Spruce Bark Biorefinery

KA103X degree Project in Chemical Engineering, First Cycle, 15 hp, 2021 School of Engineering Sciences in Chemistry, Biotechnology and Health

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Submitted:
2021-06-09

Abstract

Spruce bark (*Picea abies*) contains several fundamental main substances; lignin, non-cellulose polysaccharides, cellulose and extractives. This undergraduate study focuses on developing a process to extract each of these components from spruce bark using a biorefinery concept, with a main focus on extracting lignin without degradation. The purpose of the bark biorefinery concept is to contribute to a circular bioeconomy, by increased use of natural renewable resources. With extended research on the area, it will be possible to produce polymers, green chemicals and biofuel from the components in bark.

This report covers the extraction of the bark components with soxhlet extraction, hot-water extraction, organosolv extraction and peracetic acid delignification. The extraction was made on two samples from Norway spruce, matchstick-sized bark (MS) and 20 mesh-sized bark with a diameter of 0.8 mm (20M). The purpose was to be able to compare the efficiency of the extraction between the two samples. Afterwards, the characterization of extracts and residue was executed with carbohydrate analysis, 2D HSQC-NMR and FTIR-analysis.

The results showed that a smaller particle size led to more efficient extractions of all components as well as purer extract solutions. Lignin concentration determinations of samples at each step showed that a significant amount of lignin was lost prior to the organosolv extraction. Future research should look into ways to reduce this loss in order to increase the lignin yield. The findings in the FTIR and NMR analyses correlates with what could be seen in other reports, discussing similar subjects. For upscaling of this process, future research should go toward optimization of all extraction methods in order to make an upscaling of the process economically viable.

Keywords

Norway spruce bark, bark biorefinery, extractives, cellulose, lignin, non-cellulose polysaccharides, soxhlet extraction, hot-water extraction, organosolv extraction, PAA delignification, carbohydrate analysis, 2D HSQC-NMR and FTIR.

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Abbreviations

2D HSQC-NMR: 2D Heteronuclear Single Quantum Coherence Spectroscopy - Nuclear Magnetic Resonance Spectroscopy

20M: 20 mesh-sized bark with a diameter of 0.8 mm

FTIR: Fourier-Transform Infrared Spectroscopy

GalA: Galacturonic acid

GlcA: Glucuronic acid

HPAEC-PAD: High-Performance Anion Exchange Chromatography Pulsed Amperometric Detection

HW: Hot-water extraction

MS: Matchstick-sized bark

PAA: Peracetic acid

1. Introduction

With increasing levels of pollution filling the air and an increasing worry in the large environmental impact our current style of life is causing, research into renewable resources is in high demand. In order to reduce the negative impact as well as to ensure the future generation's assets, current methods of harvesting resources should get both cleaner and more efficient. Since the forest makes up a significant part of renewable resources that are used today, this report looks into ways to make the forest industry more efficient. This is done by looking at how the *bark*, which today is most often burned as fuel, can be used to extract valuable resources. The bark examined comes from Norway spruce, or *Picea abies*, collected in Norra Djurgården and the method used is based on a biorefinery concept.

Four different types of extraction methods were used in order to remove specific components from the bark and create pure extracts that could later be analysed. To see how the size of the bark affected the processes, two samples were prepared, one 20 mesh (20M) and one matchstick sized (MS). The extraction process that was used in this lab can be seen in *Figure 1* below. After these steps, the extracts and the bark residue were analysed using 2D HSQC-NMR, FTIR and HPAEC-PAD.

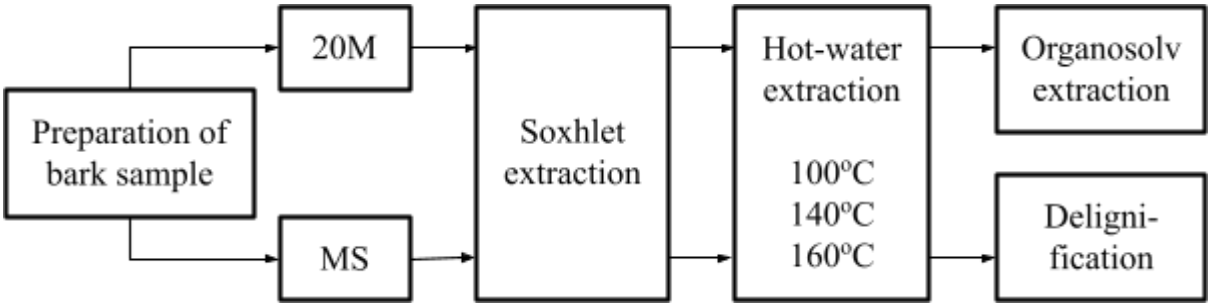


Figure 1. *Flow chart of extraction processes used in the method, where 20M is 20 mesh-sized bark and MS is matchstick-sized bark.*

1.1 Goal

The goal of this project was to test a biorefinery approach for extraction of the components in bark using only environmentally-friendly methods. In doing this, not only was the extraction of extractives, pectins, hemicellulose and cellulose analysed but focus was also put into trying to recover lignin without degradation. The project was executed on a laboratory scale while simultaneously researching the possibilities for a future up-scaling of the process.

1.2 Limitations

The project solely focused on extracting components from Norway spruce inner bark. Extracting lignin without degradation was the primary focus of the project but the other components were also analysed. The project was on a laboratory scale where two bark samples were compared which had the sizes 20M and MS. For simplicity's sake the extractives part of this report focuses on stilbene glucosides and tannins. The hot-water extraction was performed using three different temperatures.

2. Background

2.1 Bark

Bark is made up of a complex conformation of living material that is tightly packed to form a layered tissue. This complex structure gives the bark the ability to perform several varying functions on the surface of a tree stem. To give a few examples, it can help with the transportation of nutritional substances across the tree, it can aid in water storage and it can offer protection against threats like heat, cold and herbivores.[1] The formation of bark occurs at the outer parts of the tree stem's cambium, see *Figure 2.a)*. Here, cell division takes place which produces undifferentiated cells that later get sorted into different specialized functions in the tree. The inner bark is mostly made up of the living system of phloem tissue, which is the layer responsible for transportation of sugars, and consists of cells such as sieve cells, albuminous cells and parenchyma.[2] The outer bark, on the other hand, is made up of an inert system of dead, layered tissue. This is because as new cells keep getting produced in the cambium, the inner layer of phloem slowly starts getting pushed outward. Eventually the cells making up the phloem system meet the outer layer and get crushed, which ruins the functions of the cells and leaves behind a dead tissue.

