A Study on Lignin or Zein Based Hydrophobic Coatings

by

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Abstract

The two by-products lignin, from the paper industry, and zein, from starch production, have shown potential as hydrophobic barriers. Therefore, this study has aimed to produce and investigate the hydrophobicity of lignin or zein dispersions coated on paper. The lignin coatings were made from softwood (spruce) and hardwood (birch) lignin that was extracted using hydrotropic extraction (HEX) and milled wood lignin (MWL) extraction. The lignin was then dissolved in acetone and spray coated onto paper. In the zein dispersions, zein was dissolved in either ethanol (EtOH) or acetic acid (AcOH). In two of the zein coatings, glycerol was added as a plasticiser.

For the lignin coatings, no difference in hydrophobicity was found between softwood lignin and hardwood lignin. The MWL coatings gave the highest contact angle (CA), birch 107° and spruce 106°, and the lowest cobb value of around 37 g/m^2. However, none of the lignin coatings showed potential as a water vapour barriers as all the coatings had high water vapour transmission rate (WVTR) values of around 1300 g/(m^2 day). The paper that the coatings were applied to had high water CA and low moisture uptake. This makes the success of the MWL more difficult to establish.

The zein coatings did not give hydrophobic CA except for the dispersion containing 15wt% zein in EtOH (the highest value being 90°). However, it was found that some zein dispersions had a better water vapour barrier compared to the lignin dispersions and 10wt% zein in AcOH plasticised with glycerol had a WVTR value of 89 g/(m^2 day) and 10wt% zein in EtOH had WVTR value of 710 g/(m^2 day).

Populärvetenskaplig sammanfattning

Med de nya klimatomställningarna behövs nya hållbara material. En av de mest förekommande naturliga molekylerna är lignin, vilket är en av de tre huvudsakliga beståndsdelarna i växter, till exempel trä. Förutom lignin är de två andra huvudbeståndsdelarna i trä; cellulosa och hemicellulosa. Idag används framför allt cellulosa i pappersindustrin och lignin har därför länge ansetts vara en restprodukt. En annan molekyl som idag också anses vara en restprodukt är majsproteinet zein som kommer från majsstärkelsetillverkning. Dessa två molekyler har potential att användas inom förpackningsindustrin då de kan vara vattenavstötande och därför kan användas som en vätskebarriär. Därför har vi i denna studie utvunnit lignin ur björk och gran samt undersökt potentialen hos lignin och zein som vattenavstötande beläggning på papper.

För att utvinna ligninet ur gran och björk förbehandlades träflisen först med ånga i en process som kallas ångexplosion vilket framför allt avlägsnar hemicellulosan. Därefter användes två olika separationsmetoder, MWL och hydrotrop extraktion. MWL är en metod som idag används för att utvinna ett lignin som är så likt obehandlat lignin som möjligt. I en MWL extraktion låts trät ligga i varm lösning med kemikalien dioxan som löser upp ligninet. Dioxanet får sedan avdunsta och kvar är ligninet. I den hydrotropa extraktionen används hydrotropen SXS som är en molekyl som har förmågan att göra ämnen som inte är lösliga i vatten vattenlösliga. Hydrotropen gör ligninet lösligt i vatten till en viss grad och för att sedan få ut ligninet från vattnet tillsätts ännu mer vatten vilket späder ut hydrotropen och gör att ligninet inte längre är lösligt i vattnet. Därefter kan ligninet enkelt separeras ut med hjälp av ett filter.

När ligninet utvunnits blandades det med aceton och sprayades på papper. Därefter mättes vattenbarriäregenskaperna.

För majsproteinet zein som redan var utvunnet så skapades fem olika blandningar. Med dessa blandningar testades två olika koncentrationer av zein (10 vikts-% och 15 vikts-%), två olika lösningsmedel (ättiksyra och etanol) samt med och utan mjukgöraren glycerol. Dessa sprayades sedan på papper och vattenbarriärsegenskaperna analyserades.

Efter analyserna var det tydligt att MWL beläggningen var mest vattenavstötande, men att ingen av ligninbeläggningarna var bra ångbarriärer. Trots detta var det svårt att avgöra skillnaden som MWL beläggningen gjorde, då pappret redan från början var vattenavstötande. Den bästa ångbarriären var däremot zeinblandingen i ättiksyra innehållande glycerol som däremot inte var en bra vätskebarriär. Den enda zeinblandningen som gav mätvärden som kunde klassificeras som vätskeavstötande (hydrofobt) var 15 vikts-% zein i etanol.

List of abbreviations

AcOH – Acetic Acid

AFM – Atomic Force microscopy

Al-OH – Aliphatic hydroxyl group

ASL – Acid Soluble Lignin

B170 – STEX pre-treated birch extracted hydrotropically at 170 ℃

B25 – STEX pre-treated birch extracted hydrotropically at 25 ℃

BDL – Below Detection Limit

CA – Contact Angle

COOH – Carboxyl Group

CSF – Combined Severity Factor

DM – Di-iodomethane

EtOH – Ethanol

G – Guaiacyl

H – *p*-Hydroxyphenyl

HEX – Hydrotropic Extraction

MW – Molecular Weight

MWL – Milled Wood Lignin

NMR – Nuclear Magnetic Resonance

NREL – National Renewable Energy Laboratory

PDI – Polydispersity Index

Ph-OH – Phenolic Hydroxyl Group

S – Syringyl

S170 – STEX pre-treated spruce extracted hydrotropically at 170 ℃

Sa – Surface Average

SDR – Surface-Developed Interfacial Area

SRS - Sugar Recovery Standards

STEX – Steam Explosion

SXS – Sodium Xylenesulfonate

TMDP – 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane

WDI – Water Deionised

WVTR – Water Vapour Transmission Rate

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1 Introduction

With the challenges that come with climate change, the need for new sustainable materials has increased. Furthermore, to decrease the climate footprint fewer materials need to go to waste and new uses for otherwise unused materials needs to be found. In the packaging industry, new materials are needed that can replace the petroleum-based plastics that today are used as a water barrier. Lignin which is a by-product from paper production and zein which is a by-product from corn starch production have shown potential since they both have hydrophobic properties. Therefore, the aim of this study was to hydrotropically extract hydrophobic lignin as well as create different dispersions of lignin or zein that could be used to coat paper. Furthermore, this study aimed to examine the water barrier properties of said coatings.

1.1 Lignin

Lignin is one of the most abundant biopolymers available on this planet and presents a solution when the need for new raw materials has increased as petroleum is being phased out. Another benefit of lignin is that it is one of the main constituents in wood which means that it does not necessarily compete with food production and the production does not consume a large amount of added water outside of rain. Furthermore, lignin has an aromatic backbone meaning that it can be used to derive aromatic chemicals and aromatic polymers. (Duval & Lawoko, 2014)

The different applications of lignin as a polymeric material is a researched field dating three decades back. However, an issue still faced is the heterogeneous nature and high polydispersity index (PDI) of the macromolecule. Consequently, developing fractioning processes to influence reactivity and PDI that can industrially be scaled up is an important field of research. (Duval & Lawoko, 2014)

It has been observed that lignin can adsorb heavy metals (Duval & Lawoko, 2014). This must be considered when using lignin in food packaging since if the material in any stage of the manufacturing process comes in contact with heavy metals it could potentially adsorb the metals which could later leach into the food product when the pH or other parts of its environment changes.

Lignin can be used as a replacement for other biopolymers/polymers both modified and unmodified. Unmodified lignin has however shown low thermal stability and the melt processing was highly dependent on the molecular weight, thus highlighting the importance of controlling the heterogenicity. (Duval & Lawoko, 2014) Modifying lignin, for example, creating lignin nanoparticles, can lower the high PDI (M. Yang et al., 2021). Another approach to tackling this problem is by creating lignin-polymer blends with more predictable mechanical properties (Duval & Lawoko, 2014).

1.1.1 The Chemical Composition of Wood

The three main components in wood are cellulose, hemicellulose, and lignin. These three polymeric components are the reason for the mechanical properties of wood. Cellulose has a high molecular weight and crystallinity, giving the fibres of wood their strength. Hemicellulose act as a linking molecule between cellulose and lignin. The lignin, which is amorphous, is the cementing material in which the cellulose fibres are embedded. (Pandey, 1999) An average composition of wood is presented in Table 1.1 and the molecular structure of the cellulose, hemicellulose and lignin are presented in Figure 1.1.

Table 1.1. The average composition of softwood and hardwood in wt.% (Richter et al., 2019).

	Softwoods	**Hardwoods**
Cellulose [%]	47±6	45±6
Hemicellulose [%]	21±7	29±7
Lignin [%]	29±5	23±5
Extractives [%]	3.9±2.9	4.2±2.4

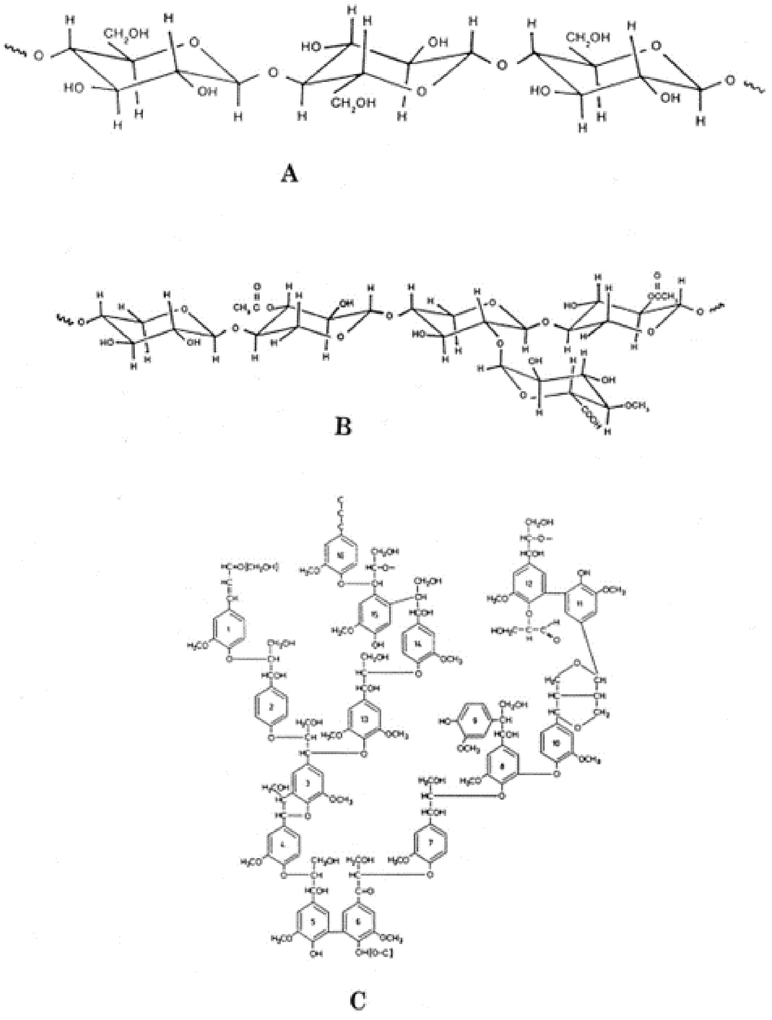

Figure 1.1. The molecular structure of the three main components in wood; (A) cellulose, (B) hemicellulose, and (C) lignin. (Pandey, 1999)

Li et al. (2009) suggest that there are more extractives in birch than in spruce. They also found that all extractives and most of the hemicellulose can be removed from the studied samples by steam explosion.

The hemicellulose in softwoods and hardwoods differ, with hardwoods containing 20-35% partly acetylated glucuronoxylan (O-acetyl-4-O-methylglucuronoxylan) whereas softwoods only contain 10% glucuronoarabinoxylan (arabino-4-O-methylglucuronoxylan). However, hardwoods only contain a small amount of glucomannan, and softwoods contain up to 18% O-acetylgalactoglucomannan. (Hon & Shiraishi, 2000)

There are several linkages between lignin and polysaccharides which are presented in Figure 1.2. Previous studies have found that the linkage between lignin and the segment of the carbohydrate molecule is a direct linkage to arabinoglucuronxylan via ester bond at benzyl or conjugated esters at γ positions. (Katahira et al., 2018)

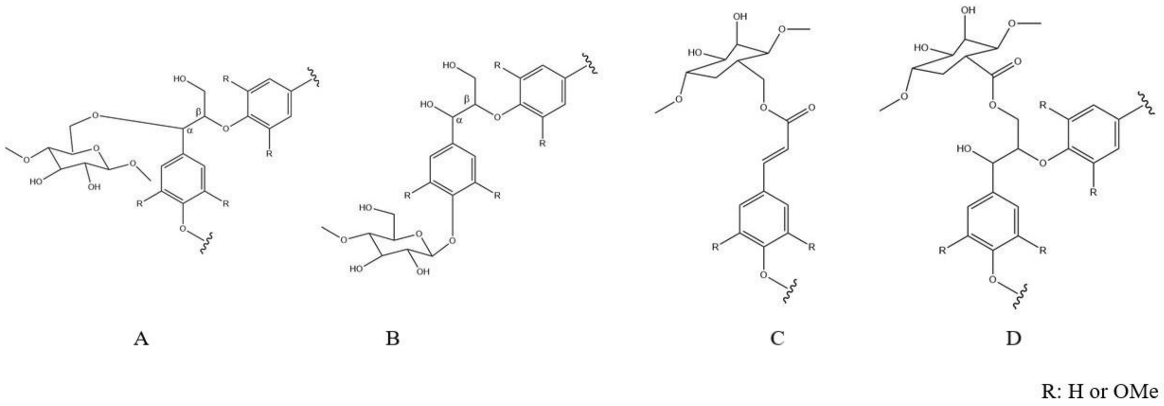

Figure 1.2. Possible lignin-carbohydrate structures; A) benzyl ether, B) phenyl glucoside bond type, C) conjugate γ-ester and D) γ-ester.

1.1.2 The Chemical Composition of Lignin

The aromatic backbone of lignin consists primarily of p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units, presented in Figure 1.3. These units are formed by the monolignols shown in Figure 1.4. The ratio of these units can differ between species, nonetheless, hardwood comprises G-units and S-units while softwood is comprised primarily of G-units. (Olsson, 2021) The most common linkage between the repeating units is the β-O-4 bond (40-60% of the linkages in softwood and hardwood) which is an ether bond between the β carbon on the aliphatic chain of one unit and the C4 carbon on the aromatic ring. Important functional groups of lignin include phenolic (Ph-OH) and aliphatic hydroxyl (Al-OH) groups. Ph-OH can be formed when a β-O-4 bond is cleaved and both Al-OH and Ph-OH affect the chemical reactivity of the lignin. (Katahira et al., 2018)

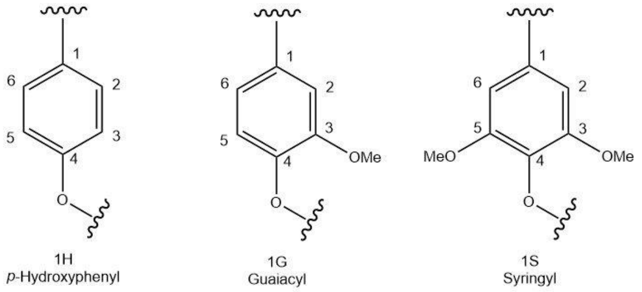

Figure 1.3. The repeating units of lignin p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units.

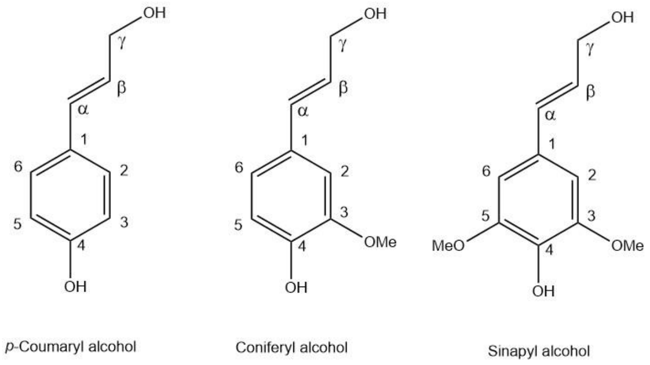

Figure 1.4. The three monolignol p-coumaryl alcohol, coniferyl alcohol and syringyl alcohol.

