500 mL round-bottom flask followed by a magnetic stirring egg and 200 mL of toluene. The flask was equipped with a reflux condenser and the mixture was heated at 110˚C using an oil bath for 2 h with vigorous stirring. The mixture was allowed to cool to room temperature. The toluene was removed by vacuum filtration and discarded. The toluene residues were removed by heating the walnut shells overnight in a vacuum oven at 80 ˚C and 50 mbar.

3.2 Typical Mild Organosolv Extraction Procedure

20 g of pretreated finely ground walnut shells were placed in a 250 mL round-bottom flask equipped with a magnetic stirring egg. Into this, 200 mL of solvent in total and 4.8 mL of (10 M) aqueous HCl solution was added and the setup was equipped with a reflux condenser. The mixture was stirred and heated with an oil bath to the desired temperature. After the required extraction time, the mixture was allowed to cool to room temperature, the extract was recovered by vacuum filtration and concentrated by rotary evaporation. The obtained solid was redissolved in a minimal amount of acetone and the lignin was subsequently precipitated by addition to 300 mL of water. 50 mL of saturated aqueous Na_2SO_4 was added to improve flocculation. The product was collected by filtration, and air-dried overnight to obtain organosolv lignin as a powder.

3.3 Preparation of UV-Vis samples

5 mg of dried lignin was added to an aspirin tube containing 4 mL of THF. 1 mL of this solution was added to another aspirin tube containing 5 mL of THF and this was repeated. Finally, 1 mL of the 3rd solution was added to 4 mL of THF and this was added into a quartz cuvette to measure the UV absorbance using a UV-Vis Spectrophotometer between 250-400 nm.

3.4 Mixing of Lignin into Pure Cream

5 g of commercial Nivea cream was placed in centrifuge tubes. 0.1, 0.5, and 1 wt% samples were prepared for each lignin and overhead stirrers were used to mix the creams with the lignin at 300 rpm for 20 h. The colours of the mixture varied from light brown/pink to medium brown as the lignin content was increased (Figure 11).

3.5 Film application

Quartz plates with the appropriate dimensions to fit into the UV-Vis spectrophotometer were cut. A film applicator was placed atop two plates and around 4 mg/cm^2 of cream was applied evenly onto a third plate using a 1 mL syringe. This plate was then passed under the film applicator. The layer was allowed to dry for 20 minutes before the transmittance was measured for the range of 250-400 nm. The same plate was used for every measurement to ensure uniformity in the thickness of each film.
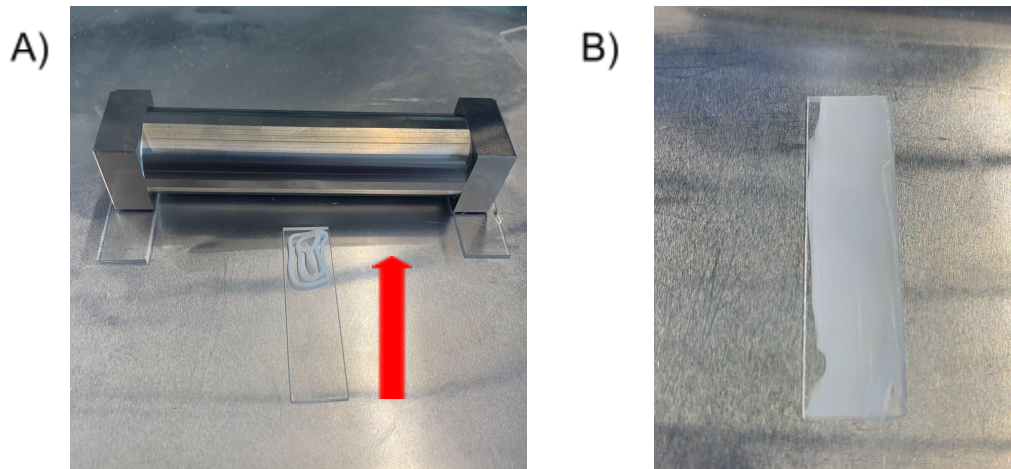

Fig. 5A/B: Use of film applicator to evenly coat quartz plates with cream mixtures

3.6 Two-dimensional nuclear magnetic resonance (2D-NMR) analysis

50 mg of dried lignin was dissolved in 0.7 mL of d6-acetone. A few drops of D_2O were added if the lignin did not fully dissolve. The mixture was put into an NMR-tube and take a 2D proton heteronuclear single quantum coherence spectra (HSQC) with an NMR spectrometer with the following parameters: (11, -1),

(160, -10), nt = 4, ni = 512. The obtained HSQC spectra were analysed by integrating the signals in the aromatic region that correspond to the three different aromatic units (protons numbered as per Figure 7).

These signals are in the region [(proton range)(carbon range)]:
$S_{2/6}$: [(6.48-6.90)(104-109)]
$S'_{2/6}$: [(7.17-7.50)(105-109)]
$S_{condensed}$: [(6.35-6.65)(106-109)]
G_2: [(6.78-7.14)(111.5-116)]
G_5: [(6.48-7.06)(115-120.5)]
G_6: [(6.65-6.96)(120.5-124.5)]
$H_{2/6}$: [(7.05-7.29)(128.5-133)]

The signals in the aliphatic region that correspond to the β-O-4 linkages were integrated. These are in the region [(proton range)(carbon range)]:
β-O-4α [(4.76-5.10)(73-77.5)]
β'-O-4α [(4.44-4.84)(81.5-86)]

The total number of linkages per 100 C9 units were based on the signal of the α proton of the linkages. The total number of linkages was calculated with the following formulae:
β-O-4 linkages = (β-O-4α + β'-O-4α) / total aromatic x 100
β-O-4 linkages = β-O-4 / total aromatic x 100
β'-O-4 linkages = β'-O-4 / total aromatic x 100

3.7 Gel permeation chromatography (GPC) analysis

2 mg of dried lignin was dissolved in 1 mL of tetrahydrofuran (THF). This mixture was filtered through a 0.45 µm syringe filter into an autosampler vial with a reduced volume inlet of 0.3 mL. A drop of toluene was added as an internal standard. The autosampler vial was sealed with a cap. 0.2 µL of the sample was injected into a THF GPC. The obtained signal was corrected for the reference signal. The elution volume was selected for the appropriate range (~200-10000 Da). The mass distribution was calculated by the software.