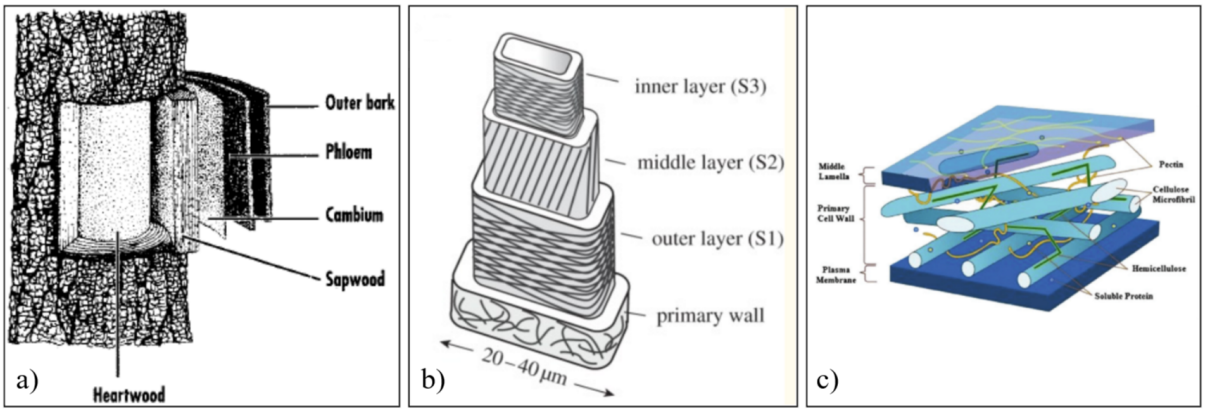


***Figure 2**. Hierarchical structure of a) a tree stem,[3] b) cells walls of a plant cell,[4] c) the primary wall of a plant cell.[5]*

There are three main substances that make up almost all of the material in a tree; cellulose, lignin and non-cellulose polysaccharides. They are located in the walls of the plant cells and offer stability and rigidity to the material.[6] The cell walls are made up of four layers with slightly different properties, see *Figure 2.b)*, which comes from the fact that they all contain different compositions of the three main substances.[4] Since these substances are all polymers, the walls are made up of a network of interactions between the chains as can be seen in *Figure 2.c)*.

Except for the three main substances, there also exist some extractives, ash, starch and metals in the tree. The composition of all these components vary greatly depending on where in the tree one looks. In general, bark has a significantly higher concentration of both extractives and ash than stem wood (32% vs. 2% for extractives[7] and 2% vs. 0.2% for ash[8]), which makes the material difficult to handle in the normal pulping process. Because of this, the bark is

often used as fuel in the pulping industry instead of as a source of useful materials. Today, about 60% of all processed bark is burnt up as fuel,[9] while the remaining 40% is used to make products such as corks, spices, gums and some medicines.

2.1.1 Extractives

Extractives in spruce are non-structural components responsible for physical and chemical properties such as color, adhesion, durability and defense against microbial invasion.[10] Extractives are put in two main categories, lipophilic and hydrophilic. The innerbark consist of approximately 1.2% lipophilic extractives and 13% hydrophilic extractives.[11] A major group of lipophiles are fatty acids whereas hydrophilic extractives consist mostly of lignans, flavonoids, stilbene glycosides and tannins.[12] This report focuses on tannins and stilbene glucosides.

Stilbene glucosides acts as metabolites in plants. Its molecular structure consists of a stilbenoid part substituted with a β-D-glucosyl, see *Figure 3*. The inner bark composition consist of 5-10% stilbene glucosides.[11] Whereas astringin, isorhapontin and piceid are major in spruce bark.[13] Their function as metabolites grant plants with antifungal, antibacterial and environmental stress protection. This is why these compounds are of commercial interest today.[14,15]

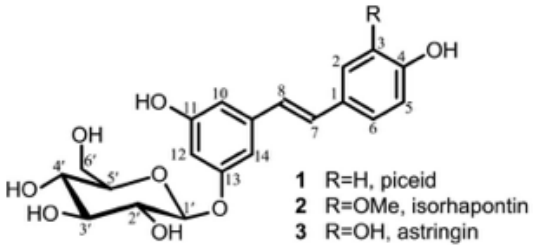


***Figure 3**. The molecular structure of the major stilbene glucoside groups.[14]*

Tannins are polyphenol with astringent properties and act as a binder and also precipitate proteins in plants. This grants plants protection against bacteria and parasitic fungi. A tannin that exists in Norwegian spruce bark is the condensed tannin.[16] It appears in adequate concentration and suppresses decomposition of the plant tissue; lignin in the bark therefore degrades slower than that of in wood.[17] Tannins also have adhesive traits towards heavy metals and thus are able to create metal complexes. Researchers propose that tannins could be used as a purifier of industrial freshwater.[18] Tannins are capable of participating in protein complex as well, which can be beneficial for the nutritional value of food.[19]

2.1.2 Non-cellulose polysaccharides

The non-cellulose polysaccharides in plant cells is the collective name of all polymers whose units consist of several different monosaccharides. This includes both hemicellulose, which is mostly composed of different sugar units, and pectins, which also consist of a significant amount of acidic sugars. These compounds make up around 20-30% of the bark dry matter[11] and hold several functions in the plant cells.

According to the Oxford English Dictionary, hemicellulose is defined as "*any... constituents of the cell walls of plants [that] are polysaccharides of simpler structure than cellulose*".[20] This comes from the fact that the polymers of hemicellulose are made up of a random sequence of monosaccharides of different sizes and side groups and because of this, the chains will not be ordered in the same, complex way as cellulose. Instead, an amorphous network will be formed in the cell walls, similar to lignin.[4] The main function of this network is to aid the cell in stability and rigidity, which is done by offering cross-links between the cellulose fibrils and the lignin matrix.[21] The polymer chains of hemicellulose are usually around 500 - 3 000 units and the most common components are the five-carbon sugar xylose and the six-carbon sugar mannose. It is present in all cell walls of the plant cells, but the highest concentration can be found at the first and second cell wall.[22]

Pectin holds a similar structure to that of hemicellulose, but its main component consists of the sugar acid galacturonic acid, GalA. It also contains high levels of the deoxy sugar rhamnose and the aldopentose sugar arabinose.[23] Pectins aids the cell by, amongst other things, providing structural integrity, protection and flexibility. It mainly occurs in the middle lamella of plant cells where it helps keep walls of adjacent cells joined together.[24]

Because of the varying structure of the chains, hemicellulose has several different applications in everything from food-additives and flavor modifiers to drug carriers and inhibitors for some viruses.[25] The primary area for research on future applications of hemicellulose is as a biodegradable alternative for fossil-based resources, mainly in the use of bioplastics. In order to achieve this, research is now being focused on trying to increase the functionalization of the polymers so that new characteristics can be implemented.[25] Pectins is a common additive in food. It is used for viscosity building, protein stabilization and as a gelling agent in foods such as jellies, yoghurts and juice.[26] In the future, applications of pectins are thought to increase in the biomedical sector as a natural and biodegradable carrier in drug delivery.[27]

2.1.3 Lignin

Lignin is a major plant-derived biopolymer which accounts for 40% of all lignocellulosic biomass energy. It is also the second most abundantly accessible natural polymer since it composes for around 15-30% of all biomass content in plants.[28] Lignin is regarded as a class of phenolic natural polymers instead of an intrinsically defined compound owing to its molecular complexity and structural diversity.[29]

Lignin has an extensive three-dimensional structure with various functional moieties such as carboxyl, carbonyl and methoxy groups. Lignin is based on repeating phenylpropane-based monolignols subunits, which are coniferyl alcohol, sinapyl alcohol, and low amounts of p-coumaryl alcohol. Lignin is used for several biological functions in plants. Firstly, it provides mechanical support for the stems and branches by acting as an adhesive for the fibers. It also acts as an adhesive for binding different cells together at the middle lamella. Secondly it causes the cell wall to become hydrophobic which makes it waterproof. And finally it shields against microbial degradation of wood. Some polysaccharide-degrading proteins excreted by microorganisms are unable to penetrate the cell wall due to the compact

structure of the lignified wood. However, there are some microorganisms with the ability to degrade lignin but it does not change that lignin acts as a barrier.[29]

Lignin in Norway spruce bark can be found in both inner and outer bark but a higher amount can be found in the outer bark. Though the true chemical aspect and amount of lignin in spruce bark is not yet determined but is estimated to be about 15%. Prior research points to the result that milled wood lignin and spruce bark lignin have very similar chemical properties.[11]

The applications for lignin are various. The heat value of 26.7 MJ/kg causes lignin to be exemplary as fuel which is presently its most common function in the paper industry. Although lignin does have other functions than only serving as fuel which makes it quite underused. For instance, it is a major component in bioplastic which is sold under the trade name "liquid wood". Lignin can also be used to synthetically create vanillin.[29]

2.1.4 Cellulose

Cellulose is one of the most common structural molecules among plants. It is an organic compound, and belongs to the category of polysaccharides.[30] Cellulose is an important component in plants[31], and works as a building block. It is the most abundant organic polymer on earth[32] and a high magnitude of the cellulose exists in the secondary plant walls.[31] The cellulose contents in spruce inner bark is about 23 %.[11]

The glucose monomer units that cellulose is made up of, forms a long-chain polymer. The cellulose molecules have the possibility to align into microfibrils, due to the fact that the monomer units in the molecule are alternately rotated 180°. The microfibrils are embedded in a matrix of hemicellulose, pectin and lignin. The fibrils can form fibril aggregates with both crystalline and non-crystalline regions.[4] *Figure 4* displays how cellulose originates from plants.