1.1.3 Steam Explosion

Steam explosion (STEX) is used as a pre-treatment for lignocellulosic biomass, such as hardwood and softwood, to remove hemicellulose. During the pre-treatment, the biomass is subjected to high-pressure steam with a high temperature that usually is ranging between 160-260°C for 1-20 minutes. The rapid decrease of the pressure that occurs when the biomass moves from the reactor will cause the biomass to explosively decompress. When the material is thermally degraded organic acids from the material can be released that can hydrolyse the intramolecular hemicellulose bonds (autohydrolysis). The bonds can also be hydrolysed by the addition of catalysts (H_2SO_4, HCl, SO_2). The hydrolysis of the hemicellulose bonds makes the hemicellulose water-soluble, and the resulting sugars can consequently be recovered in the liquid fraction after the STEX. This will lead to an enriched cellulose content of the remaining solids (Olsson, 2021).

During the STEX, the ultra-structure of the biomass changes which leads to an increase in surface area and porosity. Due to this, the material is more susceptible to future processing (Olsson, 2021). The severity of the STEX pre-treatment, such as higher temperature, affects the chemical and physio-chemical properties of lignin. Maniet et al. (2017) found that phenolic OH and -COOH groups increase while aliphatic OH decrease with increased severity of the STEX. When lignin is subjected to higher severities during the STEX there is a larger loss of β-O-4 bonds and greater depolymerization of the lignin (Maniet et al., 2017; Olsson, 2021). Furthermore, extraction yields of lignin tend to be higher for severity factors 3.2-3.8 (Maniet et al., 2017). The severity factor of the steam explosion can be calculated by the correlation between process temperature and retention time described by Jacquet et al. (2011) for a steam explosion reactor with a small volume which obeys Arrhenius law (see Equation (1)).

$$S = Log_{10}\left[t\, e^{\frac{T-100}{14.75}}\right] \tag{1}$$

Where S = severity factor, t = retention time (min) and T = process temperature (°C) (Jacquet et al., 2011).

The combined severity factor CSF accounts for the pH of the liquid fraction and is described according to the following equation (Kellock et al., 2019):

$$CSF = S - pH \tag{2}$$

Where pH is the measured pH after STEX treatment.

1.1.4 Hydrotropic Extraction of Lignin

The separation of lignin, cellulose and hemicellulose can be made through a hydrotropic process. The advantage of using this process is a purer lignin sample containing low carbohydrates and sulphur contaminations. (Ansari & Gaikar, 2014; Gabov et al., 2013) Still, some sulphuric contaminants can be present if the hydrotropic agent is sulphuric (Devendra et al., 2016). Hydrotropic processing has also been shown to result in lignin with a higher molar weight, lower PDI and a higher amount of phenolic and aromatic groups, but less carboxylic groups than other separation methods (Ansari & Gaikar, 2014; Gnana Prakash et al., 2022). This indicates that the hydrotropic process can result in more hydrophobic lignin because of the longer aromatic backbone (Liu et al., 2022; Q. Yang & Pan, 2016), thus suggesting that hydrotropic lignin can be used as a water-repelling barrier in food packaging.

Olsson (2021) developed a hydrotropic extraction (HEX) method for lignin from hardwood biomass. The biomass was first pre-treated using a steam explosion that removes the hemicellulose and opens up the structure to allow for the hydrotropic extraction i.e., solubilizes lignin and separates it from the cellulose. Afterwards, the lignin was precipitated by the addition of water which decreases the concentration of the hydrotrope and accordingly decreases the solubility of lignin. (Olsson, 2021)

Sodium Xylenesulphonate

Hydrotropes are, like surfactants, amphiphilic molecules used to solubilise substances that are not soluble in aqueous solutions. However, the difference is that hydrotropes have shorter hydrophilic tails than surfactants. (Olsson, 2021) Hydrotropes can therefore be considered weaker than surfactants. For a hydrotrope to be able to solubilize a substance, the minimum hydrotrope concentration (MHC) must be reached since below this concentration the hydrotrope cannot self-assemble. Hydrotropes are believed to assemble into sandwich-type structures (Korpinen & Fardim, 2009). A commonly used hydrotrope is sodium xylenesulphonate (SXS), shown in Figure 1.5, which is also considered a relatively green chemical. The concentration typically used for SXS extraction of lignin is between 30% and 40% (w/w). It is believed that SXS works better for extracting S units than G units which are more chemically resistant (Korpinen & Fardim, 2009).

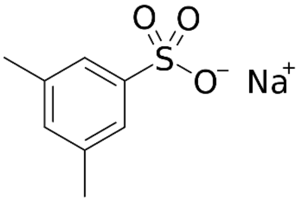

Figure 1.5. The chemical structure of SXS.

1.1.5 Milled Wood Lignin

Another method of lignin extraction is called Milled Wood Lignin (MWL) and was developed by Björkman in the 1950s. This method involves finely milling the wood followed by lignin extraction using a dioxane-water (96:4, v/v) solution. (Lundquist, 1992) It has been suggested that the lignin extracted by the MWL method is very similar to protolignin (Obst & Kirk, 1988), i.e. chemically unchanged lignin (Brunow et al., 1999). A reason for this is the low extraction temperature that limits reactions that would chemically modify the lignin (Sapouna & Lawoko, 2021). Still, MWL does not have the exact same structure as native lignin but can be used in studies as a way of examining the degree of modification of the lignin from the studied extraction method (Obst & Kirk, 1988). The MWL extraction technique has been shown to provide lignin with high molecular weight (Wegener & Fengel, 1977), which also makes MWL a good candidate for creating hydrophobic coatings.

1.1.6 Lignin in Hydrophobic Coatings

Research has previously been made on the use of kraft lignin in hydrophobic coatings. Two approaches to making the lignin coating more hydrophobic are to modify the lignin and increase its hydrophobicity through for example esterification and forming polymer-lignin blends. (Alwadani et al., 2021; Hult, Ropponen, et al., 2013; Tavares et al., 2018).

Modifications

To increase the hydrophobicity of the kraft lignin one approach is to modify the lignin by esterification (Hult, Ropponen, et al., 2013). This increases the hydrophobicity by decreasing the hydroxyl groups and thus decreasing the capacity of the lignin to form hydrogen bonds with water, decrease the glass transition temperature and increase the thermoplasticity of lignin (Alwadani et al., 2021; Hult, Ropponen, et al., 2013). The results of a study conducted by

Hult, Koivu and co-workers (2013), shown in Table 1.2, suggest that the hydrophobicity increases with the length of the carbon backbone of the fatty acid used for esterification. Still, even if the fatty acids used in Hult, Ropponen and co-workers' (2013) study have longer carbon chains than palmitic acid, the hydrophobicity is not as high. Consequently, the results of these two studies imply that esterification using saturated fatty acids results in a more hydrophobic coating than unsaturated fatty acids. Nevertheless, it is important to note that even if palmitic acid resulted in the lowest water vapour transmission rate (WVTR) it was coated on a paper with a lower WVTR than the paper in the tall oil fatty acid esterification study which, makes it more difficult to compare the vapour barriers. However, the results of the contact angle tests strengthen the hypothesis that the lignin esterified with a palmitic acid coating is more hydrophobic.

Table 1.2. Water vapour transmission rate (WVTR) and water contact angle (CA) of different lignin-containing coatings.

Coating	**WVTR coating/paperboard** [g/m^2x24h]	**Contact angle/stability time**
Kraft lignin dissolved in DMF (Alwadani et al., 2021)	-	~29 °/- (coated on glass)
TOFA modified softwood lignin, one layer (Hult, Ropponen, et al., 2013)	500/840	~80°/2 min
TOFA modified softwood lignin, two layers (Hult, Ropponen, et al., 2013)	260/840	~80°/2 min
Softwood lignin esterified with palmitic acid, one layer (Hult, Koivu, et al., 2013)	190/740	~102°/2 min
Softwood lignin esterified with palmitic acid, two layers (Hult, Koivu, et al., 2013)	130/740	~100°/2 min
Softwood lignin esterified with lauric acid, one layer (Hult, Koivu, et al., 2013)	250/740	~98°/2 min
Softwood lignin esterified with lauric acid, two layers (Hult, Koivu, et al., 2013)	150/740	~95°/2 min

Solvents

Hult, Koivu, and coworkers used deuterated chloroform ($CDCl_3$) to dissolve the esterified lignin (Hult, Koivu, et al., 2013). However, this chemical is toxic and might not, therefore, be appropriate for use in food packaging (Sigma-Aldrich, 2021). An alternative can therefore be acetone (Hult, Ropponen, et al., 2013) or ammonium hydroxide (Alwadani et al., 2021).

1.2 Zein

Zein is a by-product from the wet milling process of starch and oil production of corn (Luo & Wang, 2016). It is an alcohol-soluble protein that is present in the endosperm of corn (Lawton, 2002).

In solution, the structure of zein gets extended. The proteins are organised in oligomers which lead to higher temperature resistance, and they are resistant to treatment with reducing agents and tend to form higher molecular weight aggregates. One issue with film formation from zein is that it is brittle. (Guo et al., 2008)

Plasticisers can be added to modify the mechanical properties of the film, such as reducing brittleness, however they often also change the barrier properties of the material (Ghanbarzadeh & Oromiehi, 2008; Lai & Padua, 1997). Plasticisers are molecules that can position themselves between polymer molecules and interfere with the polymer-polymer interaction (Han, 2014). The addition of oleic acid to zein films have been seen to increase elasticity and deformation at break, however it also reduces the hydrophobicity of the surface and the roughness (Egea et al., 2022). Additionally, adding 20 % glycerol to zein films has shown to form a smooth and continuous films (Guo et al., 2008). Ghanbarzadeh & Oromiehi (2008) found that glycerol could have a better plasticising effect than olive oil, which contains a large quantity of oleic acid. The reason for this is because the oleic acid could weaken the structure of films. Still, the films plasticized by olive oil had a lower water vapour permeability than films with glycerol. (Ghanbarzadeh & Oromiehi, 2008)

The hydrophobicity of the zein film can be controlled by changing the concentration of zein in the film forming solution. Dong et al. (2013) found that 0.5 mg/ml zein in 80% ethanol-water solution gave the highest water CA. They relate the differences in the zein samples to the surface roughness of the film.

Li and co-workers (2020) found that using airglow discharge plasma the surface properties of the zein film could be altered, resulting in a lower hydrophobicity. Research that has been done on dual-layering of zein films with chitosan (Kansal, Hamdani, Ping, Sirinakbumrung, et al., 2020; Vrabič Brodnjak & Tihole, 2020) or with starch (Kansal, Hamdani, Ping, & Rabnawaz, 2020) showed excellent oil and water barrier properties.

Dong et al. (2015) found that the hydrophobicity of the zein coating was higher for coatings where the solvent evaporation was droplet-mediated, such as electrospinning or spray-coating, compared to surface-mediated evaporation, such as cast drying. This is because during droplet-mediated evaporation there is less time for α-helix to β-sheet transformation, thus leaving the hydrophobic groups of zein on the surface (Dong et al., 2015).

1.3 Coating Processing

It is important to control the viscosity of the coating. The viscosity of the coating needs to be low enough in order to be applied to the material. At the same time, if the viscosity of a Newtonian coating on a vertical surface is too low it could give rise to sagging. However, for low viscosity non-Newtonian coatings this phenomenon can be avoided if the yield strength is higher than the gravitational force. Still, it is important to remember that the viscosity changes after the coating process largely due to the evaporation of the solvent. (Tracton, 2006)

The coating method can also affect the coating’s properties. For example, spray coating can result in an uneven, orange peel-like, topography (Tracton, 2006). Uneven topography has been shown to promote a higher hydrophobicity (Heydari et al., 2013; Mohamad et al., 2013). Additionally, Luís et al. (2019) found that zein films that were moulded after a lotus leaf mould, which has a rough surface, had increased hydrophobicity. This suggests that spray coating is a suitable coating method when creating a hydrophobic barrier. Orange peel appearance can be promoted by adjusting the spraying distance. A short distance can result in air blast from the spraying gun hitting the coating and a longer distance can lead to solvent evaporation before the dispersion hits the surface, both causing orange peel appearance. (Patton, 1964) Additionally, pre landing solvent evaporation, where the spray is dried before hitting the surface, can be obtained with spray drying (Dong et al., 2015). However, it should be noted that coating at a higher process temperature has been shown to decrease the hydrophobicity of lignin coatings due to bubble formation and higher evaporation rates. (Alwadani et al., 2021).

2 Method

The creation of lignin and zein dispersion used in this study was conducted in two different ways. The lignin was extracted from dried solid fraction of the STEX-treated birch and spruce wood and then dissolved in acetone and coated onto paper. The zein was dissolved in two different solvents and coated on paper.

2.1 Lignin

The lignin was extracted, dissolved in solvent, and coated onto paper according to the process described in Figure 2.1.

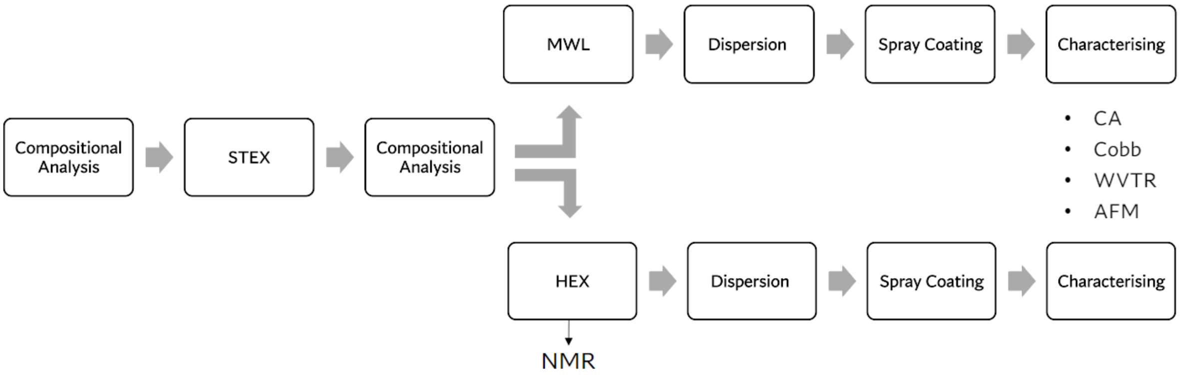

Figure 2.1. Overview of the process of making lignin coatings.

2.1.1 Steam Explosion

Firstly, 1.5 kg spruce and birch chips were each soaked in 10 L of 50 ºC deionized water (WDI) overnight. Thereafter, the water was decanted, and the two kinds of wood were treated for 5 minutes each in a 10 L steam explosion reactor heated to 190 ºC. The collected material was then pressed in a hydraulic press at 230 bar and the liquid fraction was collected and frozen. The liquid fraction was later analysed, and pH measurements were taken. The solid fraction was washed three times by soaking in 50 ºC WDI for 30 minutes followed by being pressed in a hydraulic press at 230 bar. Afterwards, the remaining solids were dried for 2 days at 45 ºC and then milled using a 1 mm grid. Finally, the milled wood was dried again and analysed and used in the HEX and MWL extraction. (Olsson, 2021)

The CSF for the woods were calculated according to equations (1) and (2) and are summarised in Table 2.1.

Table 2.1. The CSF and pH measurements of the STEX treated wood.

Wood	**CSF**	**pH**
Spruce	0.2	3.1
Birch	0.5	2.9

2.1.2 Hydrotropic Extraction

The dried solid fraction of the STEX-treated spruce and birch samples were used for the HEX. A 40% (w/w) SXS solution was made and used for batch 2 and 3, while a 34 % solution was used for the first batch. SXS-solution was added to the biomass in ratio 5.57 % (w/v) (Olsson, 2021). For the first batch, two of the samples were heated to 170°C for 1 hour in a microwave and two samples were left in ambient temperature for 1 hour. For batch 2 and 3 all samples were heated to 170°C. Afterwards, the liquid fraction was separated from the solid fraction by vacuum filtration. The liquid fraction was diluted 10 times until lignin precipitated and centrifuged. No precipitate was obtained from the spruce sample left in ambient temperature. The liquid was decanted, and the sediment was diluted with WDI and centrifuged again. The liquid was once more decanted, and the sediment stored in 45°C oven until dry. The solid fraction was washed sequentially with 0.1M NaOH, 0.05M NaOH and WDI and then stored in 45°C oven to dry for 48 hours. (Olsson, 2021)). The birch and spruce extracted at 170℃ were labelled B170 and S170 respectively and the birch extracted at 25℃ was labelled B25.