4. Results and Discussion

Extraction efficiency is an important indicator of the feasibility of implementing the process on an industrial scale. Therefore, this is used as the first criteria to ascertain the samples that were worth exploring further in order to optimize the extraction conditions. Table 1 summarizes the yields for every extraction performed. These were calculated assuming a lignin percentage of 40% for walnut shell and 13% for miscanthus[6][7]. Miscanthus biomass extraction was the only one allowed to proceed for 72 h which may account for the much higher yield compared to the other extractions. For walnut shell lignin, the ethyl acetate extraction gave the highest yield (21.5%) followed by the propyl acetate reactions using water and propanol as cosolvents which gave yields of 16.3% and 13.1% respectively. All three of these reactions result in the alkoxylation modification and this appears to improve the extraction yield due to an increase in solubility leading to the isolation of larger fragments. This is further confirmed by the GPC

results (Figure 8) that show larger M_w fragments were recovered for the acetate extractions. In general the extraction efficiencies were somewhat lower than that reported in literature for 80°C, 16 h walnut shell ethanosolv extractions but this could be explained by the higher temperature used. However, the three highest acetate extractions in particular were on par with the literature value of 21.3%.[9] Extraction 3 was repeated due to the use of a water bath leading to an inconsistent temperature overnight.

Table 1: Summary of organosolv lignin extraction yields corrected for lignin percentage by biomass source. [a]extraction efficiency = mass of lignin obtained / (lignin% x mass of starting material)

Exp no.	Source	Solvents	Extraction time/h	Temp./ °C	Mass/g	Extraction efficiency/ %[a]
1	Walnut shell	THF/H$_2$O 9:1	16	60	0.75	9.4
2	Walnut shell	THF/H$_2$O 1:1	16	60	0.12	1.5
3	Walnut shell	EtOAc	16	unknown	1.97	24.6
4	Walnut shell	Acetone/H$_2$O 1:1	16	60	0.15	1.9
5	Walnut shell	EtOAc	16	60	1.72	21.5
6	Walnut shell	PropOAc/H$_2$O 9:1	16	60	1.30	16.3
7	Walnut shell	PropOAc/H$_2$O 9:1	16	40	0.28	3.5
8	Walnut shell	PropOAc/H$_2$O 6:4	16	60	0.17	2.1
9	Walnut shell	PropOAc/propanol 9:1	16	60	1.05	13.1
10	Miscanthus	PropOAc/H$_2$O 9:1	72	60	2.04	78.5

Lignin Analysis

2D HSQC NMR was used to quantify the number of notable linking motifs in the sample to determine the degree of alkoxylation from each different solvent combination used. These results gave an idea of the possible correlation between the linkage number and the yield/colour of the lignin obtained. From the

NMR spectra of the lignins with the highest yields (example shown in Figure 6), the H/G/S ratio and amount of linkages were determined (Table 2). Due to the overlap of the β and γ-protons of the β-O-4 and the β'-O-4 linkage, only the α-protons are considered in the calculations. With regards to literature, the total number of β-O-4 linkages obtained during these extractions were on par if not higher than the range reported for 80˚C 16 h walnut shell ethanosolv extractions (58-68)[9].

Table 2: Summary of results for a selection of mild organosolv extractions on walnut shell powder (60 ˚C for 16h). [a]Determined by HSQC NMR, [b]1.3 correction factor applied.

Exp no.	Solvents	S/G/H ratio (%)[a]	Total β-O-4[a b]	β-O-4[a b]	β'-O-4[a b]
1	THF/water 9:1	55/30/15	57	57	0
5	EtOAc	61/35/4	55	21	35
6	PropOAc/H_2O 9:1	62/33/5	71	28	43
9	PropOAc/propanol 9:1	67/30/3	76	8	68

The THF extracted lignin could be considered unmodified and this is seen in the lack of β'-O-4 linkages compared to the two acetate extractions. Upon the use of acetates as an extraction solvent, the β'-O-4 linkages are indeed introduced due to the alkoxylation modification. The number of β'-O-4 linkages is the highest for the extraction using propanol as a co-solvent as there are two routes to arrive at this linking motif, either through direct alkoxylation by the propanol or via acetylation of the γ-OH which in turn releases an alcohol that reacts with the α-OH to yield the same linkage.