Figure 4. *Shows the hierarchical layers of plants, and where cellulose can be found.*[33]

Cellulose has many different applications, and the textile industry is one of the most common. Amongst that, is it also common to use cellulose in different biomedical applications. Cellulose is also a universal component within the industrial sector.[34]

Nanocellulose can be extracted from wood fibers, it has properties such as high strength, stiffness and flexibility. Nanocellulose exists in two main types; nanocrystalline cellulose (CNC) and nanofibrillated cellulose (CNF). The two types have the same chemical composition. However, they differ in certain characteristics, mainly in morphology, crystallinity and particle size.[35] It has been discovered that it might be possible to use nanocellulose in water waste treatment.[36] Another possible future application for nanocellulose is that it can act as a replacement for fossil based materials in packaging and coatings.[35]

2.2 Bark Biorefinery

Bark is a side-stream of the forest industry and the bark biorefinery concept was established to make full use of the resources and material. The technologies allow for an opportunity to transform the underutilized bark-stream into a variety of materials and products.[37] The bark can be converted into polymers, green chemicals and biofuel.[38] The bark biorefinery technology is a building block for a circular bioeconomy. Yet, no global bark biorefineries exist. However, there is an ongoing project in Sweden "Optibark", with the aim to upscale and commercialize some of the bark components. Another initiative is the Scion programme, which is taking advantage of existing expertise through collaborations to continue the research on bark biorefineries.[37]

3. Theory behind the experiment

3.1 Extraction methods

Soxhlet extraction is primarily used to remove extractives from plant material.[39, 40] The soxhlet extractor set-up, which is displayed in *Figure 5*, includes a distillation flask, a thimble, a siphon and a condenser.[40] Pressurized hot-water extraction is a method that utilizes pressurized hot-water to extract components out of plant materials at determined temperatures. Non-polar organic components are, with advantage, extracted with this extraction method.[41]

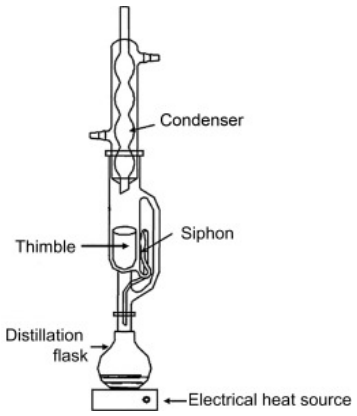


Figure 5. *Setup of the soxhlet extraction.*[42]

Organosolv lignin is extracted with a mixture of organic solvent together with a catalyst or an additive. Alcohols are the most frequently used organic solvent in the extraction of

organosolv lignin. The isolation of organosolv lignin is performed via distillation where the organic solvent is evaporated, and the lignin is recovered by precipitation.[29]

3.2 Peracetic Acid Delignification

Delignification in acidic conditions, for reaction formula see *Figure 6*, of lignocellulosic mass allows for removal of lignin from the plant tissue. This project will use peracetic acid. At low pH and high temperature, β ether bonds and side chain hydroxyl groups break primarily. Benzyl cations will condensate which increases their ionic properties thus making them more reactive. This will promote reactions with other substructures in lignin. The reaction process will result in lignin transforming into a mixture of Klason lignin and other low-molecular weight products such as phenols.[43,44] The residue will mostly consist of cellulose fibers.

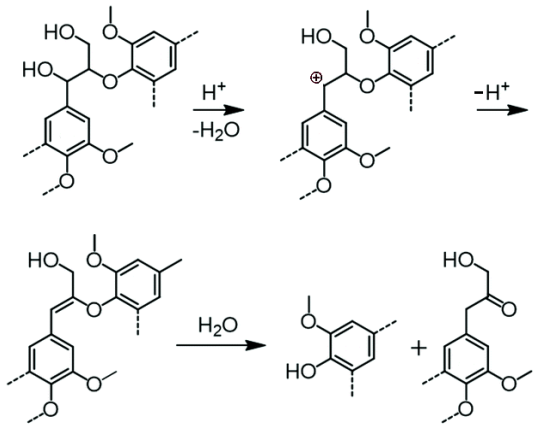


Figure 6. Shows a general reaction for an acid delignification.

3.3 NMR Nuclear Magnetic Resonance

By examination of the local magnetic field around an atomic nucleus, with nuclear magnetic resonance (NMR), it is possible to obtain information about the structure of molecules. Intermolecular interactions, the composition and the configuration of each molecule will affect the magnetic field around each nucleus. Complete information on the molecular structure will be provided by the NMR technique, which is implied by the magnetic field around each nucleus.[45]

Spinning protons and neutrons, with the quantum number (m), form the atomic nucleus of a molecule. The nuclear spin (I) of an atomic nucleus portrays its magnetic characteristics.[45] The physical phenomenon of resonance transitions builds the foundation of the NMR technique. It appears when a sample placed in an external magnetic field is exposed to electromagnetic radiation. The transitions take place between energy levels of different magnetic energy when the sample is irradiated by radiation with a frequency that matches the energy difference between the energy levels. The absorption causes the spin of the nuclei to change. The nuclei will relax and re-emit the photon and produce a signal that can be detected with a spectrometer. The signal is transformed into a NMR-spectra with Fourier

transform. The NMR-spectra is used together with a reference to determine the structure and identity of the sample.[46, 47]

3.4 Carbohydrate analysis

High-performance anion exchange chromatography (HPAEC), coupled to a Pulsed Amperometric Detection (PAD), will be used to fractionate and analyze the carbohydrate compounds from the inner bark. The method allows for separation and detection of amino sugars, mono-, oligo- and polysaccharides.[48] The weak acid characteristics of carbohydrates gives advantages to the HPAEC-method. Anions are separated with HPAEC under high pH conditions, the separation occurs as the pH exceeds the pK_a of the carbohydrate, which allows for highly selective separations. A strong anion-exchange stationary phase is used for the separation. The PAD allows for immediate identification and quantification after the separation of the carbohydrates.[48]

3.5 FTIR

Fourier transform infrared spectroscopy (FTIR), is a method that uses the absorption of infrared radiation to identify and characterize compounds, additives and contaminants in a sample.[49,50] Various atoms and molecules will absorb light of assorted wavelengths, which depends on the separation between energy levels. The energy of which radiation is absorbed is examined to specify the molecular structure and composition of each sample.[50] Only the covalent bonds in a sample will absorb radiation. The absorption causes the energy of each bond inside the molecule to change. The radiation induces a vibration which is unique for each atom. This results in unique transmittance patterns, which is used to identify and differentiate various molecules.[51]

4. Experiments

4.1 Procedures and instruments

4.1.1 Sample preparation

The bark that was used in these experiments was collected at Norra Djurgården in Sweden during July 2020. The supervisor (Barbara Reitzler) of this project collected the bark by barking fallen trees. The inner bark was first separated from the outer bark, and thereafter freeze dried to reduce the water content. Half of the freeze dried bark was milled with a Wiley mill, 3383 L70, Thomas Scientific, into a fine powder of 0.8 mm in diameter and the remaining half was divided into matchstick-sized sticks, see *Appendix 1*. The separation, freeze drying and milling of the bark was performed by the supervisor of this project in advance of the experiment.