2.1.3 MWL

3.0 g of the dried solid fraction of STEX-treated spruce and birch were respectively added to a filter. The filter was placed in a flask and 50 ml of dioxane was carefully added. Subsequently 3 ml of deionized water was added and the sample was placed in 80°C water bath for 24 hours. Thereafter the filters were removed from the flask and the solvents were left to evaporate from the flask.

2.1.4 Coating

64 ml of acetone was added to 0.84 g of the HEX-spruce and HEX-birch lignin respectively and stirred using a magnetic stirrer. The birch was left to dissolve for 3 hours while the spruce was left to dissolve for 72 hours. The B170 lignin dispersion was then sprayed on two preweighed papers using an airbrush (Biltema, Artnr. 17-372, Lund, Sweden) in three 10 ml and one 5 ml layer. The coated papers had approximately the size of 21x30 cm each. The HEX-spruce lignin dispersion was sprayed in three 10 ml layers and one 3 ml layer. S170 was sprayed onto paper in four layers with 8 ml, 12 ml, 0 ml and 3 ml respectively.

Furthermore, 48 ml acetone was added to approximately 0.06 g spruce-MWL and 62 ml acetone was added to approximately 0.15 g birch-MWL. The MWL-spruce solution was then sprayed on to a weighed paper in four coatings of 8 ml and the MWL-birch solution was sprayed on in four alternating layers of 6 ml and two alternating layers of 9 ml.

The undissolved residues where dried in 45°C oven for 2 days and weighed to gravimetrically quantify the amount of undissolved lignin.

The coated paper samples were initially air dried with taped down corners and then dried in a 45ºC oven and then weighed.

2.1.5 Characterisation

A compositional analysis of the raw material and the STEX treated material was conducted as described under the section NREL. The extracted material was analysed with ^{31}P-NMR and HSQC 2D-NMR as described below.

NREL

Before the compositional analysis of the raw material, the birch and spruce were dried in a 45 ℃ oven for 48 hours. The biomass was then milled to about 1 mm pieces and then dried for another 48 hours. The woods were then treated using Soxhlet extraction in duplicates with 5 g sample to 150 ml WDI. The Soxhlet extraction was then repeated with 150 ml (96 v/v %) ethanol.

Compositional analysis of the biomass was conducted according to the framework described by Sluiter et al. (2008) and Sluiter et al. (2012). Solids analysis was conducted on the dried raw material and the dried solid fraction of the STEX treated material (Sluiter et al., 2012).

For the solid analysis of the raw material and the STEX treated wood, 0.3 g of birch and spruce were first hydrolysed using 3 ml 72 % sulphuric acid in a 30 ℃ water bath. The samples were stirred every 5-10 minutes for one hour and then autoclaved for 1 hour in 120 ℃. Afterwards, the samples were filtered through crucibles that had previously been burned at 575 ℃ for 4 hours, rinsed with at least 5 ml WDI and put in a 105 ℃ oven overnight. The crucibles were then burned for 4 hours at 575 ℃ and afterwards weighed. The liquid fraction was analysed for acid soluble lignin (ASL) using a spectrophotometer (205 nm for birch and 240 nm for spruce) and carbohydrates using ion chromatography. For the ion chromatography analysis three standards were used (SRS-0 where no acid was added and SRS-1 and SRS-2 that had been hydrolysed and heat treated with the samples).

Liquid analysis was done on the liquid fraction of the STEX pre-treated material (Sluiter et al., 2008). 5 ml of each sample and two standards were added to autoclave safe bottles together with 0.174 ml 72 % sulphuric acid and put in a 120 ℃ autoclave for 1 hour. The samples were then filtered through pre-burned crucibles. The crucibles were then again burned at 575 ℃ for 4 hours and later weighed. The liquid fraction from the autoclaved samples, the untreated liquid fraction and three sugar standards were filtered and analysed in the Dionex for sugar content. Lastly, the untreated liquid fraction was analysed in a spectrophotometer (205 nm for birch and 240 nm for spruce) for ASL.

^{31}P-NMR

A stock solution of pyridine and $CDCl_3$ 1.6:1 (v/v) ratio was mixed. The relaxation agent was prepared by dissolving 50 mg of $Cr(AcAc)_3$ in 5 ml of stock solution. The internal standard was prepared by dissolving 140 mg of N-HDI (N-hydroxy-5-norbornene-2,3-dicarboxiamide) in 5 ml of stock solution. 20 mg sample was dissolved in a mixture of 500 µL stock solution, 100 µL of relaxation agent and 100 µL of internal standard. 100 µL of TMDP was added to the sample and mixed. The samples were transferred to NMR-tubes and run in a Bruker Avance III HD 500 MHz spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany) within an hour of the addition of TMDP.

HSQC 2D-NMR

80 mg sample was dissolved in 0.6 ml of d-DMSO. The solution was transferred to NMR-tubes and run in a Bruker Avance III HD 500 MHz spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). The data obtained using the following settings: 80 scans, 1.5 s relaxation delay, 10.3 µs pulse length, 11 ppm spectral width, 1538 FID size and frequency discrimination in F1 e/a. The data was processed in the software Topspin using the processing meth-

od by Decatur (2020). The baseline was then automatically corrected, and the axis reference was adjusted to the chemical shift of d-DMSO (δ_C / δ_H $= 39.5/2.5$). Thereafter the peaks were picked and integrated.

The data from the HSQC 2D-NMR the amount of C_9 units in each sample was quantified. For the spruce samples the G_2 linkage reflects a C_9 unit since only G units are available. C_9 unit for birch samples was calculated according to equation (3) (Sette et al., 2011) since both S and G units are available in the sample.

$$\mathrm{C}_9 = 0.5\ \mathrm{S}_{2,6} + \mathrm{G}_2 \tag{3}$$

2.2 Zein

An overview of the process of making the zein coatings can be viewed in Figure 2.2.

Figure 2.2. Overview of the process of making zein coatings.

2.2.1 Dispersion

10% and 15% (w/w) zein and solvent dispersions were created by mixing zein with ethanol or acetic acid. Both solvents had a concentration of 90% (v/v). When zein and solvent had been added the dispersion was mixed with a magnetic stirrer until all the zein was dissolved. To four of the dispersions, glycerol (0.3 g/g zein) was added, and the dispersions were mixed at 60°C for 30 minutes.

2.2.2 Coating

Before the coating process, the paper sheets were weighed in order to obtain the coating weight. Afterwards, all the respective zein dispersions were sprayed on using a spray gun (Biltema, Artnr. 17-372, Lund, Sweden) in four layers of 3 ml dispersion each except for sheet 1 which only had 2 layers of 3 ml. The layers were alternated between vertical and horizontal spraying strokes and the paper was allowed to dry in a fume hood for at least 15 minutes between the layers. Next, the papers were allowed to air dry with taped down corners to avoid curling until they were put in a 45 C oven. Lastly, the coated papers were weighed and sent to Tetra Pak for characterisation.

An expected coating weight was calculated by letting 1 ml dispersion dry in a fume hood for 2 days and then weighed to get the density of the coating.

2.3 Characterisation of Coated Samples

After the dispersions were coated onto paper the samples were sent to Tetra Pak for analysis of hydrophobicity and surface morphology. The following analyses were conducted on the samples:

2.3.1 Water Contact Angle

Water contact angle was measured by Tetra Pak using a Krüss MSA instrument and following Krüss own method (*Mobile Surface Analyzer-MSA One-Click SFE*, n.d.) using WDI and DM. Ten drops per sample were measured.

2.3.2 Cobb test

Cobb test was conducted by Tetra Pak according to ISO standard method ISO 535:2014 Paper and board – Determination of water absorptiveness. The sample was weighed, and 100 cm^2 of sample was then put under 100ml of WDI for 3 minutes. Then the water was removed, the surface blotted to remove excess water, and the sample was weighed again to calculate the amount of moisture that was absorbed.

2.3.3 WVTR

Analysis was conducted by Tetra Pak in an Mocon Permeation Analyzer, with the barrier facing high humidity. The samples were measured at 23°C and 50% RH using a mask to minimize measuring area of the samples to 5 cm^2. Readings were taken after 2 days.

2.3.4 AFM

The analysis was conducted by Tetra Pak on a Nanoscope V with Multimode 8 instrument with a ScanAsyst-Fluid+. 2nm tip radius probe. An area of 10x10 µm was scanned with 2048 samples/line. The data was analysed using the NanoScope Analysis software.

3 Results & Discussion

One of the objectives of this study was to hydrotropically extract lignin from birch and spruce. Lignin was extracted with a variating yield 2-29% for spruce and 16-29% for birch. However, hemicellulose was still present in the samples. The other objective was to create hydrophobic coating dispersions of lignin and zein. It was found that MWL lignin gave the highest CA and lowest cobb values indicating a higher hydrophobicity. Whereas zein in AcOH plasticised with glycerol gave low WVTR values.

3.1 Lignin

The effect of STEX treatment on birch and spruce was studied with compositional analysis and thereafter the biomass was hydrotropically extracted. It was found that MWL lignin provided the most hydrophobic barrier properties, however it was difficult to establish the hydrophobicity of the coating due to the high hydrophobicity of the uncoated paper itself.

3.1.1 Compositional Analysis

As seen in Figure 3.1 and Figure 3.2, the results of the compositional analysis show that the sample had a low lignin yield. One explanation for this is the noticeable loss of mass during the STEX. For both spruce and birch only about 20 wt% of the material was retained after the treatment. A large part of the material was left inside the steam explosion tank and reactor. Additionally, some material was lost to the liquid fraction. Despite this, the total lignin yield was approximately 23 wt% and 32 wt% for spruce and birch respectively. This indicates that other constituents had favourably been removed. It can be seen in Figure 3.2 that the ASL yield is close to 22 wt% for spruce. A higher ASL content can also be found in the liquid fraction (Table 3.1). This, relatively high ASL content together with the low β-O-4 percentage in Table 3.6, indicate a larger depolymerisation of spruce than birch. This, despite earlier studies finding that syringyl rich lignin is more easily depolymerised than guaiacyl rich lignin (Shimizu et al., 1998). The pH of the liquid fraction was 3.11 and 2.87 for spruce and birch respectively. Li and co-workers found that a higher pH suppresses repolymerisation, which could result in birch being repolymerized to a larger extent thus lowering the ASL yield and a resulting in a high Klason lignin yield (J. Li et al. 2007). A higher Klason lignin content can also be found in the liquid fraction of birch (Table 3.1). Another explanation for the high Klason lignin yield in birch might the formation of pseudo lignin. This is further supported by the low xylan yield (see Figure 3.3) (Aarum et al., 2018).

The low ASL content after the STEX, might furthermore be a consequence of the STEX treatment not being conducted at a high severity. The combined severity factor (CSF) is 0.2 and 0.5 for spruce and birch, respectively (Table 2.1). Consequently, the lignin was not degraded as much as it would have in a higher severity and less ASL was formed. The spruce CSF value is similar to the ones found by Kellock et al. (2019) for 180°C steam treatment for 10 minutes.

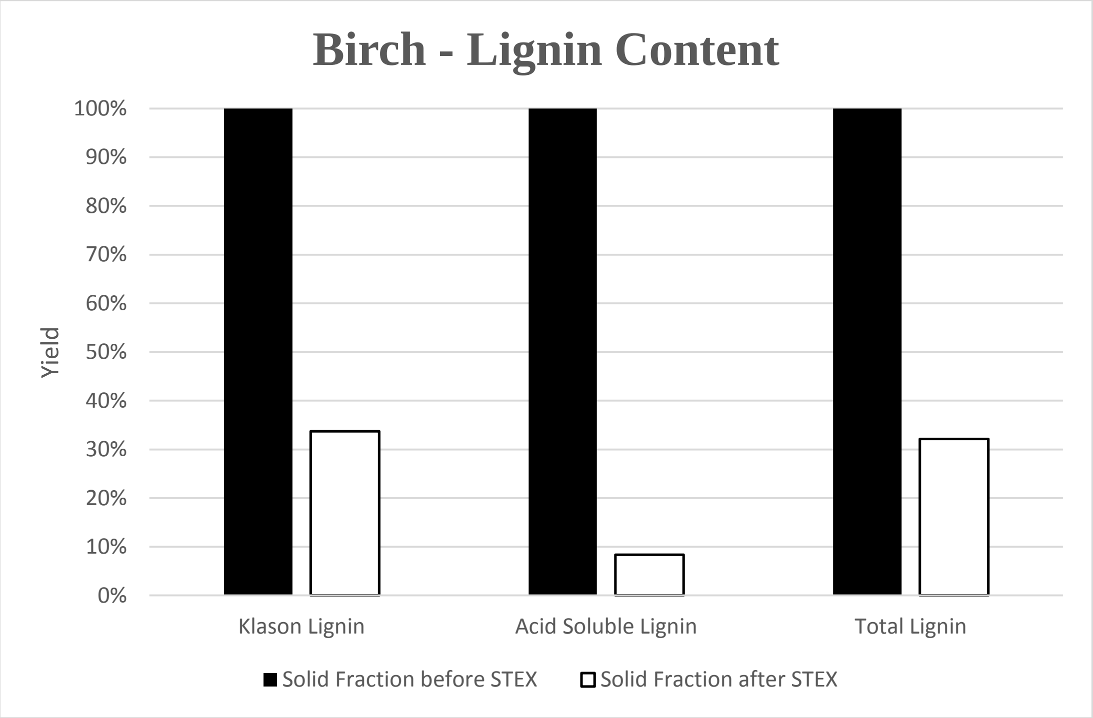

Figure 3.1. Shows the yield of Klason lignin and acid soluble lignin as well as the total lignin in % for the birch before and after STEX.

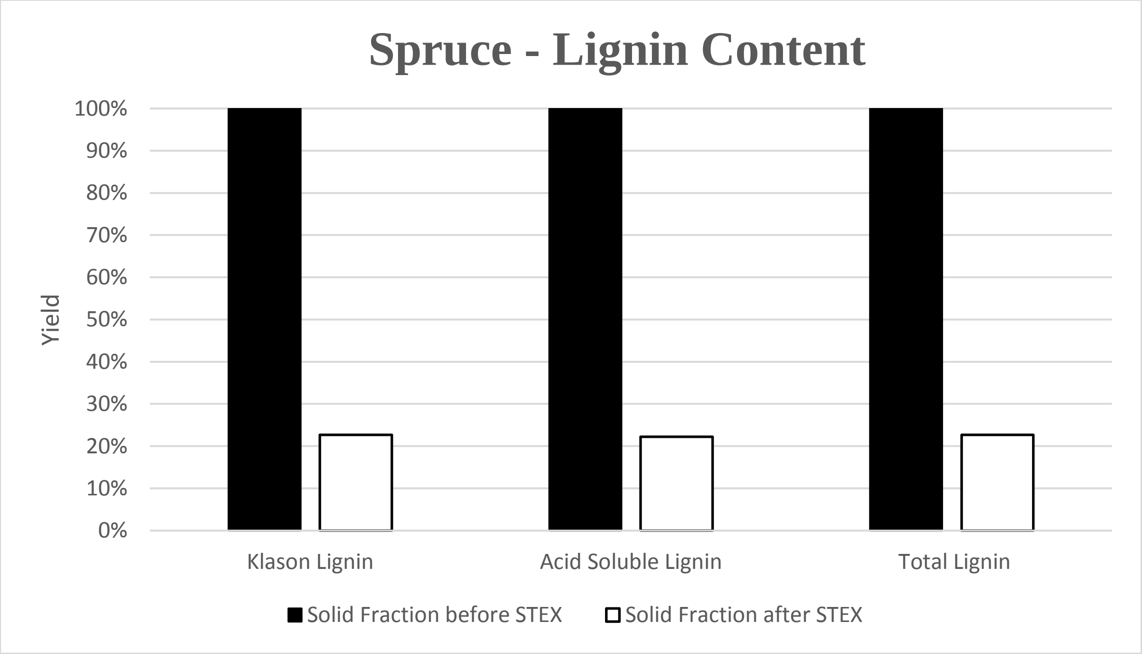

Figure 3.2. Shows the yield of Klason lignin and acid soluble lignin as well as the total lignin in % for the spruce before and after STEX.