Figure 6 shows the 2D-NMR results for Experiment 6 as an example. The presence of the signal at [4.5, 82] can be attributed to the β'-O-4α linkage. The signal at [4.9, 72] is due to the β-O-4α linking motif and its relatively smaller integral value confirms that a majority of the linkages were alkoxylated during the extraction. Although unexplored in literature, the signal at [1.75, 20] is due to the protons on the acetyl group, confirming that acetylation has taken place during this extraction. This is further consolidated by the lack of such a signal in this region for the unmodified THF extraction sample (Appendix 1).
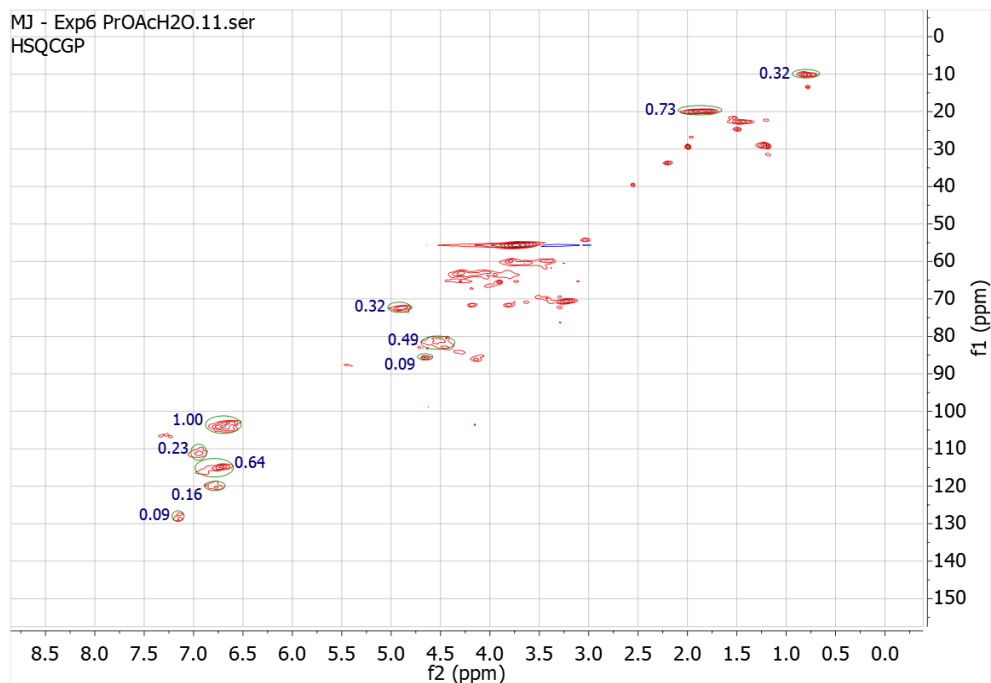
Figure 6: 2D HSQC spectra (d6-acetone) spectrum for propyl acetate/H₂O extracted lignin from walnut powder (60 ℃ for 16h).
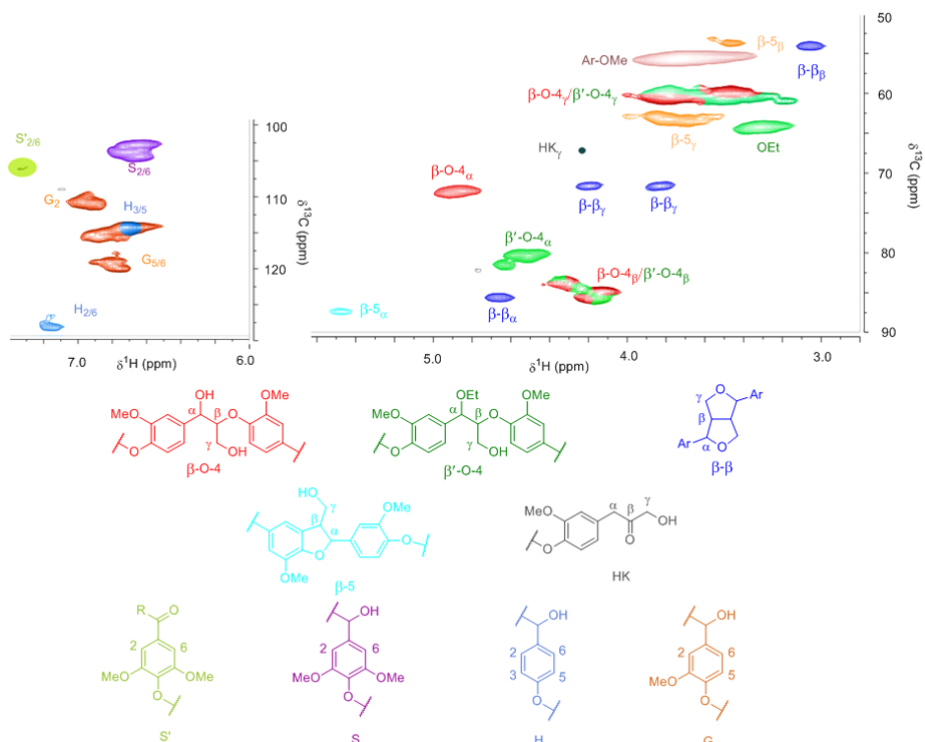

Figure 7: HSQC analysis of lignin. Identification of all lignin linkages measured with 2D-HSQC of lignin obtained from walnut shells using mild treatment (reproduced from Ref 8).