4.1.2 Soxhlet extraction

The prepared 20M and the MS bark, 6.001g and 5.264 g respectively, were placed in separate cellulose thimbles and then covered with a filter paper. Boiling stones were placed in flasks and weighed. 200 ml solvent (90:10 acetone:water (v/v)) was poured into both flasks.

The thimble containing the sample was first placed in the soxhlet apparatus. The heater was then turned on and the solvent soon reached its boiling point at approximately 60 ℃. The vapors of the solvent reached the condenser which caused the vapor to condense and drip down into the thimble which contained the sample. The solvent caused the sample to release some of its extractive content in each cycle. The solvent, which contained parts of the total extractive content, permeated out of the thimble and slowly filled up the siphon until it reached the top, which caused the liquid to empty out into the flask. The extractive content was, at the end of the extraction, all collected in the flask. Which was then transferred to the rotary evaporation instrument. The experiment ran for 40 cycles for both bark samples.

The solvent was later separated from the residue by rotary evaporation set to 50 ℃. The flask was placed in a fume hood for further air drying after the evaporation of the solvent solution was complete.

4.1.3 Hot-water extraction

The residual bark samples from the soxhlet extraction were reused for these extractions.

The first step of the extraction was to weigh and prepare the 20M and MS samples. 4.660g of the 20M sample was placed in a dionium cell and 4.820g of the MS sample was placed in a stainless steel cell. The cells together with three glass flasks were loaded into the solvent extractor instrument (ASE350 Accelerated Solvent Extractor, Dionex, USA). Milli-Q water was used as solvent for both samples. The instrument ran the extraction in 3 cycles at 20 minutes each for each of the three temperatures 100ºC, 140ºC and 160 ºC. Afterwards, the pH of the extract for each temperature was measured using a pH-meter.

The bark samples were thereafter stored for further extraction. The pressurized hot-water extracts and the solvent were poured into separate round bottom flasks and plastic test tubes for the 20M and MS samples respectively. The extracts were thereafter freezed with liquid nitrogen and freeze dried. After being fully dried, the extracts were weighed and saved for analytical determination.

4.1.4 Organosolv extraction

The residual bark of the 20M sample from the hot-water extraction was reused for this extraction.

The organosolv extraction was executed only on the milled bark after the hot-water extraction with the instrument ASE350 Accelerated Solvent Extractor, Dionex, USA. The solvent used was 70:30 ethanol:water (v/v), 1.5 wt % sulphuric acid and the extraction was run at a

temperature of 160°C. Two extract samples were created, one with 9 cycles and one with 6 cycles. After the extraction was done, some extra Milli-Q water was added to both samples for the lignin to start precipitating and the remaining ethanol was evaporated using a Rotary evaporator. The supervisor completed the remaining steps of centrifugation of extract solution to separate precipitated lignin and water-soluble components, filtration of precipitated lignin and the freeze-drying of the extracts and the residue.

4.1.5 Peracetic acid delignification

The residual bark of the MS sample from the hot-water extraction was reused for this extraction.

The sample preparation started with a weighing of the residue obtained from the hot-water extraction, which had a total weight of 2.638 g. A solution of 10wt% peracetic acid (PAA) was then prepared. The pH of the solution was adjusted to 4.1 with NaOH. The bark sample was placed in a round-bottom flask together with 60 ml of the solution and a magnetic stirrer. The flask and its content were then placed in an oil bath and heated to 60 ºC. After one hour of stirring, the solution was filtered using a Büchner funnel. The residue was again placed in the round-bottom flask, and 60 ml of the PAA-solution was added. This procedure was repeated for three cycles.

4.2 Characterization

4.2.1 NMR Nuclear Magnetic Resonance

2D HSQC-NMR analysis was performed on all of the six hot-water extraction samples and the lignin sample from the organosolv extraction. Each of the samples were prepared by dissolving the freeze-dried extracts with 600 µl dimethyl sulfoxide, DMSO. The samples were placed in the NMR instrument (Bruker NMR spectrometer Avance III HD 400 MHz) and run with 44 scans for all hot-water extracts and 88 scans for the lignin sample, with DMSO as reference.

4.2.2 Carbohydrate analysis

Nine samples, which are displayed in *Table 1*, were prepared for the carbohydrate analysis. To do this, 200 mg of each sample was mixed together with 3 ml of 72% H_2SO_4 in separate 100 ml bottles and placed in a vacuum desiccator for 1 hr. Afterwards the samples were taken out of the desiccator, stirred and put back in the desiccator for another 20 min.

Table 1. *Samples prepared for carbohydrate analysis.*

H.W. extract	*Raw bark 1*	*Raw bark 2*	*Acetone MS*	*Acetone 20M*	*Organo solv 1*	*Organo solv 2*	*Delig. 1*	*Delig. 2*

A reference solution was prepared by mixing together 8 different monosaccharides, the exact amount of each monosaccharide is listed in *Table 2*. This reference solution was then mixed with 3 ml of 72% H_2SO_4 and 84 ml Milli-Q water.

The sample mixtures were each diluted with 84 ml Milli-Q water. All 10 samples were then sealed and put into an autoclave at 125 ºC for 60 minutes. While this was happening, aluminum was wrapped around a glass-fiber filter for each sample and each of these filters with aluminum were weighed.

Table 2. *Mass of monosaccharides used for reference solution*

	Ara	**Rha**	**Gal**	**Glc**	**Xyl**	**Man**	**GalA**	**GlcA**
Mass [mg]	40.0	10.3	25.8	150.7	50.9	25.3	40.9	10.5

After the autoclave was finished, the reference solution was poured into a 100 ml volumetric flask and the liquid level was brought up to the mark with Milli-Q water. The reference was then distributed between 6 different 100 ml volumetric flasks with 1, 2, 4, 8, 12 and 14 ml of solution respectively through pipetting and their liquid levels were brought up to the mark with Milli-Q water.

A 3-piece funnel with a glass-fiber filter was used to filter all nine of the sample solutions and 5 ml of boiling Milli-Q water was added twice in order to wash out any remaining material from the filter. The nine sample solutions were then poured into separate 100 ml volumetric flasks and diluted with Milli-Q water until they reached the 100 ml mark. The 3-piece funnel was used to wash each filter from the sample solutions under vacuum with 100 ml boiling Milli-Q water followed up with 100 ml cold Milli-Q water. After the washing, the filters were wrapped in aluminum foil and dried overnight in an oven. The following day, all filters were weighed in order to determine the amount of Klason lignin trapped inside.

A 10x dilution of each sample solution was made in separate 100 ml volumetric flasks. The sample solutions were filtered using a syringe filter (0.2 μm, nylon) to each fill an IC vial for the High-performance anion-exchange chromatography Coupled with Pulsed Amperometric Detection (HPAEC-PAD) ICS 3000 (Dionex, Thermo Scientific, USA). The diluted reference sample was filtered into 6 different IC vials with one syringe filter per reference solution. 10 ml from each of the nine diluted sample solutions were pipetted into separate glass vials.