The carbohydrate yields are presented in Figure 3.3 and Figure 3.4. From the compositional analysis it can be seen that spruce contains significantly more mannan than birch and that birch contain more xylan than spruce, which is confirmed by literature (Hon & Shiraishi, 2000; Ramos, 2003). For spruce, which had a lower initial xylan content, but a higher initial mannan content, the lowest carbohydrate yield is the mannan yield. The removal of these sugars from the solid fraction into the solid fraction is confirmed in Table 3.1. It was confirmed that the polysaccharide content has decreased in both birch and spruce after the STEX. The glucan content largely represents the cellulose content of the wood, and the higher yield of glucan shows that STEX mainly removes hemicellulose. The total carbohydrate yields for spruce and birch respectivly are ~17% and ~16%. However, the total yields of all carbohydrates not counting glucan are ~14% and ~6%. This confirms that the STEX mainly removes hemicellulose. The difference between birch and spruce can be explained by the fact that C5 sugars (e.g. xylose) are less temperature resistant, and more susceptible to acid hydrolysis, than C6 sugars (Aarum et al., 2018; Ramos, 2003). Because of the different hemicellulose compositions of softwood and hardwood, where softwood contains mainly C6 sugars and birch contains mostly C5 sugars, birch requires a lower severity during the STEX than spruce (Ramos, 2003). This can also be confirmed by the lower pH of the liquid fraction of birch than the liquid fraction of spruce.

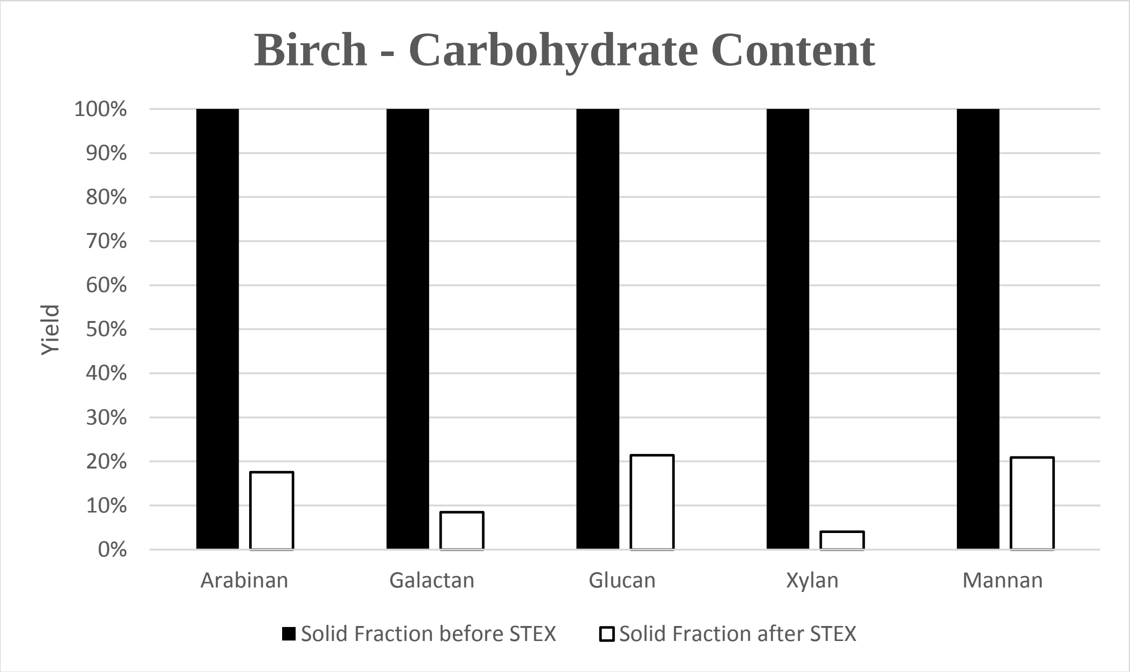

Figure 3.3. Shows the hemicellulose and cellulose yield in the untreated (before STEX) and treated (after STEX) birch.

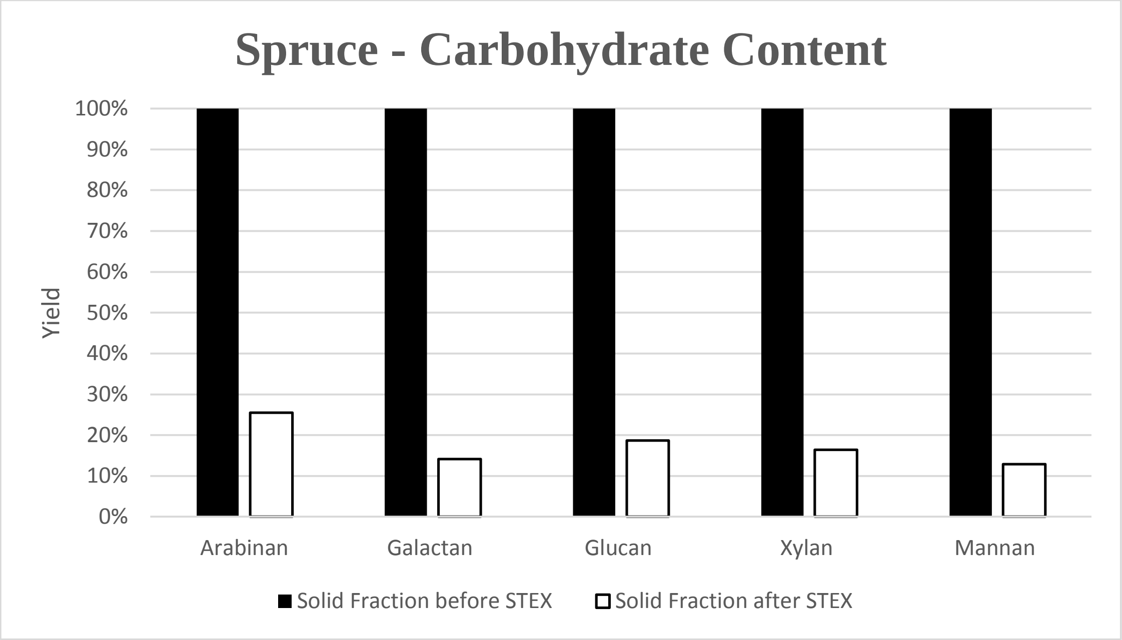

Figure 3.4. Shows the hemicellulose and cellulose yield in the untreated (before STEX) and treated (after STEX) spruce.

Table 3.1. Shows the composition of carbohydrates and lignin in the liquid fraction left from the STEX. The concentrations are given in the unit g/L.

Wood	**Klason lignin [g/L]**	**ASL [g/L]**	**Arabinan [g/L]**	**Galactan [g/L]**	**Glucan [g/L]**	**Xylan [g/L]**	**Mannan [g/L]**
Spruce	0.788	3.002	0.006	0.072	0.165	0.154	0.638
Birch	1.445	1.228	0.005	0.062	0.126	0.702	0.416

3.1.2 Hydrotropic Extraction

Three hydrotropic extractions were performed at 170 ℃ and the yields are presented in Table 3.2. For both the second and third extraction, birch showed a significantly higher yield. This can be explained by birch containing both G and S units whereas spruce only contains G units which is more chemically resistant and harder to extract using SXS. For the first extraction spruce acquired a higher yield than birch. However, the difference is not substantial and the experiments were not made in duplicates. The experimental errors, such as sample sticking to the centrifuge vessels, are the same for all extractions. It cannot be concluded that the yield was not dependent on the SXS concentration. The difference in yield was minimal for the different woods.

Hydrotropic extractions were also made at ambient temperature for both spruce and birch. No lignin could be extracted from spruce at this temperature and very little lignin was extracted from birch. This indicates that hydrotropic extraction requires higher temperature to be efficient. The extract from birch was then examined using HSQC 2D-NMR to confirm that the extracted material was lignin (see 3.1.4 NMR).

Table 3.2. Extraction yields of the three HEX extractions performed at 170 ℃for spruce and birch.

Extraction	Wood	SXS concentration [w/w]	Lignin concentration after STEX [mg/g dry wood]	Weight wood before HEX [g]	Weight Extracted [g]	Yield
1	**Spruce**	34 %	350.4	8.3918	0.84	29 %
1	**Birch**	34 %	342.3	8.4191	0.7225	25 %
2	**Spruce**	40 %	350.4	8.3576	0.0726	2 %
2	**Birch**	40 %	342.3	8.3569	0.462	16 %
3	**Spruce**	40 %	350.4	13.9486	0.55	11 %
3	**Birch**	40 %	342.3	13.9337	1.36	29 %

As seen in Table 3.3, a little less than half of the samples were not soluble in acetone. This left the dispersion turbid as the insoluble substances were dispersed in the acetone. Even if the insoluble particles sedimented they were more difficult to avoid when pipetting the last part of the dispersion. This raises the question of the importance of purification. Cellulose is only slightly soluble in acetone (Naz et al., 2016). Additionally, lignin is not completely soluble in pure acetone (Domínguez-Robles et al., 2018). This indicate that these constituents are present in the insoluble fraction. However, this needs to be examined further. Moreover, other solvents might affect the weight of the insoluble substances.

Table 3.3. Weight of the insoluble residues and the dissolved lignin in the S170 and B170 acetone dispersions.

Sample	Weight Insoluble [g]	Material added [g]	Weight dissolved [g]
S170	0.3668	0.84	0.4732
B170	0.3915	0.84	0.4485

3.1.3 Milled Wood Lignin

As seen in Table 3.4, the extraction yield from the MWL extraction is very low at 3% and 7% for spruce and birch respectively. The expected yield according to literature is between 20-30% (Obst & Kirk, 1988). One explanation for the low yield compared to literature values is the different dioxane:water ratio was used, (50:3) vs (96:4) according to literature (Obst & Kirk, 1988). Another reason is the difference in pre-treatment method. The wood in this study was pre-treated using STEX at a low severity (190 ℃). Consequently, this might have led to the pre-treatment process not being as effective and not opening up the structure enough for the MWL extraction. Nevertheless, the yield was still lower for spruce than for birch which can be explained by spruce primarily containing the chemically resistant G units (Korpinen & Fardim, 2009). As seen in Figure 3.5, the birch samples are significantly darker than the spruce samples. This further confirms the lower extraction yield besides the gravimetric measurements. Still, a higher lignin yield might not be the only explanation for the colour difference since birch contains extractives that might be the source of the pigment (J. Li et al., 2009).

Table 3.4. Extraction yields from the MWL extracted from lignin pre-treated with STEX.

Wood	**Lignin concentration after STEX [mg/g dry wood]**	**Weight wood before MWL [g]**	**Weight Extracted [g]**	**Yield**
Spruce	350.4	6.0048	0.0626	3 %
Birch	342.3	6.0343	0.1451	7 %

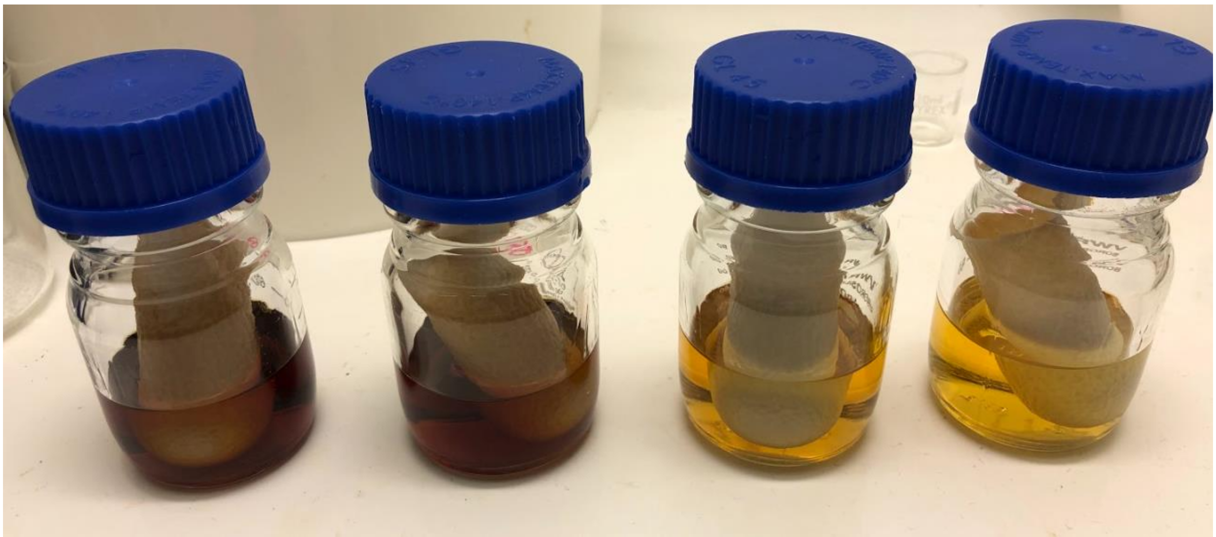

Figure 3.5. Two first samples from the left: STEX pre-treated MWL extracted birch. The two samples to the right: STEX pre-treated MWL extracted spruce samples

Residues insoluble in acetone in the MWL dispersions are presented in Table 3.5. As seen, about 25-36% of the samples were not soluble in acetone, indicating that the extracted samples contained other substances that were also soluble in dioxane, but not in acetone. However, the insoluble residues were conglomerated and not easily sprayed onto the paper and adding a purification step might not be necessary. The expected carbohydrate residues when using a 96:4 (96%) dioxane:water ratio method is around 10%. Furthermore, earlier studies show that using a 9:1 (90%) dioxane:water ratio gives an even higher carbohydrate concentration. (Obst & Kirk, 1988) This indicates that using a 50:3 (~94%) dioxane:water ratio will result in a carbohydrate concentration larger than 10 %, since more water is used than for the method suggested by Obst & Kirk (1988). As discussed in *3.1.2 Hydrotropic Extraction*, it is likely that the insoluble residues are carbohydrates as well as a small amount of lignin.

Table 3.5. Weight of the insoluble residues and the dissolved lignin in the MWL acetone dispersions.

Sample	**Weight Insoluble [g]**	**Material added [g]**	**Weight dissolved [g]**
MWL spruce	0.0228	0.0626	0.0398
MWL birch	0.0358	0.1451	0.1093

3.1.4 NMR

In the HSQC 2D-NMR analysis of the HEX lignin it was found that SXS was still present in the extracted lignin which could have affected the hydrophobicity of the coatings. In the ^{31}P NMR it was found that there were more hydroxyl functional groups present in the birch sample compared to spruce. The presence of COOH in the birch sample indicates that hemicellulose is still present that lead to autohydrolysis during the HEX resulting in the release of AcOH.

HSQC 2D-NMR

In the HSQC 2D-NMR spectrum the characteristic β-O-4 linkages could be found for all the samples as shown in Table 3.6. The peaks were assigned according to values in literature (del Río et al., 2012; Maniet et al., 2017; Wen et al., 2014). However, the amount of β-O-4 linkages in obtained samples were significantly lower than those in previous studies (Sette et al., 2011). The β-5 linkage (phenyl coumaran) could only be found for the B170 and S170 sample. The amount of this linkage was similar to that of previous studies (Sette et al., 2011). For all the samples residues from the SXS (δ_C/δ_H = 120-135/6.9-7.7) could be found (see Figure 3.6, Figure 3.7 and Figure 3.8). The spectrum of the SXS was predicted using MestreNova, see Figure A-1 in Appendix A.

Interestingly, the β-O-4 and β-5 values found for the S170 are similar to the ones found in a previous study for spruce that was pre-treated with AcOH as an acid catalyst and then STEX-treated at 210°C for 5 minutes (Caputo et al., 2022). This could suggest that the HEX increase the degree of delignification.

For the birch samples the S/G ratio was found to be much larger for the lignin extracted at 25°C, 3.25 compared to 0.83 for B170 (see Table 3.6). Subsequently the values for B25 is much higher than literature values 1.38-1.67 (Sette et al., 2011; Wen et al., 2014) and B170 is lower. This suggest G units are affected by the extraction process, since the S/G ratio would decrease if more G units were extracted. This compared to the spectrums of B170 (Figure 3.6) and that of B25 (Figure 3.8) supports this argument since there are only β-O-4 linkages connected to a S unit for the B25 but no β-O-4 linkage connected to a G unit. The failed attempt to hydrotropically extract lignin from spruce at 25 ℃ could further confirm the chemical resistans of the G units.

Table 3.6. Amount of inter-unit linkages as % of C_9 units in lignin from the different woods that were HEX at different temperatures.