Gel permeation Chromatography (GPC) was used to determine if there is a link between fragment size and the yield/colour of the samples. Most M_w values obtained (Figure 8) were somewhat lower but comparable to the literature values for 80˚C 16 h walnut shell ethanosolv extractions (M_w=2099 Da)[9]. The results pictured indicate that larger lignin fragments are obtained when using acetates as an extraction solvent, likely because solubility is improved as a consequence of the acetylation. Focusing on yield, there is a pattern with the highest M_w samples (i.e., EtOAc, PropOAc) having larger extraction efficiencies. Despite this, there is no clear correlation between colour and the molecular weight. Although the two propyl acetate extractions produced two of the lighter lignin samples, the THF extracted lignin (smallest M_w) was similar to both of these in terms of colour, so it is difficult to draw the conclusion that lighter lignins are linked to larger fragments from these results alone.
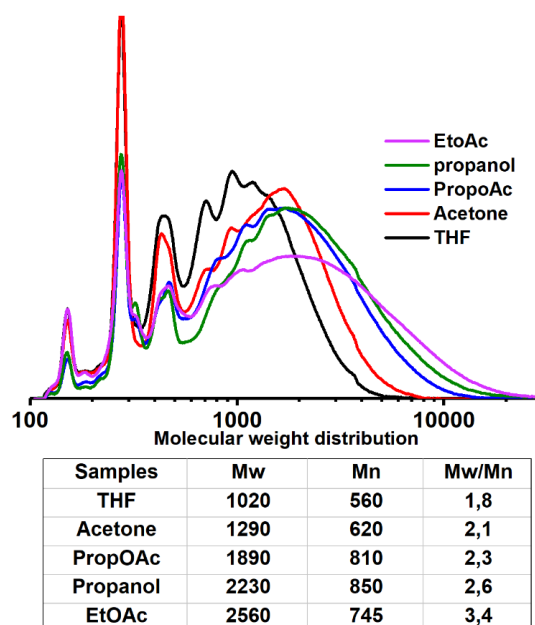

Samples	Mw	Mn	Mw/Mn
THF	1020	560	1,8
Acetone	1290	620	2,1
PropOAc	1890	810	2,3
Propanol	2230	850	2,6
EtOAc	2560	745	3,4

Figure 8: GPC results for for a selection of mild organosolv extractions on walnut shell powder (60 ˚C for 16h)

Extracted Lignin UV-Vis analysis

UV-Vis analysis was used on the lignin samples to give an initial indication of the most suitable candidates to incorporate into the sunscreen because a sample that performs poorly at this initial test is unlikely to improve the UV absorbance of pure cream significantly. Upon analysis of the newly extracted lignin samples, lignin extracted from using propyl acetate and propanol as cosolvents was superior in terms of UV absorbance in the UV-C range. As the other two acetate extracted lignin performed the worst, this may mean the presence of the alcoholic cosolvent and by extension the much higher number of β'-O-4 linkages (Table 2) may improve UV absorbance in this range. For the walnut shell source, the unmodified THF extracted lignin has the best absorbance in the UV-B range, although this difference is quite small so it is difficult to link the unmodified nature of this lignin to its UV performance in this range.
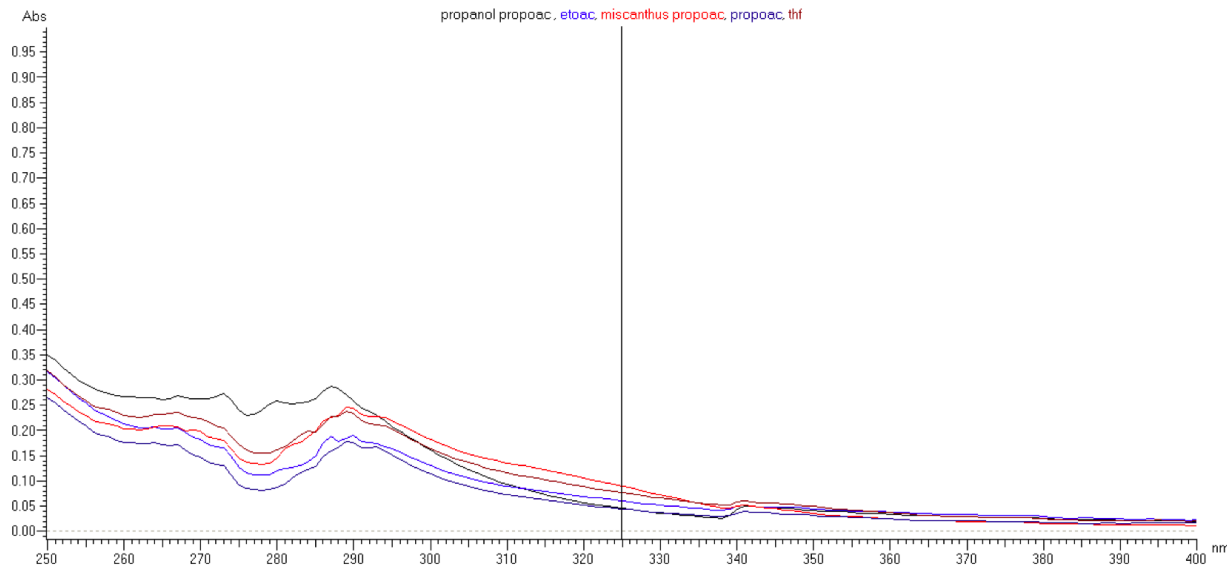
Figure 9: UV absorbances of the organosolv extracted lignin