4.2.3 FTIR

The FTIR spectra of the samples were recorded with a Spectrum 100 FT-IR spectrometer (Perkin Elmer, USA) equipped with a red golden gate accessory unit for ATR. 16 scans were recorded for each sample with a resolution of 4 cm^{-1}. The samples were raw bark from the starting material, acetone extract of the soxhlet extraction of the 20M sample, bark residue of the organosolv of 20M, bark residue of the PAA delignification of MS and HW100, HW140 and HW160 are the extracts of 20M for the hot-water extraction at respective temperature.

5. Results

5.1 Soxhlet extraction

Results from the soxhlet extraction of the 20M and MS samples are shown in *Figure 7*. Values show weight of extract in comparison to starting mass of bark samples, which was 6.001 for 20M and 5.264 for MS.

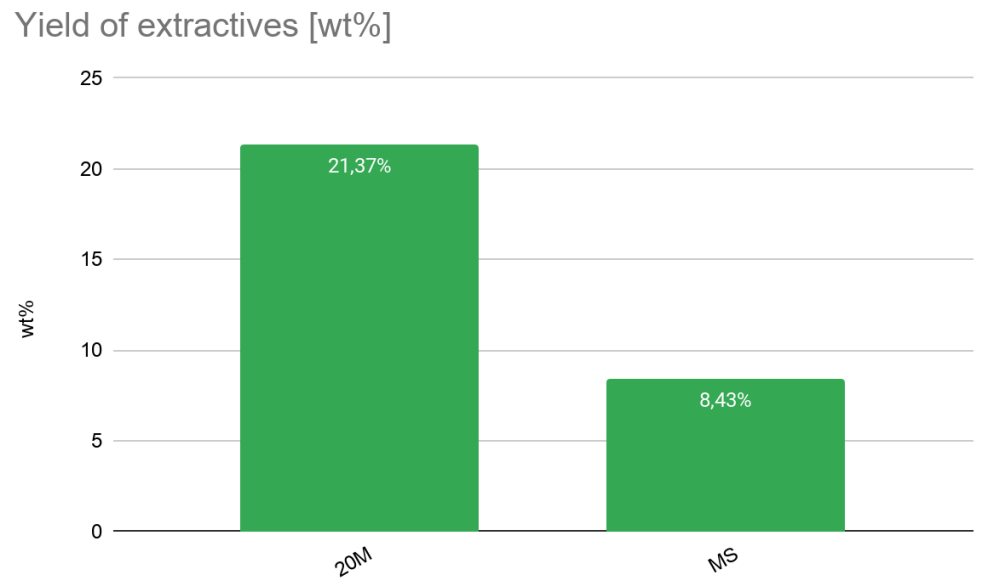


Figure 7. *Amount of extractives extracted from both 20M and MS bark samples.*

5.2 Hot-water Extraction

Figure 8, below, shows measured pH-values of all extract solutions received from the pressurized hot-water extraction.

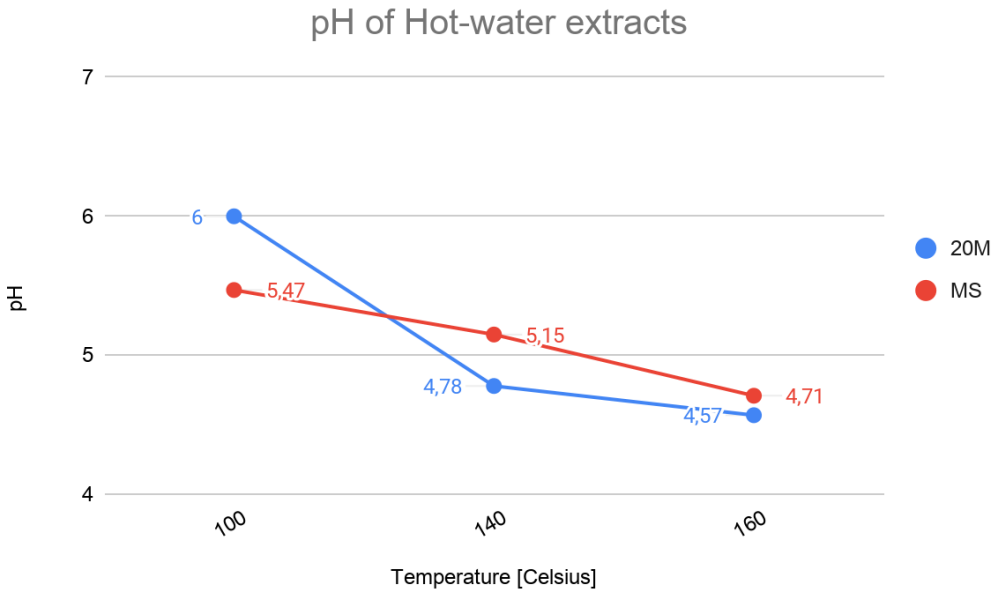


Figure 8. *pH-values measured from samples of the pressurized hot-water extractions.*

5.3 Carbohydrate analysis

See *Figure 9* and *10* below for results from determination of lignin concentration for all steps of both 20M and MS bark samples. Values normalized to the amount of lignin in the initial raw bark sample, which was at 33.25 wt%.

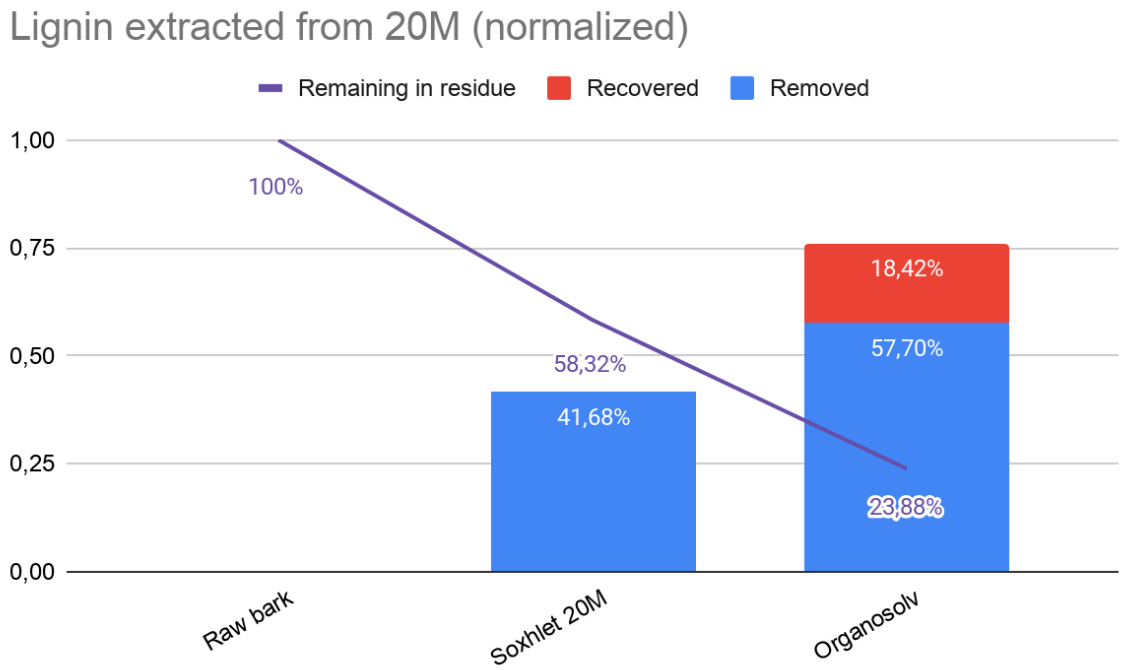


Figure 9. *Amount of lignin extracted in each step of the extraction process for the 20M sample. Values normalized to the weight of lignin in the initial raw bark.*