	β-*O*-4	**β-5**	**β-1**	**β-β**	**5-5'-*O*-4**	**S/G**
B170	18.8	9.6	–	4.0	Trace	0.83

S170	7.8	10.4	–	Trace	Trace	–
B25	12.5	–	–	–	–	3.25

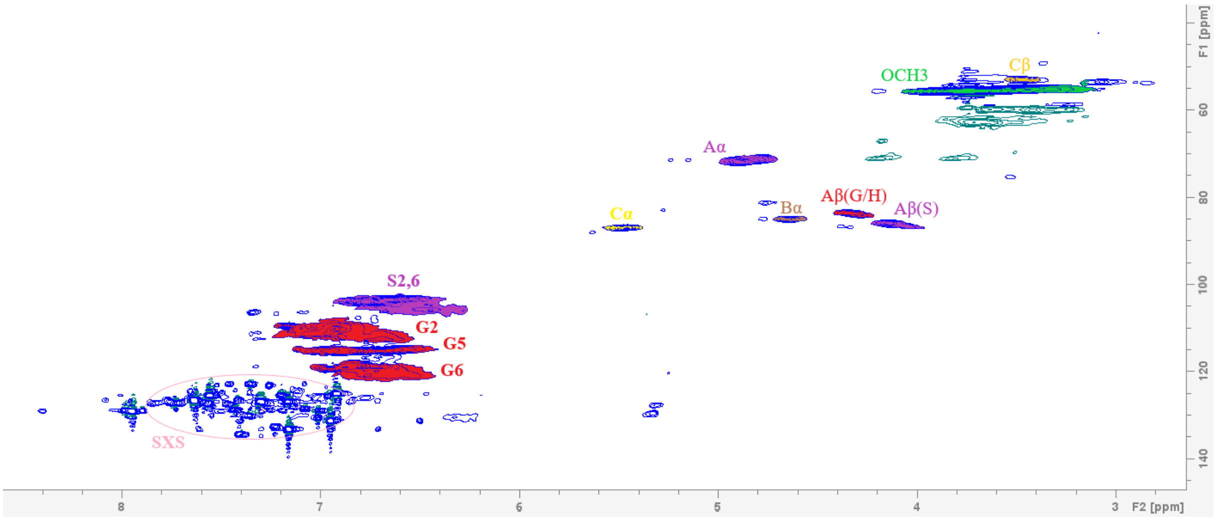

Figure 3.6. 2D-NMR spectrum of HEX-birch extracted at 170 ℃

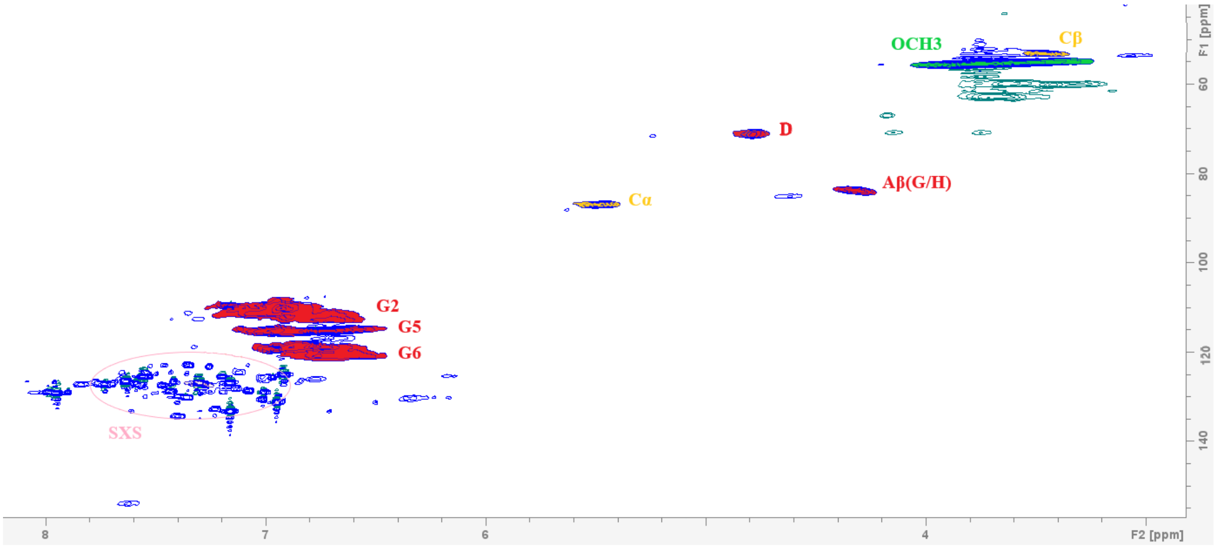

Figure 3.7. 2D-NMR spectrum of HEX-spruce extracted at 170 ℃

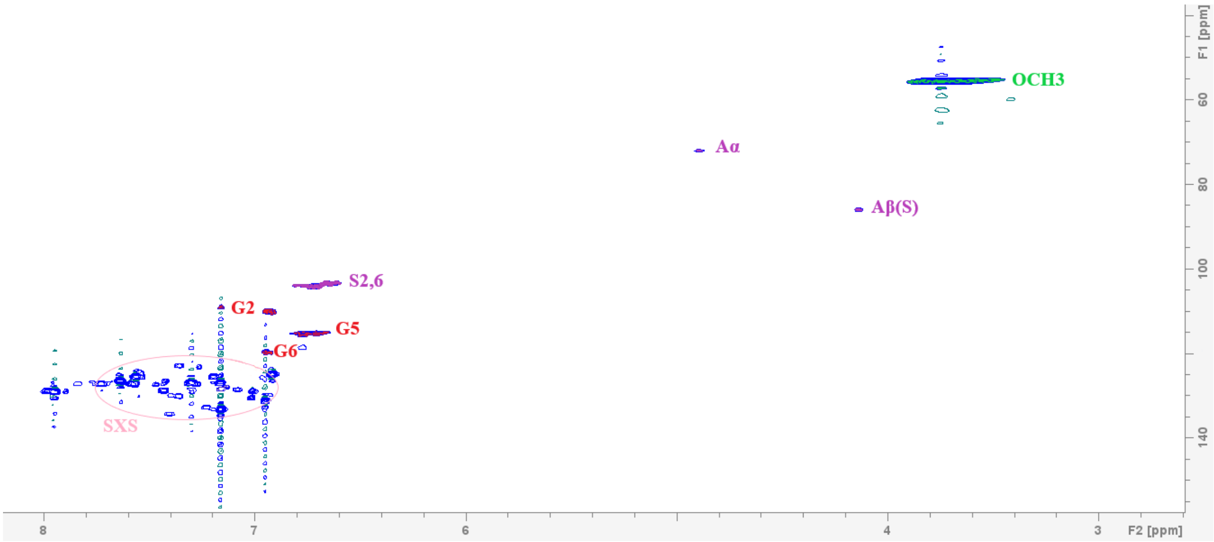

Figure 3.8. 2D-NMR spectrum of HEX birch extracted at 25 ℃

Table 3.7. The labels used in the HSQC 2D-NMR spectrum in Figure 3.6, Figure 3.7, and Figure 3.8. These values are compared to literature values (del Río et al., 2012; Maniet et al., 2017; Wen et al., 2014).

Label	**Unit**
Cβ	Cβ-Hβ in phenylcoumaran
OCH3	C-H linkage
D	Cα-Hα in β-O-4 linked to G
Aα	Cα-Hα in β-O-4 linked to S
Aβ(G/H)	Cβ-Hβ in β-O-4 linked to G
Bα	Cα-Hα in β-β’ resinol substructure
Aβ(S)	Cβ-Hβ in β-O-4 linked to S
Cα	Cα-Hα in phenylcoumaran
S2,6	C2,6-H2,6 in S
G2	C2-H2 in G
G5	C5-H5 in G
G6	C6-H6 in G
SXS	SXS

^{31}P NMR

^{31}P NMR was used to examine the hydroxyl group concentrations of the extracted lignin. The obtained spectrums can be seen in Figure 3.9 and Figure 3.10. The peaks were assigned according to literature values (Meng et al., 2019; Pu et al., 2011). In accordance with an earlier study (Gnana Prakash et al., 2022), the acquired results (Table 3.8) show a very low to no carboxyl group content after the HEX. In total, birch had a higher hydroxyl group concentration than spruce. The hydroxyl groups might slightly increase the hydrophilicity of the lignin; however, the hydrophobicity is dominated by the molecular weight (MW). The higher Ph-OH concentration might also be an indication of depolymerisation since it can be formed when β-O-4 bonds are cleaved (Katahira et al., 2018), which means that the MW of the lignin might be lower, and a less hydrophobic lignin might consequently be acquired (Q. Yang & Pan, 2016). Still, hydroxyl groups could have a positive effect on the hydrophobicity if the hydroxyl groups were to be modified by the addition of hydrophobic groups, e.g., long hydrocarbon chains.

The reason for the peak at 134.7 in Figure 3.9 could be due to release of organic acids from the biomass due to hemicelluloses left in the solid fraction of STEX treated birch. The high temperature (170 ℃) leads to autohydrolysis of the biomass which releases organic acids such as acetic and formic acid which possibly solubilises the hemicellulose (Devendra et al., 2016).

Table 3.8. Shows the hydroxyl group concentrations of the spruce and birch lignin samples after HEX at 170 ℃obtained by ^{31}P NMR. The values are calculated from the integrals obtained from the spectrums in Figure 3.9 and Figure 3.10.

Spruce		**Birch**	
Sample weight [g]	0.0196	**Sample weight [g]**	0.0198
Al-OH concentration [mmol/g]	1.48	**Al-OH concentration [mmol/g]**	1.70
Ph-OH concentration [mmol/g]	1.02	**Ph-OH concentration [mmol/g]**	1.32
COOH concentration [mmol/g]	BDL	**COOH concentration [mmol/g]**	0.0723
Total OH concentration [mmol/g]	2.50	**Total OH concentration [mmol/g]**	3.09

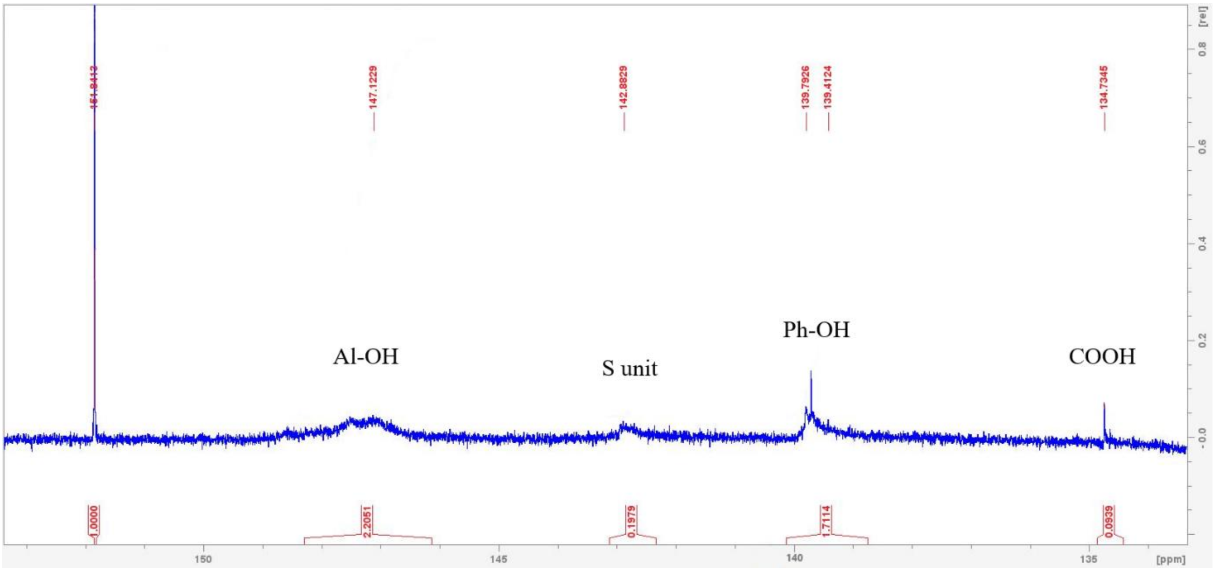

Figure 3.9 Presents the ^{31}P NMR spectrum for B170.

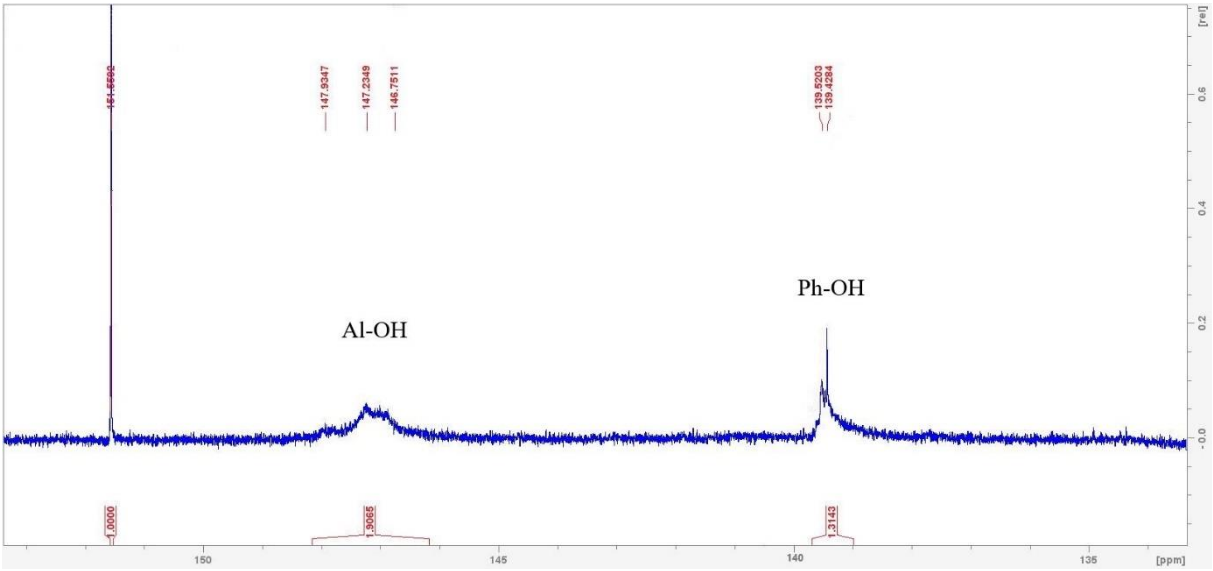

Figure 3.10. Presents the ^{31}P NMR spectrum for S170.

3.1.5 AFM

All the samples that were investigated with AFM showed a variating surface average (Sa)which indicates an uneven surface topography which could be correlated to the better water barrier properties (Heydari et al., 2013; Mohamad et al., 2013). However, from the AFM images, it was not possible to conclude if there were any pinholes in the coating. Also, the lignin fibres were too large to investigate on the nanoscale. Therefore, further studies on the surface morphology on a different scale might be of more interest and give more information about the coating.

Looking at the values in Table 3.9, it is possible that the fibres in the paper rise when wetted, resulting in a higher variability of Sa for the coated samples compared to those of the reference sample. Another possibility is that the large difference in Sa is because of the unevenness in the coating. However, it has not been established how the solvents affect the coated paper, and it is therefore difficult to say if the high surface roughness is due to lignin in the coating or due to fibre rising as a result of wetting of the paper surface.

Table 3.9. The surface average (Sa) and surface-developed interfacial area ratio (SDR) for the lignin and reference samples.