Externally supplied lignin UV-Vis analysis

To gain an impression of which lignins were best at absorbing UV light regardless of source, different samples obtained using a variety of organosolv extraction conditions and biomass (i.e., beech, poplar) were analysed alongside the extractions performed for this paper.
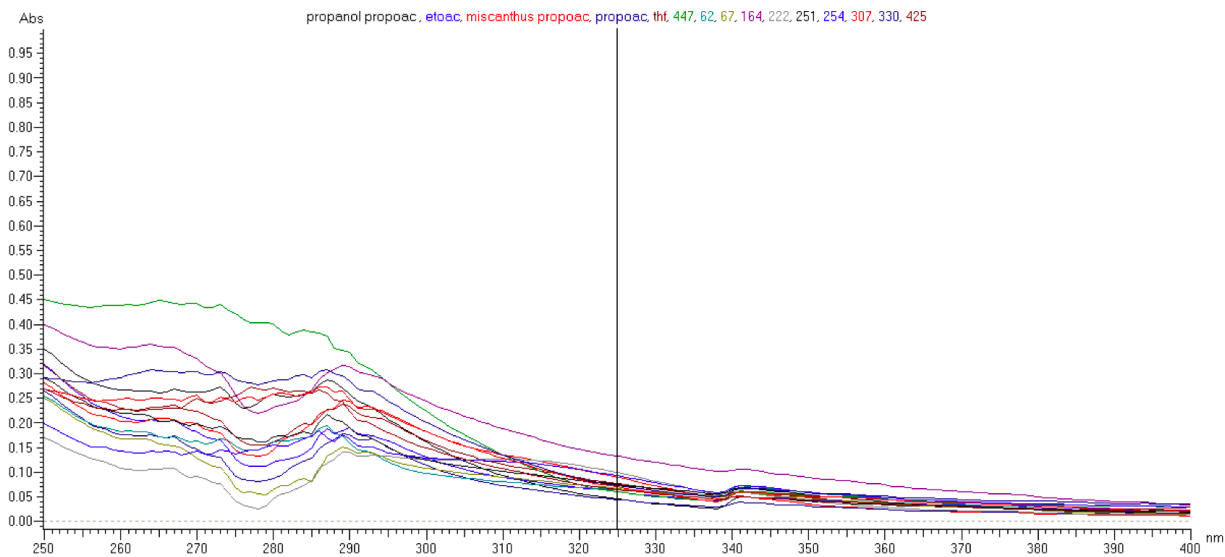

Figure 10: UV absorbances of externally supplied lignin

Figure 10 shows that the UV absorbances of the lignin extracted from walnut is relatively lower than the samples derived from other biomass sources. In particular, sample 447 (extracted from poplar) gave the highest absorbance UV-C out of all the lignin. It is worth noting that the extraction solvents used for this sample were the same solvents used to prepare the sample with the highest absorbance seen in Figure 9

for the walnut shell lignin - propyl acetate and propanol. This further strengthens the theory that an acetate/alcohol cosolvent mixture yields lignin with better UV absorbing properties.

Lignin added to pure cream

A few of the best performing lignin samples were selected to mix with pure cream in an effort to understand if the medium and concentration plays a role in the UV blocking trends. Figure 11 allows an intuitive comparison of the cream and lignin blends by eye, although the difference is subtle. All three THF extracted lignin blends appear the lightest compared to their counterparts extracted with ethyl acetate and propyl acetate.
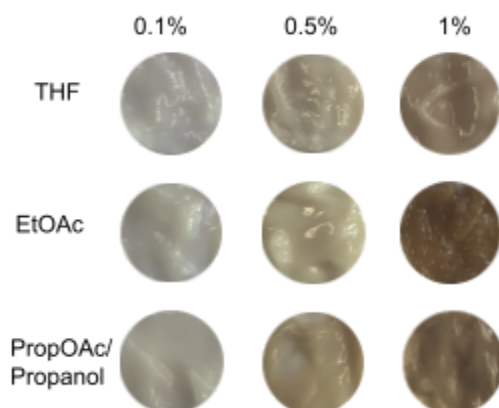

Figure 11: Colour comparison of lignin cream blends

First, pure cream was used as a blank before measuring the transmittance of the blended cream mixtures. From Figure 12, it is clear that for the creams blended with THF lignin, there is a significant difference between the 0.1% blend and the 0.5% blend. Going from 0.5% to 1% has a very slight difference in transmittance in the UV-C range and this is also seen in the mixtures using ethyl acetate extracted lignin in Figure 13. In the visible range, the THF blends have the highest transmittance compared to the other two blends, indicating that it is qualitatively the lightest coloured cream mixture.
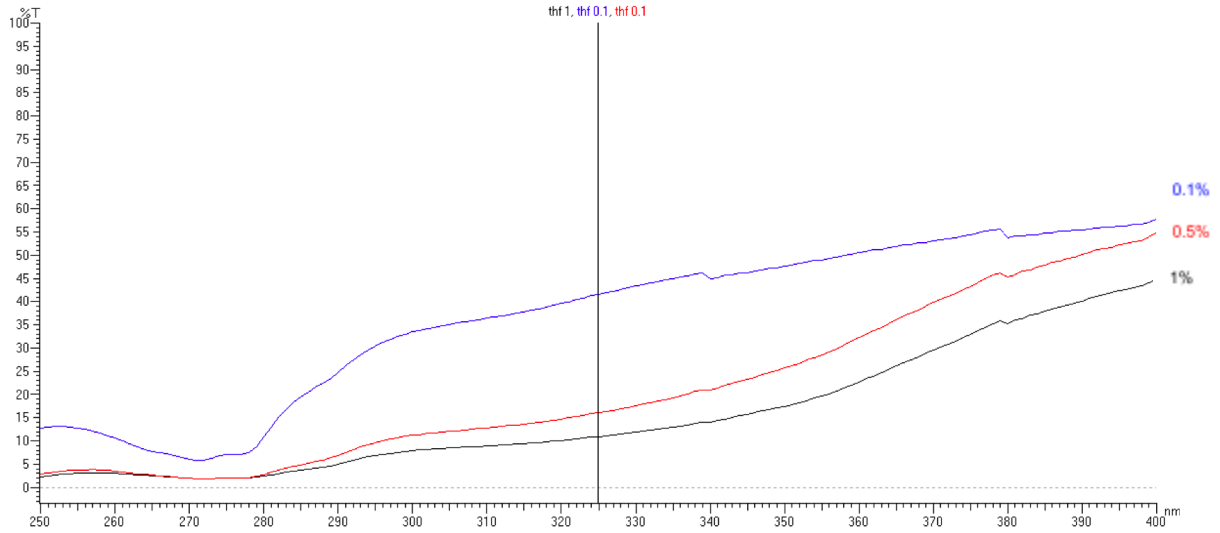
Figure 12: UV-Vis graph displaying the transmittance of cream blended with THF extracted walnut shell lignin