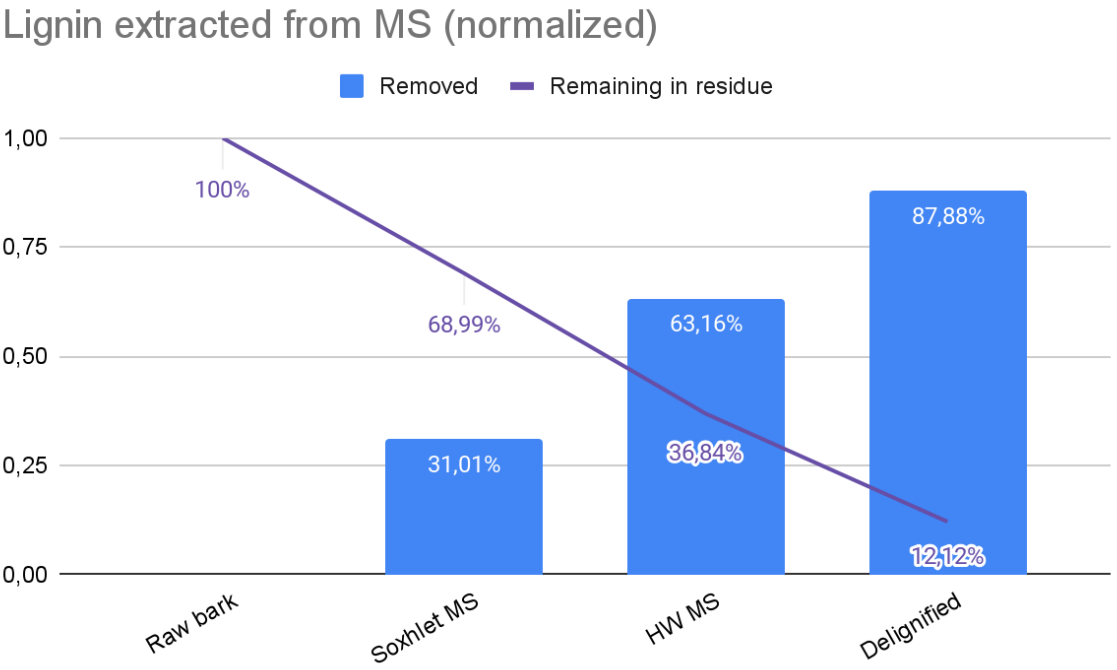


Figure 10. *Amount of lignin extracted in each step of the extraction process for the MS sample. Values normalized to the weight of lignin in the initial raw bark.*

Results achieved from carbohydrate analysis can be seen in *Figure 11* and *12*. Graphs show sugar and lignin composition of each bark sample. Percentages show weight of samples in comparison to weight of initial raw bark sample, which was at 6.001g for 20M and 5.264g for MS.

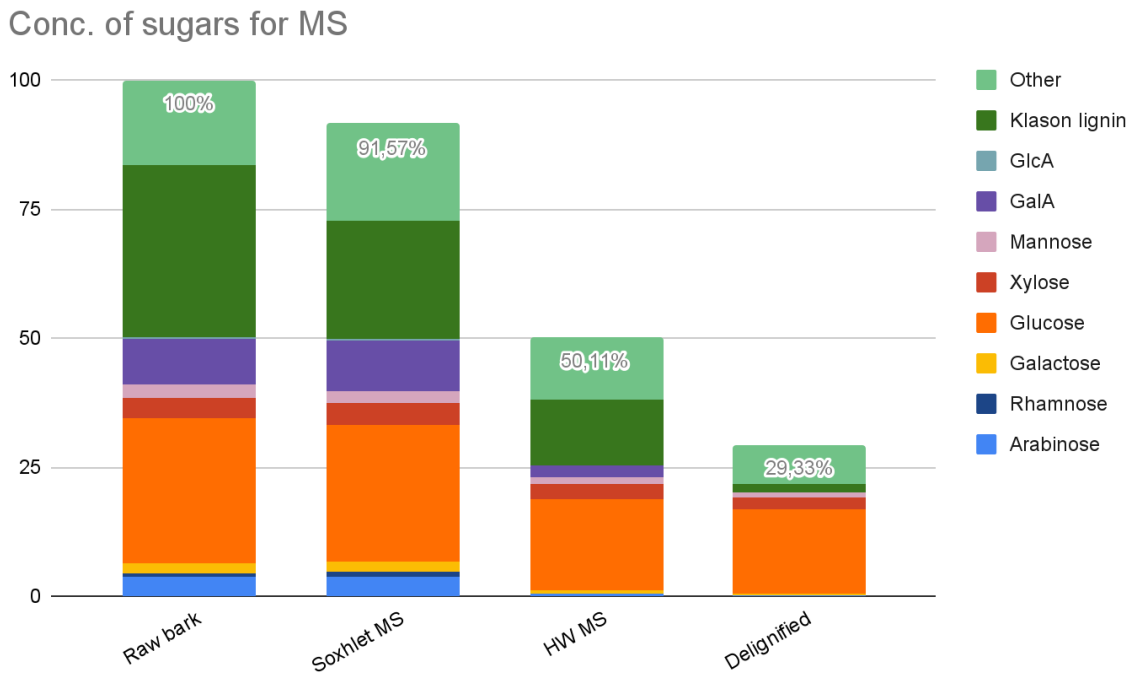


Figure 11. *Results from carbohydrate analysis showing concentration of sugars and Klason lignin at each step of the process for MS samples. Values normalized to the weight of the initial raw bark.*

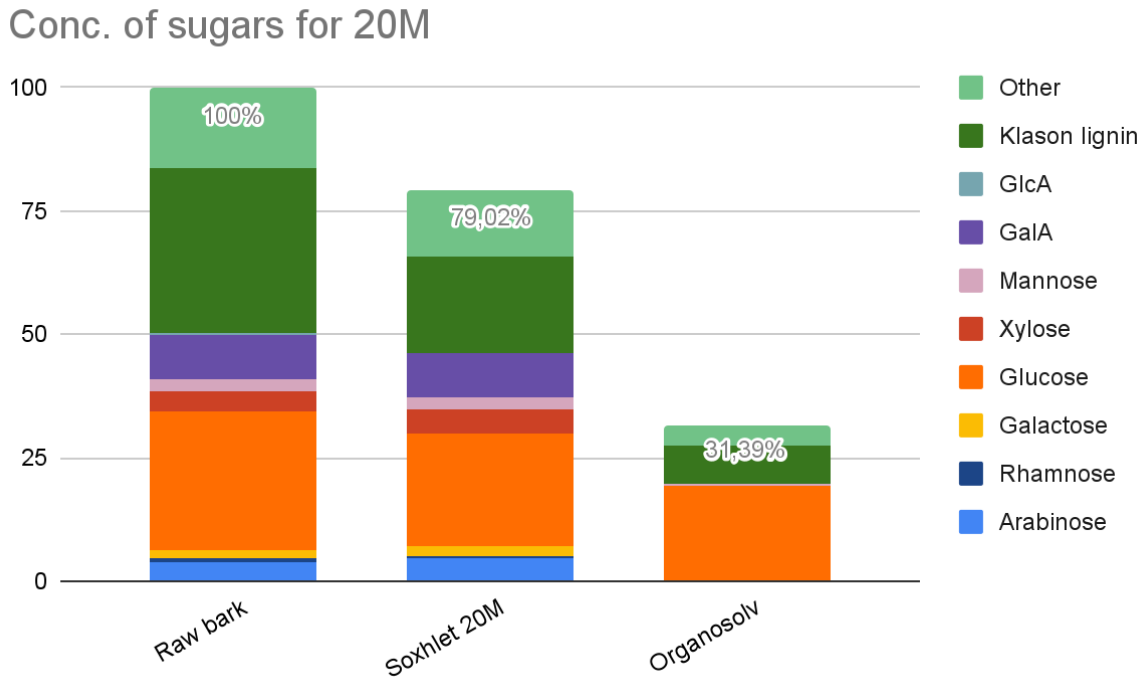


Figure 12: *Results from carbohydrate analysis showing concentration of sugars and Klason lignin at each step of the process for 20M samples. Values normalized to the weight of the initial raw bark.*