	B170		S170		Spruce MWL		Reference	
Sample nr.	12		15		17		–	
Sa [nm]	237	94.3	127	313	452	178	189	196
SDR [%]	11.3	12.7	20.7	16	58.2	12.3	35.7	24.5

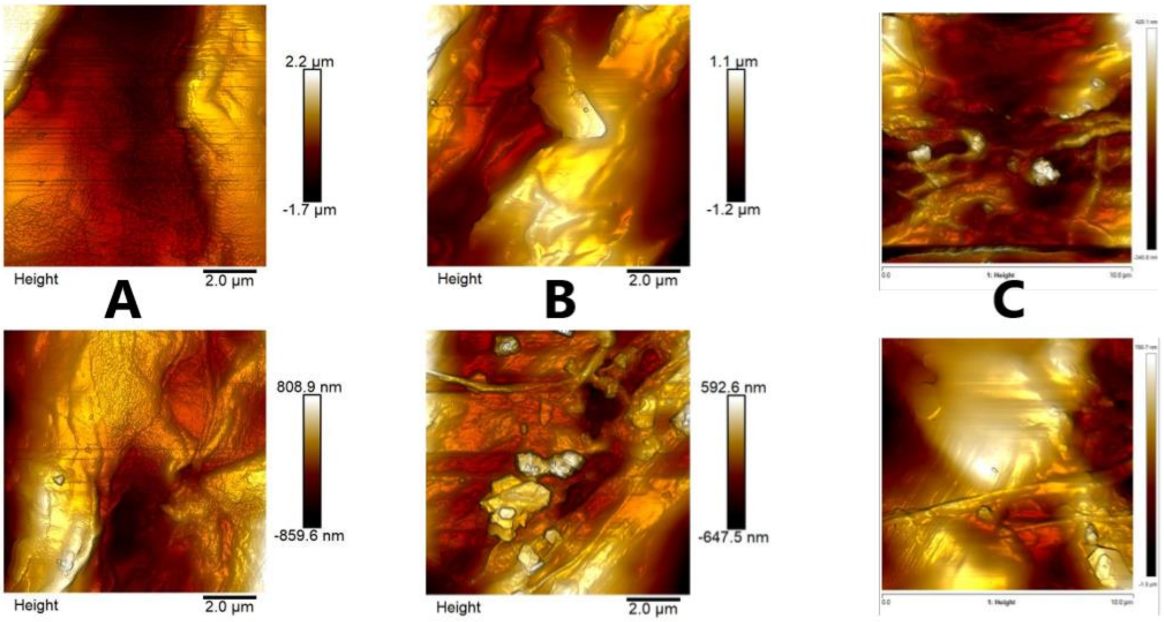

Figure 3.11. The AFM images of A) Spruce MWL, B) Spruce HEX at 170 °C and C) Birch HEX at 170 °C.

3.1.6 Hydrophobic Coating Properties

As seen in

Table 3.10, the reference (uncoated) samples have the highest water CA. The MWL samples show the best cobb and CA values. Furthermore, the CA and moisture uptake values show no significant difference between spruce and birch in the HEX-lignin and MWL, pointing towards that the difference in using softwood or hardwood in waterproof barriers is not significant. However, HEX-lignin is much more available than MWL and the use of MWL in packaging might not be industrially viable. One reason for this is the long extraction time of the MWL extraction process (Wegener & Fengel, 1977). Furthermore, dioxane is both expensive and toxic (Sigma-Aldrich, n.d.). Still, the positive results of MWL, which is structurally close to native lignin (Brunow et al., 1999; Obst & Kirk, 1988), highlights the many possibilities for the use of lignin in water-proof barriers. The reason for this is that the hydrophobicity is dominated by the aromatic backbone of lignin and the hydrophobicity is therefore also negatively correlated with the depolymerisation of lignin (Q. Yang & Pan, 2016). This underlines the importance of optimizing the many parameters of the extraction, e.g., temperature and residence time, to obtain as hydrophobic lignin as possible. Another parameter that might have negatively affected the HEX-lignin is the presence of SXS in the dispersions. It is likely that SXS, which is a hydrotrope, made the coating more hydrophilic which suggests that purification by the removal of SXS from the lignin might be a viable way to increase the coating's water-proof properties.

The negative coating weight observed in some samples can be explained by the inexact scale used to weigh the samples in a combination with the low weight of coating added. Since the scale used did not have a high enough sensitivity the errors become extra apparent when the weight of added lignin was very small which was the case for the MWL sample where only a small amount of lignin was available due to the extraction method having a low yield. A possible source of errors in the CA measurements is the uneven coating where it was not possible to avoid measuring on stains which locally had thicker coatings, see Figure B-2.

Not all coatings were tested for WVTR, but as is shown in

Table 3.10, the WVTR is very high for all tested lignin coatings. This indicates that these lignin coatings are not appropriate as water vapour barrier even if they show potential as barrier for water in its liquid state. The coatings show very high values in comparison with earlier studies (Hult, Koivu, et al., 2013; Hult, Ropponen, et al., 2013) and it should be considered that possible explanations are the low weights of the coatings and the uneven application which leaves very thin patches. The thin coating might in the worst-case scenario even result in pinholes in the coating which eases the water vapour transmission.

Additionally, the hydrophobic properties of the paper need to be taken into consideration. These properties make it difficult to conclude whether the hydrophobic properties of the MWL coatings is due to the hydrophobicity of the lignin or the paper. The good barrier properties of the MWL might thus be a consequence of the thin layer of MWL applied. In other words, if the MWL coating was not thick enough, the properties measured might primarily have been the properties of the paper. This also makes the MWL and the HEX lignin difficult to compare, since the coating weight of the HEX samples are much higher, and the paper properties might consequently not dominate as much for these coatings. Finally, the conclusion that can be drawn is that for this (hydrophobic) paper the lignin coatings have decreased the liquid barrier properties. The low moisture uptake of the bare paper in contrast to that of the coated papers could be explained by the moisture uptake of the coatings in addition to the moisture uptake of the papers. For less hydrophobic papers, however, these coatings might improve the hydrophobic barrier. The success of lignin coatings as hydrophobic barriers have been reported in earlier studies (Hult, Koivu, et al., 2013; Hult, Ropponen, et al., 2013). Still, this should be studied further, and the water barrier properties should be established on paper sheets with low hydrophobicity.

Table 3.10. Coating properties of the lignin coatings. The coating weight in g as well as in g/m^2 is presented together with the hydrophobic properties; contact angle for both water and DM as well as the moisture uptake in g/m^2. The expected coating weight is the weight of the

coating if all the sprayed sample landed on the paper. The reference samples refer to uncoated paper. WVTR was measured at 23ºC and 50% RH.

	B170		**Birch MWL**	**S170**		**Spruce MWL**	**Reference**	
Sample nr.	**12**	**13**	**14**	**15**	**16**	**17**	**Ref1**	**Ref2**
Coating Weight [g]	0.12	0.11	-0.2	0	-0.15	-0.16		
Expected Coating Weight [g]	0.22	0.22	0.11	0.24	0.24	0.04		
Coating Weight [g/m^2]	1.9	1.7	-3.2	0.0	-2.4	-2.5		
CA (Water) [°]	79.04 ±4.24	83.09 ±4.66	106.66 ±2.15	75.83 ±4.46	78.17 ±5.35	105.69 ±3.28	112,02 ±2,19	114,86 ±2,83
CA (DM) [°]	31.83 ±7.02	33.49 ±6.87	37.24 ±6.75	39.38 ±6.46	33.85 ±6.45	33.74 ±6.86	33,87 ±7,24	38,01 ±5,89
Moisture Uptake [g/m^2]	42.23	41.45	37.47	45.57	42.98	36.75	35.91	
WVTR [g/(m^2 day)]	1400			1275		1250		

3.2 Zein

The zein coatings were characterised using atomic force microscopy (AFM), CA, cobb test and WVTR.

3.2.1 AFM

The AFM analysis of the zein coatings showed that samples 2 and 10 had a relatively smooth surface compared to sample 8 and the lignin coatings (see Figure 3.12). The relative smoothness of samples 2 and 10 might be correlated with the low values of the WVTR.

In the images of samples 2 and 10, it was also possible to identify pinholes that were present in the coating, see Figure 3.12. There are also notably fewer pinholes in the images taken of sample 10 than the image of sample 2, which might be the reason for the lower WVTR value.

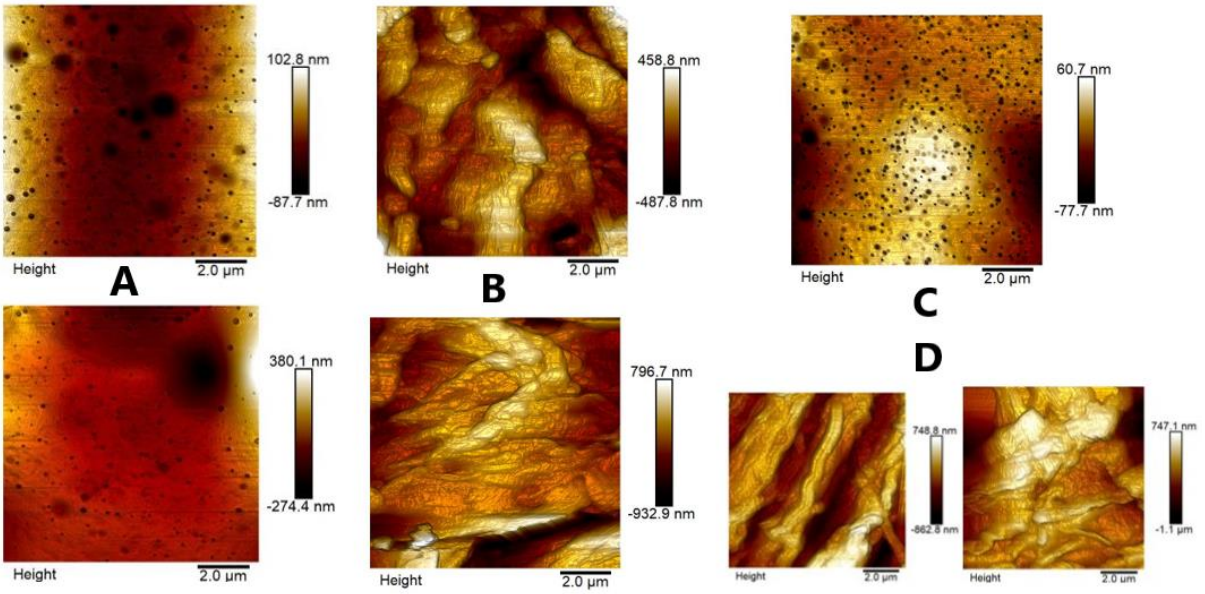

Figure 3.12. The AFM images of A) 10wt% zein in AcOH plasticized with glycerol (sample 10), B) 10wt% zein in AcOH (sample 8), C) 10wt% zein in EtOH (sample 2) and D) uncoated reference samples.

Comparing the values of sample 8 to that of the reference sample, the sample 8 seem to have similar surface roughness. One possibility is that the thickness of the coating of sample 8 is much thinner which could result in a topography that is more similar to that of the uncoated paper. Another reason could be that the solvent affects the paper which could result in fibre rising. However as was discussed in *3.1.5 AFM*, it is difficult to say how the solvent affected the surface as this was not investigated.

Table 3.11. The surface average (Sa) and surface-developed interfacial area ratio (SDR) for the zein and reference samples.

	EtOH + 10% Zein		**AcOH + 10% Zein**		**AcOH + 10% Zein + Glycerol**		**Reference**	
Sample nr.	**2**		**8**		**10**		**−**	
Sa [nm]	14.1	−	109	174	27.4	46.9	189	196
SDR [%]	1.21	−	14	35.7	0.942	1.59	35.7	24.5

3.2.2 Hydrophobic Coating Properties

As seen in Table 3.12, the highest water CA was achieved with the coating containing 15wt% zein. A possible explanation is the uneven surface formed when the 15wt% zein dispersion was spray coated on to the surface which promotes a high water CA. However, it also obtained the highest moisture uptake, making it unsuitable as a water barrier. The high moisture uptake could be explained by the presence of pinholes. Because of the higher viscosity of the 15wt% zein dispersion, it was more difficult to spray and the coating might consequently have become more unevenly spread. This would also explain why sample 4 and 5 show vastly different moisture uptakes. It must therefore be considered whether spray coating is the optimal application method for this dispersion.

The CA for the 10% zein in EtOH and AcOH (Table 3.12), both these samples had values similar to those seen in a previous study. In their study Shi et al. (2009) found that zein dispersed in EtOH and AcOH gave CA values of 73.2 ± 0.7° and 79.4 ± 2.7° respectively.

Looking at the coating weight for the samples there is a large difference between the measured value and the expected value (see Table 3.12). As mentioned previously in *3.1.6 Hydrophobic Coating Properties*, this might be because of the inexact scale that was used for the measurements. However, comparing the expected values for lignin in Table 3.10 with the expected values for zein in Table 3.12, it can be seen that the weight for the zein coatings are higher. A larger coating weight could mean that the barrier properties of the paper are less dominant as this could imply a thicker coating. However, it must be taken into consideration that the thickness of the coating does not necessarily correlate with the coating weight (Lavoine et al., 2014).

The two coatings containing 10 % zein without plasticiser showed the lowest moisture uptake as well as similar CA around 75 °. The results in Table 3.12 indicate that the importance of the solvent is more apparent when glycerol is added. The ethanol dispersion with added glycerol has a significantly higher water CA than the acetic acid dispersion containing glycerol. This could be explained by the protonation of zein in AcOH, which partially unfolds the protein *(Y. Li et al., 2012)*. However, the arrangement and cross section of these coatings needs to be studied further to understand glycerol's effect on the CA and moisture uptake.

The coating of sample 10 shows great potential as a vapour barrier and presents a significantly lower WVTR than coating 8 and 2. This, despite presenting a low water CA. This potentially highlights the importance of using a plasticiser. Because even if the glycerol is hydrophilic, a possible explanation for these great results could be that glycerol is not distributed evenly throughout the coating but is rather concentrated on the surface thus lowering the CA. An earlier study confirm that glycerol has the tendency to migrate to the surface in zein films (Parris & Coffin, 1997). Another study confirm that a low concentration of plasticiser decrease the WVTR as a result of the increased mobility in zein and the decrease in free volume (Ghanbarzadeh et al., 2007). Furthermore, as seen in the AFM, the plasticiser contributes to a smoother coating, possibly because of a higher mobility in the zein, and because of the distribution potentially is superficial, vapour is transmitted at a lower rate.

The water vapor barrier is significantly better for zein dissolved in EtOH than in AcOH. This can be explained by how the zein is arranged in the different solvents. Comparing the CA for the different solvents in the 10wt% zein dispersions there is not a significant difference between using EtOH or AcOH as solvent, however zein dissolved in AcOH gives the lowest moisture uptake. The addition of glycerol to the 10wt% zein in EtOH sample does not result in a much different CA or moisture uptake. However, the addition of glycerol to the 10wt% zein in AcOH sample significantly alters the CA and moisture uptake compared to the sample without plasticiser. This suggests that the presence of glycerol and AcOH changes the tertiary structure of the zein coating. However, further studies needs to be made to confirm this.

To finish, it cannot be concluded whether or not the hydrophobic paper affects the results of these coatings, but the results are comparable with literature data (Shi et al., 2009), suggesting that these values might not be dominated by the hydrophobic paper.

Table 3.12. Coating properties of the different zein coatings. The coating weight in g, g/m^2 and an expected value calculated from the density of the coating is presented together with the hydrophobic properties; contact angle for both water and DM as well as the moisture uptake in g/m^2. Reference values of the uncoated paper can be found in

Table 3.10. WVTR was measured at 23ºC and 50% RH.

	EtOH + 10% Zein			**EtOH + 15% Zein**		**EtOH + 10% Zein + Glycerol**		**AcOH + 10% Zein**		**AcOH + 10% Zein + Glycerol**	
Sample nr.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Coating Weight [g]	0.01	0.2	0.15	0.24	0.26	0.25	0.26	0.25	0.3	0.51	0.47
Expected weight [g]	0.52	1.05	1.05	1.54	1.54	1.28	1.28	1.33	1.33	1.62	1.62
Coating Weight [g/m^2]	0.2	3.2	2.4	3.8	4.1	4.0	4.1	4.0	4.8	8.1	7.5
CA (water) [º]	74.48 ±3.04	75.74 ±2.31	76.51 ±1.91	90.18 ±14.33	86.26 ±5.27	70.95 ±2.21	71.96 ±2.64	76.46 ±3.38	71.65 ±6.48	57.59 ±5.91	56.44 ±5.53
CA (DM) [º]	37.74 ±3.77	38.10 ±3.34	35.38 ±5.05	30.27 ±6.61	28.57 ±6.46	44.85 ±1.93	44.80 ±1.66	41.47 ±3.03	43.14 ±2.04	53.12 ±1.80	56.40 ±2.84
Moisture Uptake [g/m^2]	36.99	35.27	37.91	60.41	97.74	44.62	47.27	32.17	34.09	45.76	46.36
WVTR [g/(m^2 day)]		710						1136		89	

4 Conclusions

The paper that the dispersions were coated on was already hydrophobic. As a result, no improvement in the liquid barrier properties was made for any of the coatings. The paper was not, however, measured for WVTR and the role of the paper as a vapour barrier was not established.For the zein and HEX coatings a sufficiently high coating weight was applied for a large difference in water barrier properties compared to the paper. This cannot be concluded for MWL, since the coating weight was low. Consequently, it cannot be established whether or not the good liquid barrier properties should be contributed to the MWL or the paper.