For the cream blended with lignin extracted using ethyl acetate, the transmittance is higher overall in the UV range but lower in the visible range, indicating that it is darker in comparison to the THF lignin blends as well as worse at UV-blocking.
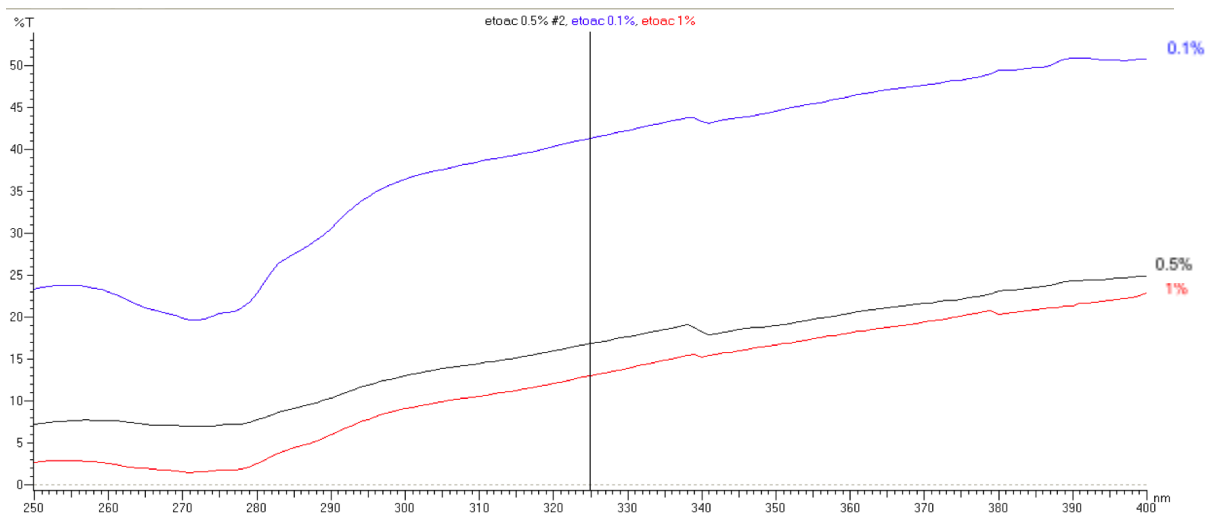

Figure 13: UV-Vis graph displaying the transmittance of cream blended with ethyl acetate extracted walnut shell lignin

The mixtures made using the best performing lignin out of all the samples, extracted using propyl acetate and propanol from poplar biomass, showed the trends seen in Figure 14. As expected, these blends showed the lowest transmittance out of the blends. However, all three blends for this lignin showed the lowest transmittance in the visible range as well, indicating that they are objectively the darkest mixtures even if they do not appear much darker to the naked eye.
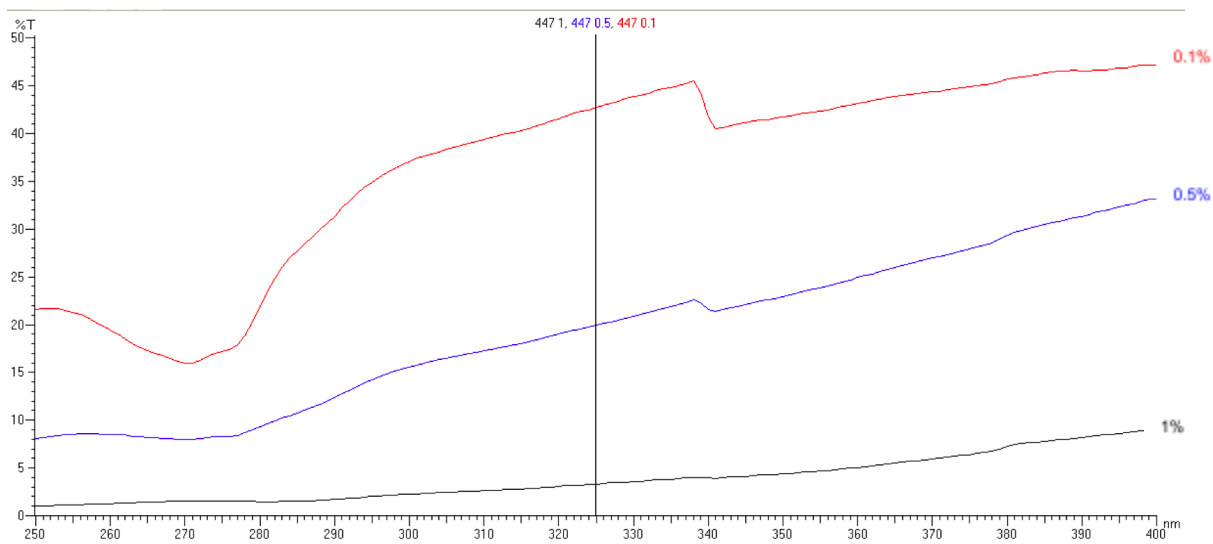
Figure 14: UV-Vis graph displaying the transmittance of cream blended with propyl acetate/propanol extracted poplar lignin

SPF

The Sun Protection Factor (SPF) of each blend was calculated using the following formula:[10]

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where EE(λ) is the erythemal effect spectrum, I(λ) is the solar intensity spectrum, Abs is the absorbance of the blend and CF is the correction factor (10). The results are summarised in Table 3.