5.4 NMR-Analysis

The spectras of the NMR-analysis for respective extraction is presented in the *Appendix* at, *Appendix 2, 4, 6, 8, 10 & 12* with the exception of the organosolv lignin spectra in *Figure 13***.** In *Figure 13* peaks of interest have been marked and are explained in *Table 3*. The structures corresponding to the assigned peaks are shown in *Appendix 14.*

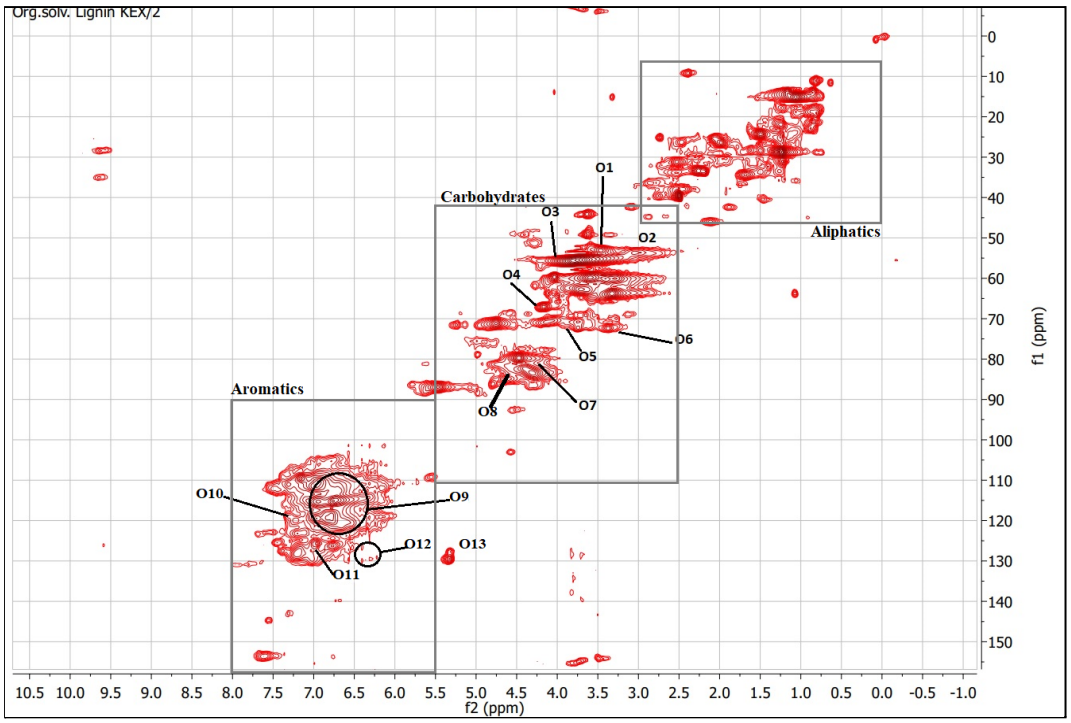


Figure 13. *2D HSQC-NMR spectra of the organosolv lignin. Assignments are explained in Table. 3*

Table 3. *Assignment of the peaks in the Figure 13 organosolv NMR-spectra.*

Symbol	1H	13C	Assignment	References
O1	3.5	53	Phenylcoumaran	52
O2	2.7-3.2	55	Resinol	52
O3	3.2-4.5	55	β–O–4′ alkyl-aryl ethers	52,53
O4	4.0-4.2	66	Hibberts Ketone [Syringyl]	53
O5	3.5-4.3	71	Phenylcoumaran	53
O6	3-3.5	69-74	Xylans	44,52
O7	4-4.5	79-86	β–O–4′ alkyl-aryl ethers	52,53
O8	4.6	86	Resinol	52,54
O9	6.3-7.2	110-120	Guaiacyl	27,28
O10	7.25	120	Stilbene	44,53
O11	6.9	125	Stilbene	44,53
O12	6.3-6.6	135-140	Extractives	56
O13	5.3	135-140	Fatty acid	53,56

5.5 FTIR-Analysis

FTIR-analysis was used as a step of the characterization of the samples. Results received from the analysis are displayed in *Figure 14*. *Table 4* displays the peak assignment of the FTIR spectra.

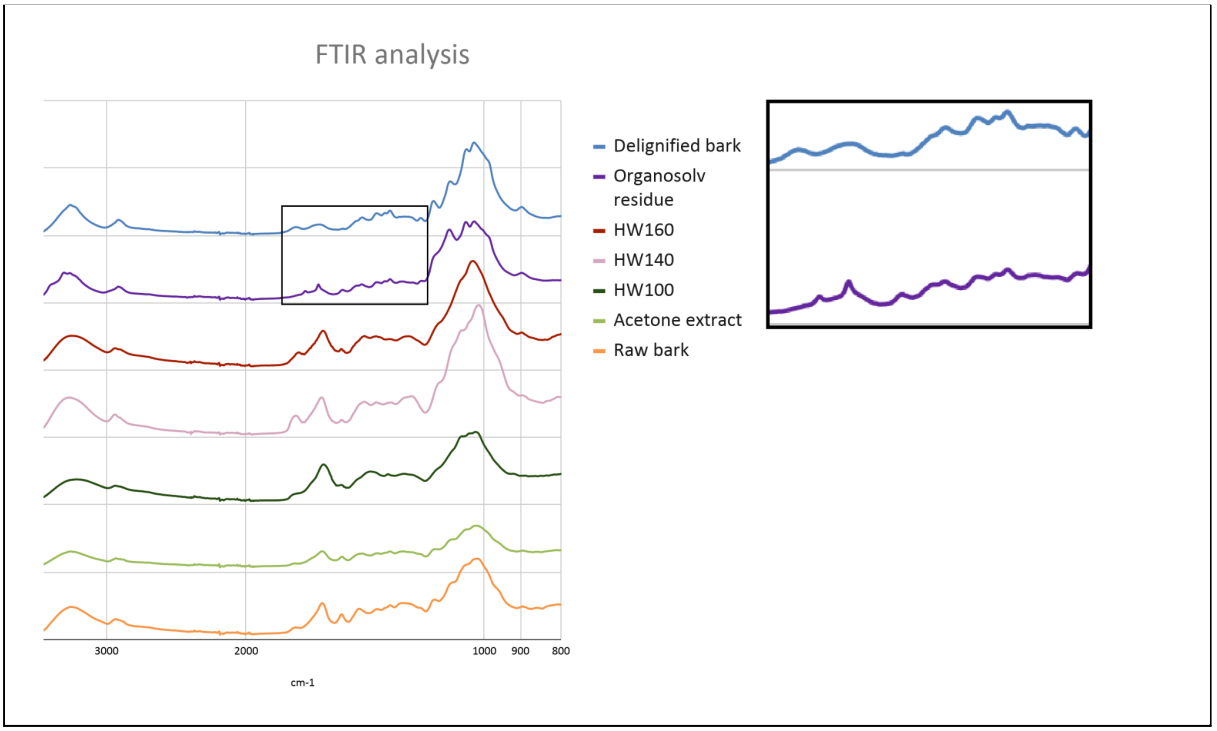


Figure 14. (Spectra to the left) FTIR spectra of the *Raw bark, Acetone extract, Residue, Delignified bark, hot-water extracts for respective temperatures.* (Spectra to the right) A "zoom-in" on the peaks corresponding to lignin, around $1600 - 1200\ cm^{-1}$.