MWL shows potential which highlights the importance of the extraction process. Extracting a lignin with a longer aromatic backbone can result in a barrier with similar characteristics as the MWL. Less harsh conditions might be a solution; however, this study shows that HEX at ambient temperature is not viable. Another downside of the HEX lignin is the presence of the hydrotrope. An improved removal of the SXS might potentially provide a more hydrophobic lignin coating since the hydrotrope is amphiphilic. Removal of other impurities in the sample might also improve the hydrophobic properties.

There does not seem to be a large barrier difference between birch and spruce, indicating that the difference in barrier properties between hardwood and softwood is not important. The large difference seems to be in extraction yield, where hardwood is easier to extract.

For zein, there is not a significant difference between the solvents except for when glycerol is added. Glycerol as a plasticiser provides a less hydrophobic barrier, but still a lower WVTR.

Furthermore, the analysis of the lignin coatings in nanoscale might be too small since the fibres are too large, and it might be of bigger interest to investigate the surface on microscale to see if the topography of the coating has any correlation with the water and water vapour barrier properties.

In the AFM analysis of the zein coatings, it was showed that 10wt% zein in EtOH and 10wt% zein in AcOH plasticised with glycerol dispersions gave smooth topography on the coated paper with pinholes present. It is possible that the smooth coating correlated with the lower WVTR for these samples.

From this study it was concluded that the zein coatings formed a better water vapour barrier, while the lignin coatings acted as a better water barrier.

5 Future Recommendations

From the conclusions drawn in this report, the following recommendations for future work were reached:

Since the results suggest that the extraction process had an impact on the hydrophobicity of the lignin coating the extraction method should in the future be optimized to extract a lignin closer to protolignin. Furthermore, it could be of interest to investigate the importance of molecular weight of the lignin for the water barrier. Therefore, it is recommended to conduct a size exclusion chromatography of the extracted lignin to see how the extraction method affects the molecular weight. Another strategy could also be to modify the lignin, making it more hydrophobic. However, for food packaging there are regulations on what modifications are allowed and this needs to be kept in mind.

The role of different solvents for lignin should be studied, for example ammonium hydroxide that has shown good potential in earlier studies. It should also be looked into purification of the hydrotropic lignin, since the 2D NMR spectrums showed that the SXS remained in the samples. Since the SXS is amphiphilic and soluble in aqueous solutions, the effect of SXS on water barrier properties should also be studied. The other residues in the lignin samples that were not soluble in acetone should also be characterized in a future study.

It could be seen in the zein coatings that the plasticizer was very important to achieve good WVTR results. In the future, it should therefore be investigated if a less hydrophobic plasticizer, e.g., oleic acid, that can also aid the coating as a liquid barrier. Additionally, it would be interesting to study the interaction between the plasticizer, zein and the solvent. This could for example be studied by examining the cross-section of the coating to studying migration of the plasticizer in the coating and how the plasticiser is dispersed, e.g., if there is a concentration gradient. The role of a hydrophobic plasticizer should also be studied for lignin, since very high WVTRs were measured for these coatings.

To further understand the difference in water barrier properties between these coatings it should be considered to make freestanding films or films on less hydrophobic paper. It should also be studied how different solvents affect the hydrophobic properties of the paper.

Moreover, the application method should be optimized. For spray coating, parameters that could be varied are the spraying distance, spraying temperature, and pressure of the spray gun. Additionally, spray drying could potentially be a good candidate for the low viscosity dispersions. For the higher viscosity dispersions other coating methods, such as casting, could also be used. In the beginning of this study 15wt% zein dispersions were made for both solvents both with and without glycerol. However, only the properties of the 15wt% zein in EtOH was studied because the high viscosity made the dispersion difficult to spray onto the paper. Lastly, the importance of coating weight and the existence of pinholes should be examined further. It is also recommended that the topography of the lignin samples should be studied on a larger scale than on a nanoscale.

6 Bibliography

Aarum, I., Devle, H., Ekeberg, D., Horn, S. J., & Stenstrøm, Y. (2018). Characterization of Pseudo-Lignin from Steam Exploded Birch. *ACS Omega*, *3*(5), 4924–4931. https://doi.org/10.1021/acsomega.8b00381

Alwadani, N., Ghavidel, N., & Fatehi, P. (2021). Surface and interface characteristics of hydrophobic lignin derivatives in solvents and films. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *609*, 125656. https://doi.org/10.1016/j.colsurfa.2020.125656

Ansari, K. B., & Gaikar, V. G. (2014). Green hydrotropic extraction technology for delignification of sugarcane bagasse by using alkybenzene sulfonates as hydrotropes. *Chemical Engineering Science*, *115*, 157–166. https://doi.org/10.1016/j.ces.2013.10.042

Brunow, G., Lundquist, K., & Gellerstedt, G. (1999). Lignin. In E. Sjöström & R. Alén (Eds.), *Analytical Methods in Wood Chemistry, Pulping, and Papermaking* (pp. 77–124). Springer. https://doi.org/10.1007/978-3-662-03898-7_4

Caputo, F., Al-Rudainy, B., Naidjonoka, P., Wallberg, O., Olsson, L., & Novy, V. (2022). Understanding the impact of steam pretreatment severity on cellulose ultrastructure, recalcitrance, and hydrolyzability of Norway spruce. *Biomass Conversion and Biorefinery*, 1–13.

Decatur, J. (2020). *HSQC and HMBC for topspin*. https://nmr.chem.columbia.edu/sites/default/files/content/HSQC%20and%20HMBC.pdf

del Río, J. C., Rencoret, J., Prinsen, P., Martínez, Á. T., Ralph, J., & Gutiérrez, A. (2012). Structural Characterization of Wheat Straw Lignin as Revealed by Analytical Pyrolysis, 2D-NMR, and Reductive Cleavage Methods. *Journal of Agricultural and Food Chemistry*, *60*(23), 5922–5935. https://doi.org/10.1021/jf301002n

Devendra, L. P., Kiran Kumar, M., & Pandey, A. (2016). Evaluation of hydrotropic pretreatment on lignocellulosic biomass. *Bioresource Technology*, *213*, 350–358. https://doi.org/10.1016/j.biortech.2016.03.059

Domínguez-Robles, J., Tamminen, T., Liitiä, T., Peresin, M. S., Rodríguez, A., & Jääskeläinen, A.-S. (2018). Aqueous acetone fractionation of kraft, organosolv and soda lignins. *International Journal of Biological Macromolecules*, *106*, 979–987. https://doi.org/10.1016/j.ijbiomac.2017.08.102

Dong, F., Padua, G., & Wang, Y. (2013). Controlled formation of hydrophobic surfaces by self-assembly of an amphiphilic natural protein from aqueous solutions. *Soft Matter*, *9*, 5933–5941. https://doi.org/10.1039/C3SM50667C

Dong, F., Zhang, M., Tang, W.-W., & Wang, Y. (2015). Formation and Mechanism of Superhydrophobic/Hydrophobic Surfaces Made from Amphiphiles through Droplet-Mediated Evaporation-Induced Self-Assembly. *The Journal of Physical Chemistry B*, *119*(16), 5321–5327. https://doi.org/10.1021/acs.jpcb.5b00011

Duval, A., & Lawoko, M. (2014). A review on lignin-based polymeric, micro- and nano-structured materials. *Reactive and Functional Polymers*, *85*, 78–96. https://doi.org/10.1016/j.reactfunctpolym.2014.09.017

Egea, M. B., Oliveira Filho, J. G. de, Braga, A. R. C., Leal, M. C. B. D.-M., Celayeta, J. M. F., & Lemes, A. C. (2022). 18—Zein-based blends and composites. In S. Mavinkere Rangappa, J. Parameswaranpillai, S. Siengchin, & M. Ramesh (Eds.), *Biodegradable Polymers, Blends and Composites* (pp. 511–526). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-823791-5.00009-0

Gabov, K., Fardim, P., & Júnior, F. G. da S. (2013). Hydrotropic Fractionation of Birch Wood into Cellulose and Lignin: A New Step Towards Green Biorefinery. *BioResources*, *8*(3), Article 3.

Ghanbarzadeh, B., Musavi, M., Oromiehie, A. R., Rezayi, K., Razmi Rad, E., & Milani, J. (2007). Effect of plasticizing sugars on water vapor permeability, surface energy and microstructure properties of zein films. *LWT - Food Science and Technology*, *40*(7), 1191–1197. https://doi.org/10.1016/j.lwt.2006.07.008

Ghanbarzadeh, B., & Oromiehi, A. R. (2008). Biodegradable biocomposite films based on whey protein and zein: Barrier, mechanical properties and AFM analysis. *International Journal of Biological Macromolecules*, *43*(2), 209–215. https://doi.org/10.1016/j.ijbiomac.2008.05.006

Gnana Prakash, D., Gopinath, K. P., Prasanth, S. M., Harish, S., Rishikesh, M., Sivaramakrishnan, R., & Pugazhendhi, A. (2022). Extraction methodology of lignin from biomass waste influences the quality of bio-oil obtained by solvothermal depolymerization process. *Chemosphere*, *293*, 133473. https://doi.org/10.1016/j.chemosphere.2021.133473

Guo, H. X., Heinämäki, J., & Yliruusi, J. (2008). Stable aqueous film coating dispersion of zein. *Journal of Colloid and Interface Science*, *322*(2), 478–484. https://doi.org/10.1016/j.jcis.2007.11.058

Han, J. H. (2014). Chapter 9 - Edible Films and Coatings: A Review. In J. H. Han (Ed.), *Innovations in Food Packaging (Second Edition)* (pp. 213–255). Academic Press. https://doi.org/10.1016/B978-0-12-394601-0.00009-6

Heydari, G., Thormann, E., Järn, M., Tyrode, E., & Claesson, P. M. (2013). Hydrophobic Surfaces: Topography Effects on Wetting by Supercooled Water and Freezing Delay. *The Journal of Physical Chemistry C*, *117*(42), 21752–21762. https://doi.org/10.1021/jp404396m

Hon, D. N.-S., & Shiraishi, N. (2000). *Wood and Cellulosic Chemistry, Second Edition, Revised, and Expanded*. CRC Press.

Hult, E.-L., Koivu, K., Asikkala, J., Ropponen, J., Wrigstedt, P., Sipilä, J., & Poppius-Levlin, K. (2013). Esterified lignin coating as water vapor and oxygen barrier for fiber-based packaging. *Holzforschung*, *67*(8), 899–905. https://doi.org/10.1515/hf-2012-0214

Hult, E.-L., Ropponen, J., Poppius-Levlin, K., Ohra-Aho, T., & Tamminen, T. (2013). Enhancing the barrier properties of paper board by a novel lignin coating. *Industrial Crops and Products, 50*, 694–700. https://doi.org/10.1016/j.indcrop.2013.08.013

Jacquet, N., Quiévy, N., Vanderghem, C., Janas, S., Blecker, C., Wathelet, B., Devaux, J., & Paquot, M. (2011). Influence of steam explosion on the thermal stability of cellulose fibres. *Polymer Degradation and Stability*, *96*(9), 1582–1588. https://doi.org/10.1016/j.polymdegradstab.2011.05.021

Kansal, D., Hamdani, S. S., Ping, R., & Rabnawaz, M. (2020). Starch and Zein Biopolymers as a Sustainable Replacement for PFAS, Silicone Oil, and Plastic-Coated Paper. *Industrial & Engineering Chemistry Research*, *59*(26), 12075–12084. https://doi.org/10.1021/acs.iecr.0c01291

Kansal, D., Hamdani, S. S., Ping, R., Sirinakbumrung, N., & Rabnawaz, M. (2020). Food-Safe Chitosan–Zein Dual-Layer Coating for Water- and Oil-Repellent Paper Substrates. *ACS Sustainable Chemistry & Engineering*, *8*(17), 6887–6897. https://doi.org/10.1021/acssuschemeng.0c02216

Katahira, R., Elder, T. J., & Beckham, G. T. (2018). Chapter 1. A Brief Introduction to Lignin Structure. In G. T. Beckham (Ed.), *Energy and Environment Series* (pp. 1–20). Royal Society of Chemistry. https://doi.org/10.1039/9781788010351-00001

Kellock, M., Maaheimo, H., Marjamaa, K., Rahikainen, J., Zhang, H., Holopainen-Mantila, U., Ralph, J., Tamminen, T., Felby, C., & Kruus, K. (2019). Effect of hydrothermal pretreatment severity on lignin inhibition in enzymatic hydrolysis. *Bioresource Technology*, *280*, 303–312. https://doi.org/10.1016/j.biortech.2019.02.051

Korpinen, R., & Fardim, P. (2009). Lignin extraction from wood biomass by a hydrotropic solution. *O Papel (Brazil)*, *70*(5), 69–82.

Lai, H.-M., & Padua, G. W. (1997). Properties and Microstructure of Plasticized Zein Films. *Cereal Chemistry*, *74*(6), 771–775. https://doi.org/10.1094/CCHEM.1997.74.6.771

Lavoine, N., Desloges, I., Khelifi, B., & Bras, J. (2014). Impact of different coating processes of microfibrillated cellulose on the mechanical and barrier properties of paper. *Journal of Materials Science*, *49*(7), 2879–2893. https://doi.org/10.1007/s10853-013-7995-0

Lawton, J. W. (2002). Zein: A History of Processing and Use. *Cereal Chemistry*, *79*(1), 1–18. https://doi.org/10.1094/CCHEM.2002.79.1.1

Li, J., Gellerstedt, G., & Toven, K. (2009). Steam explosion lignins; their extraction, structure and potential as feedstock for biodiesel and chemicals. *Bioresource Technology*, *100*(9), 2556–2561. https://doi.org/10.1016/j.biortech.2008.12.004

Li, J., Henriksson, G., & Gellerstedt, G. (2007). Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. *Bioresource Technology*, *98*(16), 3061–3068. https://doi.org/10.1016/j.biortech.2006.10.018

Li, Y., Bai, Y., Huang, J., Yuan, C., Ding, T., Liu, D., & Hu, Y. (2020). Airglow discharge plasma treatment affects the surface structure and physical properties of zein films. *Journal of Food Engineering*, *273*, 109813. https://doi.org/10.1016/j.jfoodeng.2019.109813

Li, Y., Li, J., Xia, Q., Zhang, B., Wang, Q., & Huang, Q. (2012). Understanding the Dissolution of α-Zein in Aqueous Ethanol and Acetic Acid Solutions. *The Journal of Physical Chemistry B*, *116*(39), 12057–12064. https://doi.org/10.1021/jp305709y

Liu, X., Liu, Q., Wang, S., Liu, Z., Yang, G., Wang, H., Xiong, W., Li, P., Xu, F., Xi, Y., & Kong, F. (2022). Effect of functional group and structure on hydrophobic properties of environment-friendly lignin-based composite coatings. *International Journal of Biological Macromolecules*, *215*, 132–140. https://doi.org/10.1016/j.ijbiomac.2022.06.055

Luís, Â., Domingues, F., & Ramos, A. (2019). Production of Hydrophobic Zein-Based Films Bioinspired by The Lotus Leaf Surface: Characterization and Bioactive Properties. *Microorganisms*, *7*(8), 267. https://doi.org/10.3390/microorganisms7080267

Lundquist, K. (1992). Wood. In S. Y. Lin & C. W. Dence (Eds.), *Methods in Lignin Chemistry* (pp. 65–70). Springer. https://doi.org/10.1007/978-3-642-74065-7_4

Luo, Y., & Wang, T. (2016). Chapter 9—Pharmaceutical and Cosmetic Applications of Protein By-Products. In G. Singh Dhillon (Ed.), *Protein Byproducts* (pp. 147–160). Academic Press. https://doi.org/10.1016/B978-0-12-802391-4.00009-4

Maniet, G., Schmetz, Q., Jacquet, N., Temmerman, M., Gofflot, S., & Richel, A. (2017). Effect of steam explosion treatment on chemical composition and characteristic of organosolv fescue lignin. *Industrial Crops and Products*, *99*, 79–85. https://doi.org/10.1016/j.indcrop.2017.01.015

Meng, X., Crestini, C., Ben, H., Hao, N., Pu, Y., Ragauskas, A. J., & Argyropoulos, D. S. (2019). Determination of hydroxyl groups in biorefinery resources via quantitative 31P NMR spectroscopy. *Nature Protocols*, *14*(9), Article 9. https://doi.org/10.1038/s41596-019-0191-1

Mobile Surface Analyzer-MSA One-Click SFE. (n.d.). Retrieved 10 January 2023, from https://www.kruss-scientific.com/en/products-services/products/msa-one-click-sfe?documentKey=products

Mohamad, A. johari, Zhu, X., Liu, X., Pfleging, W., & Torge, M. (2013). *Effect of surface topography on hydrophobicity and bacterial adhesion of polystyrene*. 228–233. https://doi.org/10.1109/3M-NANO.2013.6737421

Naz, S., Ahmad, N., Akhtar, J., Ahmad, N. M., Ali, A., & Zia, M. (2016). Management of citrus waste by switching in the production of nanocellulose. *IET Nanobiotechnology*, *10*(6), 395–399. https://doi.org/10.1049/iet-nbt.2015.0116

Obst, J. R., & Kirk, T. K. (1988). Isolation of lignin. In *Methods in Enzymology* (Vol. 161, pp. 3–12). Academic Press. https://doi.org/10.1016/0076-6879(88)61003-2

Olsson, J. (2021). *Fractionation of hardwood using steam explosion and hydrotropic extraction: Process development for improved fractionation* [Doctoral Thesis (compilation)]. Chemical Engineering, Lund University.