Table 3: Summary of SPF calculations for lignin cream blends

Extraction solvent	SPF 0.1%	SPF 0.5%	SPF 1%
THF	4.6	9.2	10.7
EtOAc	4.4	8.6	10.4
PropOAc/propanol	4.2	8.0	15.4

Sample 447 (using propyl acetate and propanol) performs the best overall with an SPF of 15.4 at only 1 wt%. Looking at Figure 11, there is not much colour trade-off for this high UV blocking performance as it is not significantly darker than the other blends of the same concentration. This is superior to the values obtained in literature wherein 10% of a organosolv lignin cream blend improved its SPF to 8.1 and this may be attributed to the lignin itself because the same brand of pure cream was used for these extractions.[11]

5. Conclusion

Various lignin extraction methods were evaluated for optimal yield and colour performance. Considering yield and colour, ethyl acetate is the most optimal extraction solvent as it had the highest extraction efficiency at 21.5% whilst being one of the lighter lignin samples out of the extractions performed. Regarding sunscreen performance, the propyl acetate and propanol extracted lignin appears the best candidate at 1 wt% with an SPF of 15.4. There is promise in these methods of extraction for further exploration such as looking into synergistic effects when combining these lignin with sunscreens instead of just pure creams. Another point to investigate would be the effect of using different creams on the lignin dispersion and by extension the UV performance of the blends. Furthermore, it is evident from this work that the extraction conditions can be further tuned to optimize the performance of the end-product as there is a definite link between the two.

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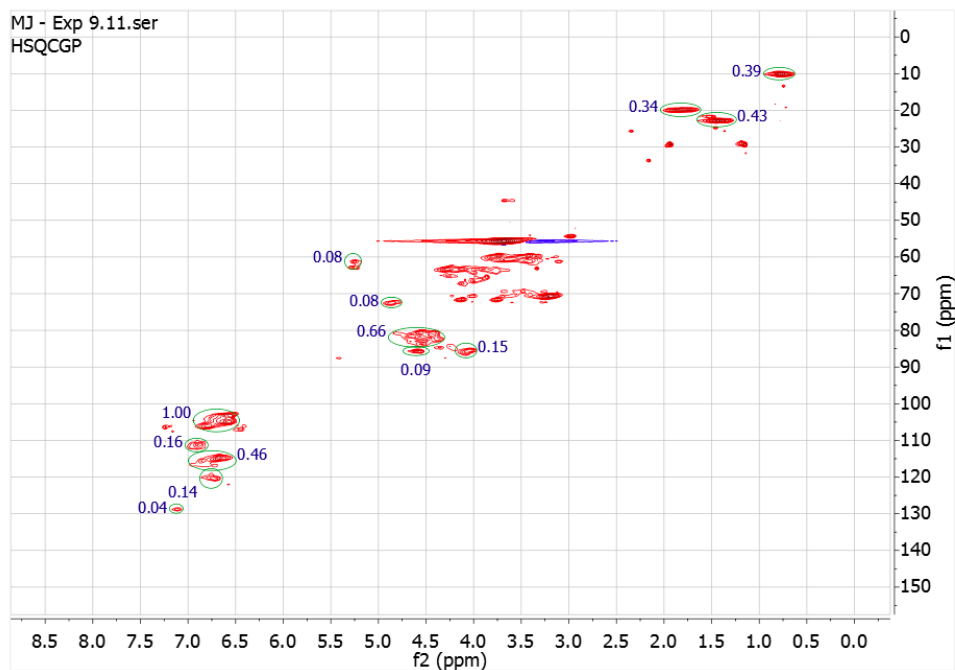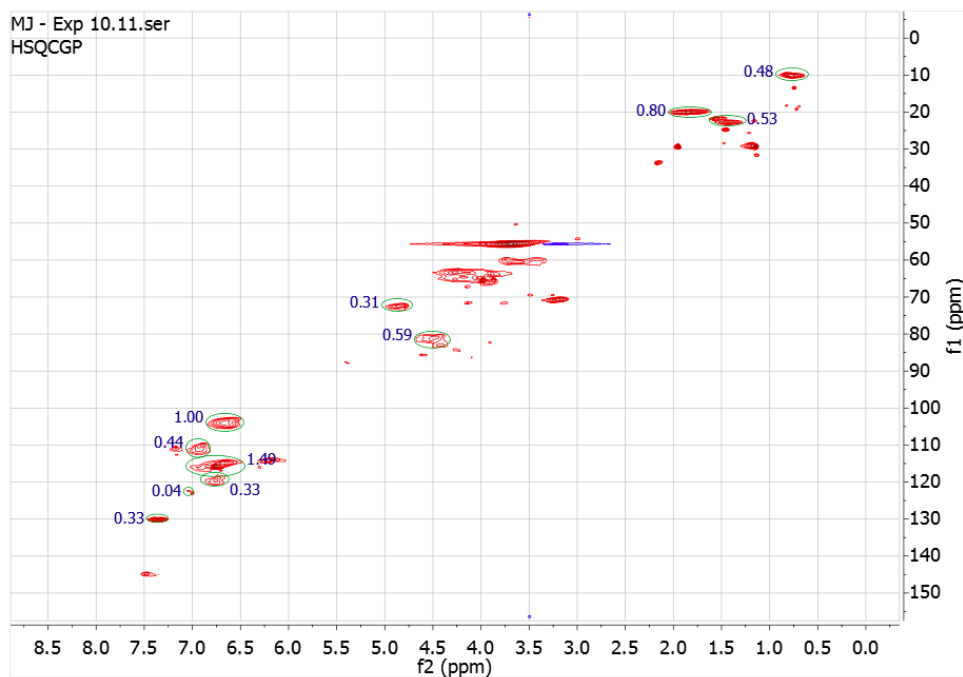
Appendix
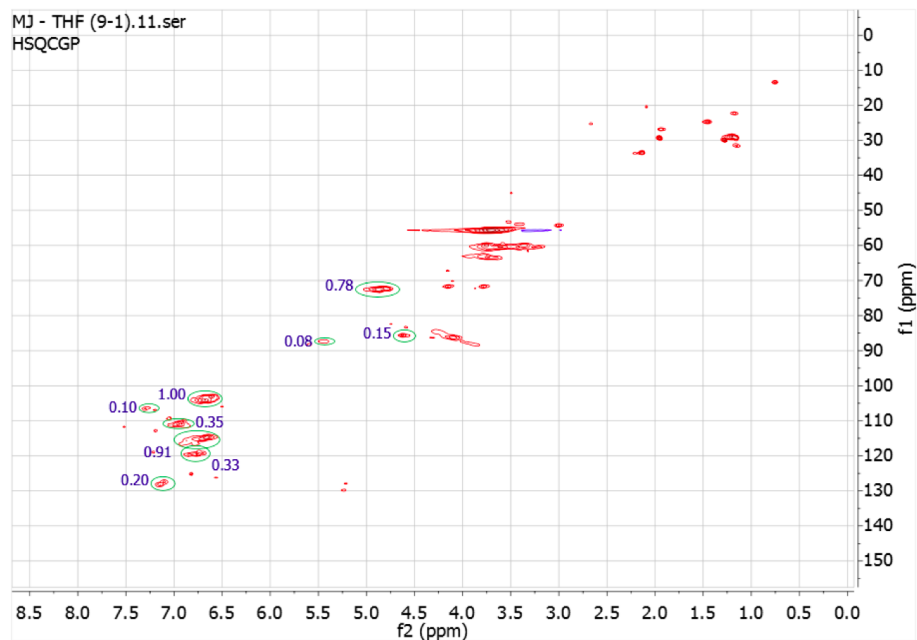
Appendix 1: 2D HSQC spectra (d6-acetone) spectrum for 9:1 THF/H₂O extracted lignin from walnut powder (60 °C for 16 h)
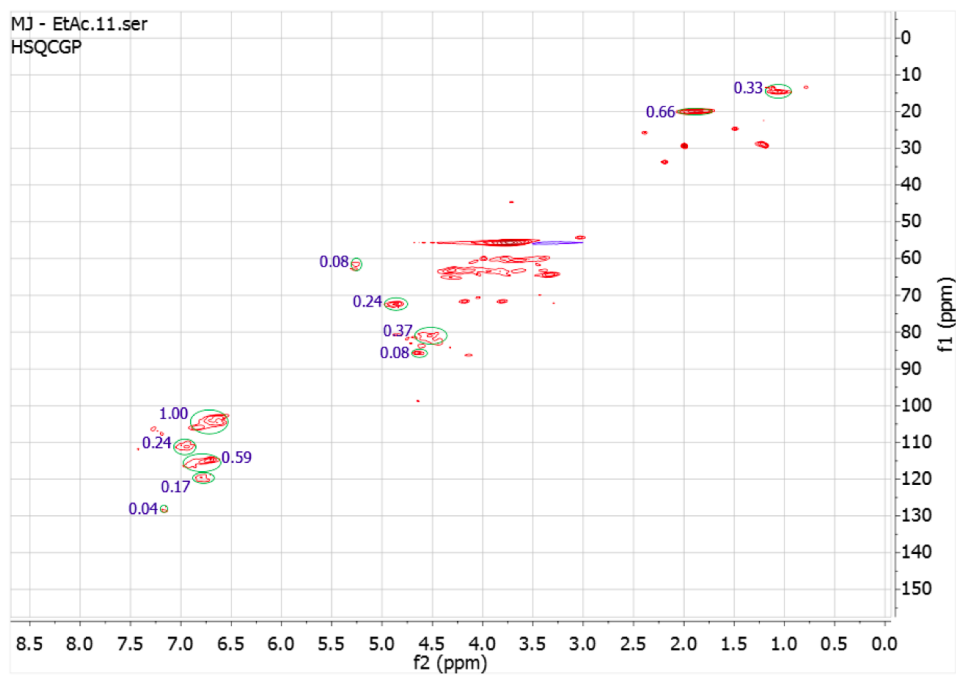
Appendix 2: 2D HSQC spectra (d6-acetone) spectrum for ethyl acetate extracted lignin from walnut powder (60 ℃ for 16 h)

Appendix 3: 2D HSQC spectra (d6-acetone) spectrum for 9:1 propyl acetate/propanol extracted lignin from walnut powder (60 ℃ for 16 h)

Appendix 4: 2D HSQC spectra (d6-acetone) spectrum for 9:1 propyl acetate/H_2O extracted lignin from miscanthus (60 ℃ for 72 h)