Pandey, K. K. (1999). A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. *Journal of Applied Polymer Science*, *71*(12), 1969–1975. https://doi.org/10.1002/(SICI)1097-4628(19990321)71:12<1969::AID-APP6>3.0.CO;2-D

Parris, N., & Coffin, D. R. (1997). Composition factors affecting the water vapor permeability and tensile properties of hydrophilic zein films. *Journal of Agricultural and Food Chemistry*, *45*(5), 1596–1599.

Patton, T. C. (1964). *Paint Flow and Pigment Dispersion*. Interscience Publishers.

Pu, Y., Cao, S., & Ragauskas, A. J. (2011). Application of quantitative 31P NMR in biomass lignin and biofuel precursors characterization. *Energy & Environmental Science*, *4*(9), 3154–3166. https://doi.org/10.1039/C1EE01201K

Ramos, L. P. (2003). The chemistry involved in the steam treatment of lignocellulosic materials. *Química Nova*, *26*(6), 863–871. https://doi.org/10.1590/S0100-40422003000600015

Richter, F., Atreya, A., Kotsovinos, P., & Rein, G. (2019). The effect of chemical composition on the charring of wood across scales. *Proceedings of the Combustion Institute*, *37*(3), 4053–4061. https://doi.org/10.1016/j.proci.2018.06.080

Sapouna, I., & Lawoko, M. (2021). Deciphering lignin heterogeneity in ball milled softwood: Unravelling the synergy between the supramolecular cell wall structure and molecular events. *Green Chemistry*, *23*(9), 3348–3364. https://doi.org/10.1039/D0GC04319B

Sette, M., Wechselberger, R., & Crestini, C. (2011). Elucidation of Lignin Structure by Quantitative 2D NMR. *Chemistry – A European Journal*, *17*(34), 9529–9535. https://doi.org/10.1002/chem.201003045

Shi, K., Kokini, J. L., & Huang, Q. (2009). Engineering Zein Films with Controlled Surface Morphology and Hydrophilicity. *Journal of Agricultural and Food Chemistry*, *57*(6), 2186–2192. https://doi.org/10.1021/jf803559v

Shimizu, K., Sudo, K., Ono, H., Ishihara, M., Fujii, T., & Hishiyama, S. (1998). Integrated process for total utilization of wood components by steam-explosion pretreatment. *Biomass and Bioenergy*, *14*(3), 195–203. https://doi.org/10.1016/S0961-9534(97)10044-7

Sigma-Aldrich. (n.d.). *1,4-Dioxane*. Retrieved 23 January 2023, from https://www.sigmaaldrich.com/SE/en/product/sial/76887

Sigma-Aldrich. (2021). *SAFETY DATA SHEET - Chloroform*. https://www.sigmaaldrich.com/SE/en/sds/SIAL/288306

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton, D. (2008). *Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples: Laboratory Analytical Procedure (LAP); Issue Date: 12/08/2006* (p. 14).

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2012). *Determination of Structural Carbohydrates and Lignin in Biomass: Laboratory Analytical Procedure (LAP)*. National Renewable Energy Laboratory. https://www.nrel.gov/docs/gen/fy13/42618.pdf

Tavares, L. B., Ito, N. M., Salvadori, M. C., dos Santos, D. J., & Rosa, D. S. (2018). PBAT/kraft lignin blend in flexible laminated food packaging: Peeling resistance and thermal degradability. *Polymer Testing*, *67*, 169–176. https://doi.org/10.1016/j.polymertesting.2018.03.004

Tracton, A. A. (2006). *Coatings Technology Handbook* (3rd ed.). CRC Press.

Vrabič Brodnjak, U., & Tihole, K. (2020). Chitosan Solution Containing Zein and Essential Oil as Bio Based Coating on Packaging Paper. *Coatings*, *10*(5), Article 5. https://doi.org/10.3390/coatings10050497

Wegener, G., & Fengel, D. (1977). Studies on milled wood lignins from spruce part 1. Composition and molecular properties. *Wood Science and Technology*, *11*(2), 133–145. https://doi.org/10.1007/BF00350991

Wen, J.-L., Yang, S., Sun, S.-L., Yuan, T.-Q., & Sun, R.-C. (2014). *Structural characterization and thermal properties of enzymatic hydrolysis lignins* (pp. 91–110).

Yang, M., Zhang, X., Guan, S., Dou, Y., Gao, X., & Miao, L. (2021). Green preparation of lignin nanoparticles in an aqueous hydrotropic solution and application in biobased nanocomposite films. *Holzforschung*, *75*(5), 463–473. https://doi.org/10.1515/hf-2020-0021

Yang, Q., & Pan, X. (2016). Correlation between lignin physicochemical properties and inhibition to enzymatic hydrolysis of cellulose: Lignin Properties and Enzymatic Hydrolysis. *Biotechnology and Bioengineering*, *113*(6), 1213–1224. https://doi.org/10.1002/bit.25903

Appendix A: 2D NMR Spectrum of SXS

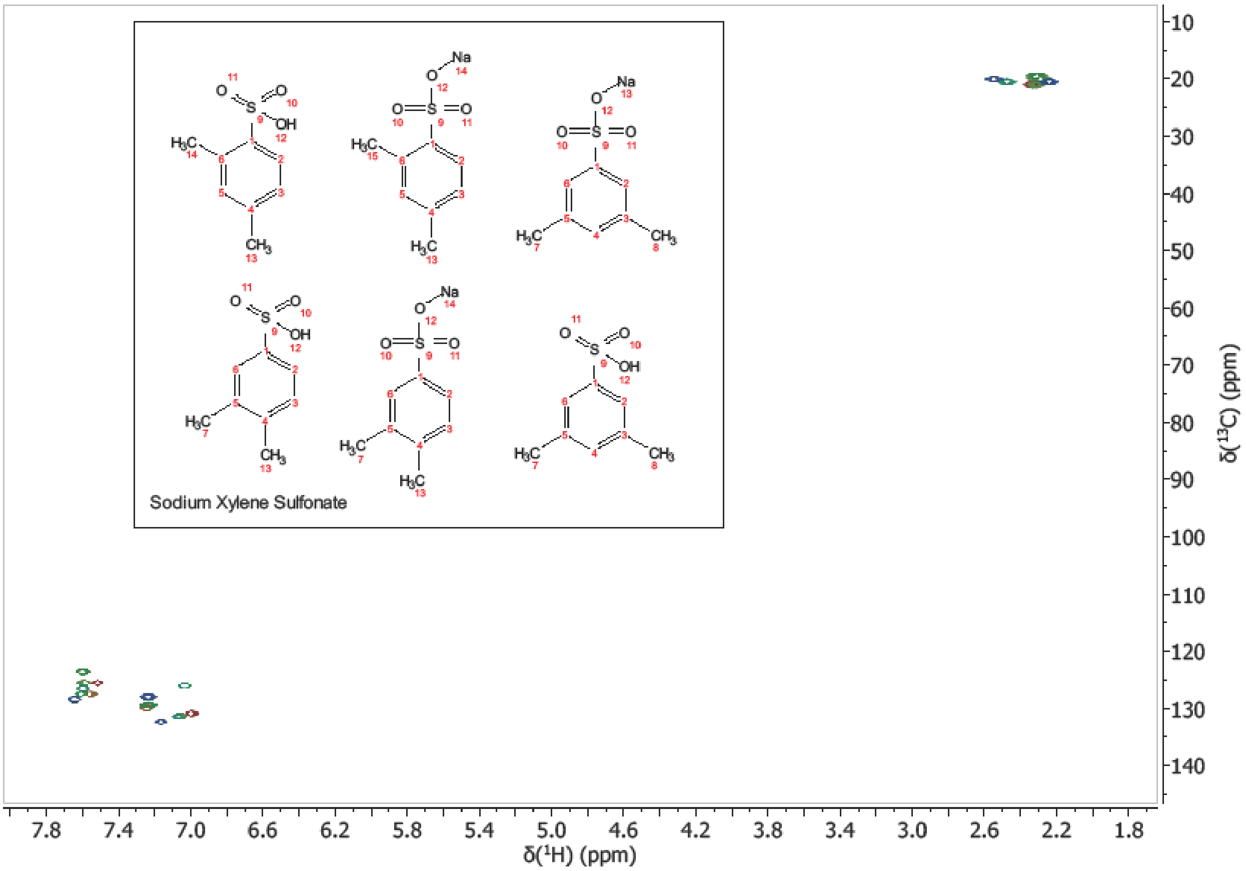

Figure A-1. Shows the predicted 2D NMR spectrum for SXS.

Appendix B: Water Barrier Testing - Lignin

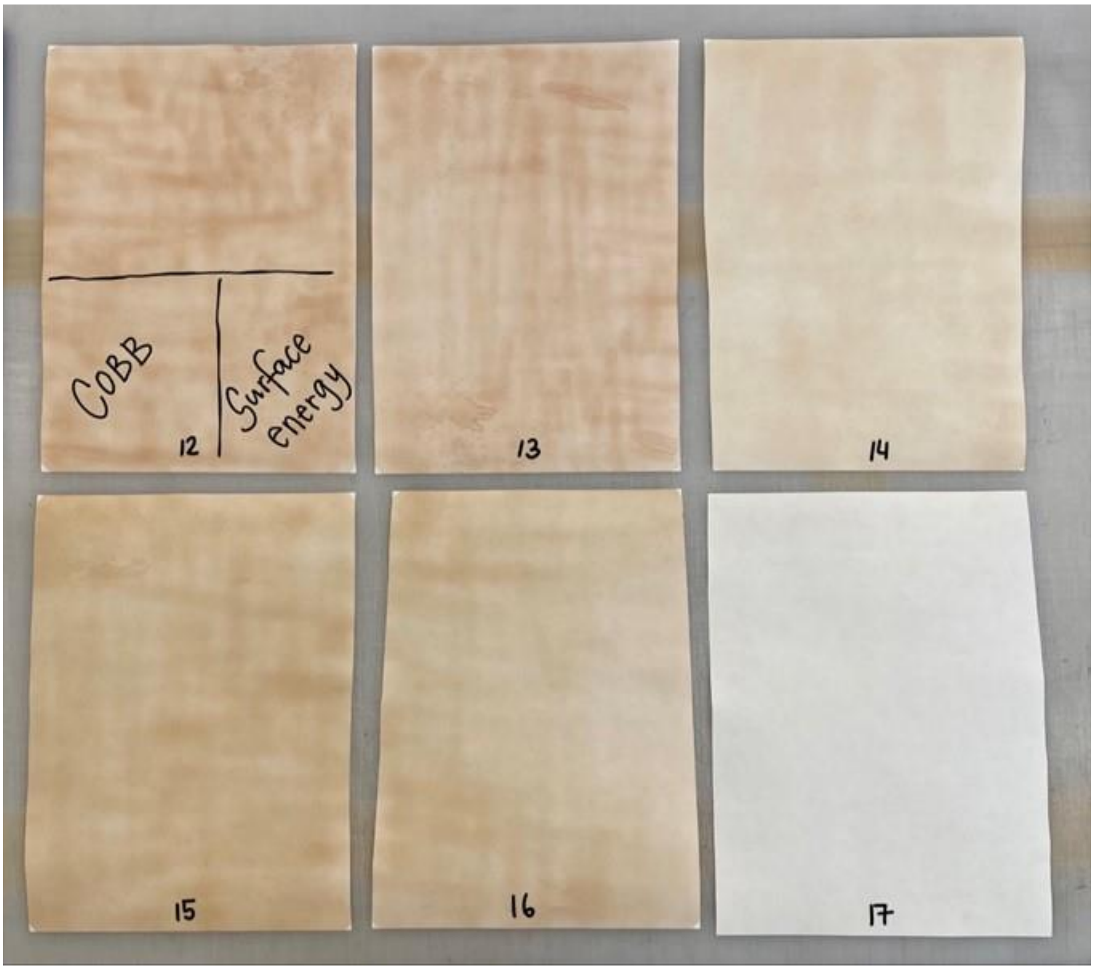

Figure B-1. Shows the lignin-based coatings. Sample 12 and 13 are 170B, sample 14 is MWL birch, sample 15 and 14 are 170S and sample 17 is MWL spruce.

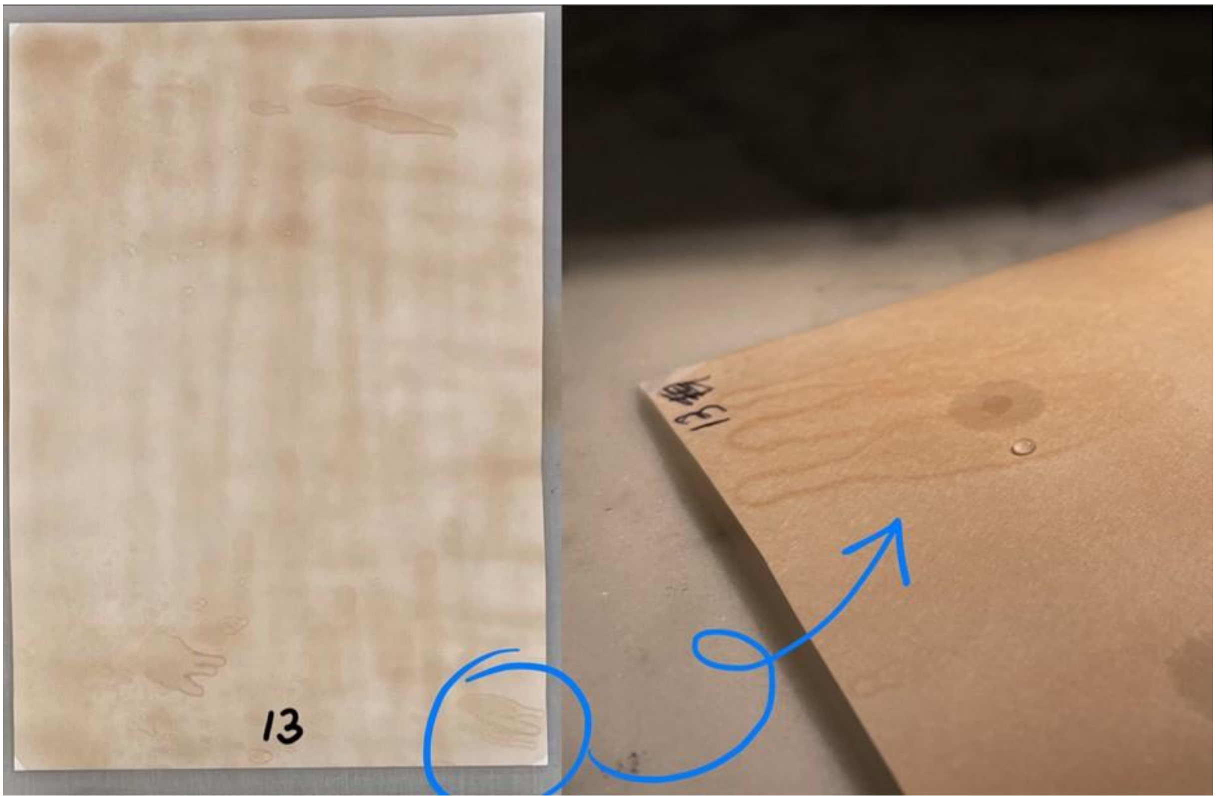

Figure B-2. Shows the water and DM CA measurements on coating stains.