Table 4. *Assignment of the FTIR spectra in Figure 14.*

Delignified bark	Organosolv residue	$HW\ 160^{\circ}C$	$HW\ 140^{\circ}C$	$HW\ 100^{\circ}C$	Peak Assignment	Attributed to [57–62]
3320	3336	3333	3350	3269	O-H stretching	Phenolic, aliphatic structures
2886	2893	2932	2932	2923	C-H stretching	Aromatic methoxy groups, aliphatic methyl groups of side chains
1716	1682	1714	1733		C=O stretching	Hemicellulose
1619	1620	1599	1605	1596	C=C aromatic skeletal vibration and C=O stretching	Lignin
		1516	1513	1514	C=C aromatic skeletal vibration	Lignin
	1459				C-H bending	Lignin, Hemicellulose
1425	1420	1416	1416		C-H bending	Lignin, Hemicellulose
1364	1367	1371	1369	1389	C-H bending	Cellulose, Hemicellulose
1314	1317	1317	1312	1322	C-H wagging	Cellulose, Syringyl derivate
	1270			1262	C-O stretching and C-H bending	Lignin
		1246	1235		C-O stretching	Lignin, Xylan
1158					C-O-C asymmetric vibration	Cellulose, Hemicellulose
1103	1107				Ring asymmetric valence vibration	Cellulose, Hemicellulose
1039	1041		1073		C-O stretching	Cellulose, Hemicellulose
1029	1031	1032	1016	1045	C-O stretching	Cellulose, Hemicellulose
896	892	897	896		C-H wagging	Cellulose, Hemicellulose, Pectin

6. Discussion

6.1 Extractives

For the yield of the extractives, see *Figure 7*, one can observe that the 20M yield of about 21% is somewhat close to the actual amount of extractives in bark which should be about 30%. In addition, the MS yield of about 9% is significantly lower than what is expected. The likely cause for this is the difference in size for the samples. It is easier to extract the components from the sample with smaller particle sizes, since it is easier for solvents to penetrate the sample.

A significant amount of the lignin was extracted, results for the extraction can be seen in *Figure 9* for the 20M sample and *Figure 10* for the MS sample. About 40% of the initial amount of the 20M lignin and about 30% of the initial amount of the MS lignin were extracted. This is partially due to the condensed extractives making the level of lignin in the bark appear higher than it actually is.[63] During the acid hydrolysis in the carbohydrate analysis, some of the extractives were condensed and bound to the lignin. During the acetone extraction, the extractives will no longer be condensed and no longer be bound to the lignin. The initial amount of lignin most likely contained condensed extractives.

6.2 Hot-water Extraction

The hot-water extraction, to determine the sugar composition, was performed only on the MS sample. As one can observe from *Figure 11* the normalized amount of sugar decreases from around 50% to around 25% during the hot-water extraction. The composition of sugars in the bark residue before hot-water extraction consists mostly of glucose and minor amounts of xylose, mannose, GalA and arabinose. Afterwards, the amount of glucose remained approximately the same while a lot of GalA (73%), mannose (43%) and xylose (30%) were removed. A majority of the galactose (67%), rhamnose (100%) and arabinose (86%) were also removed. The minor presence of rhamnose in the sample after hot-water extraction, imply that the pectin was efficiently extracted from the bark. The extraction of xylose and mannose from hemicellulose were not as efficient as the extraction of pectin. About 70% of the xylose and 57% of the mannose remained in the sample after the extraction. The amount of glucose remaining in the bark alludes to the fact that cellulose was unaffected by this extraction method at all three of the temperatures used.

These results are as expected, since the molecular weight of hemicellulose- and pectin polymers are lower than that of cellulose. In addition, their bonds to the cell walls are weaker than that for cellulose. This implies that it is possible to extract hemicellulose and pectin at lower temperatures than cellulose. However, about 30% of the initial lignin content has been extracted, which is implied by the result of the carbohydrate analysis, shown in *Figure 10.* This is also evident from the NMR analysis, which show guaiacyl signals, see *Appendix 4, 6, 8, 10 & 12*. This could be explained by the fact that nonpolar components are extracted through the hot-water extraction. Lignin is a nonpolar molecule, and will most likely be affected.

The hot-water extraction gave information about the quality of yield as well as abundance of various sugars from the lignocellulosic raw material. The 2D HSQC-NMR spectra, seen in *Appendix 2-13*, that equate to the hot-water extraction show a clear decrease in the presence of extractives correlating to an increase in temperature. Although, when investigating the data from *Appendix 2, 4 & 6* and *Appendix 8, 10 & 12* a more successful extraction has been performed with the 20M sample. Signals corresponding to stilbene are still prominent in the MS sample even at higher temperature, *Appendix 6*, thus indicating on the latter.

As the temperature increases, more signals of pectin could be spotted. Although, even at 100°Csome signals correlating to pectin substructures could be observed for the 20M sample and not the MS sample, *Appendix 3 & 8*. This was as expected. With the increasing temperature more peaks connecting to xylose, mannose, galactose, arabinose and rhamnose appear, *Appendix 4, 5, 7 & 8*. This is due to the fact that hemicellulose and pectin are more soluble in water at higher temperatures. Also, *Figure 8* shows that the pH of hot-water extracts decreases with increasing temperature. Acetic- and uronic acids are cleaved from plant tissue at higher temperatures and thus decrease the pH.[11] As the system becomes more acidic, hydrolysis on pectin- and hemicellulose substructures was more prone to happen. This could perhaps explain the observations seen in *Figure 14*; The peak at $1073\,cm^{-1}$in HW 140°Cdisappears in HW 160°C. These findings agree with results from similar studies.[11,64]

6.3 Organosolv extraction

Figure 9 shows that a yield of about 18% of the initial lignin amount was achieved for the organosolv treatment of the 20M bark residue. Comparison to other reports using similar methods shows that this value can vary greatly between about 10%[65] up to 60%.[66] The reason for this seems to lie in the pretreatment of the sample before organosolv extraction is done. Performing an extraction of either extractives or a hot-water treatment before organosolv seems to reduce the amount of organosolv lignin obtained. This assumption correlates with what can be seen of the lignin concentration before organosolv extraction in *Figure 9*, where over 40% of the initial lignin has already been extracted, although parts of this could also come from interference with condensed extractives.

As shown in *Figure 13* and *Table 3* the organosolv lignin shows many signals coming from guaiacyl, *O9*, and a significantly lesser amount corresponding to syringyl, *O4*, see *Appendix 14*. Whereas both guaiacyl and syringyl stand for the peak in *O3*. Other typical substructures of lignin could be seen among the peaks; *O1*, *O2*, *O3* and more, see *Table 3*. Also, signals correlated with stilbene were shown in the spectra. This goes in agreement with earlier studies that showed that hydroxystilbene glucosides such as astringin, isorhapontin and piceid, are incorporated in lignin.[14] On the other hand, the peaks could also mean that these represent extractives since smaller peaks as seen in *O12* seems to correlate with similar experiments that state that the peaks come from extractives.[56]

Another peculiarity that appeared in the spectra were the peaks originating from the xylose. Studies have shown that these sugars along with lignin can form lignin-carbohydrate complexes. Or, perhaps it could be that there are still some sugars left in the bark, even after the hot-water extraction.[67] Figure 12 shows that indeed low relative concentrations of mannose and xylose still existed in the organosolv sample after extraction, about 7% of the initial concentrations for both sugars remained. Possible reasons for this could be the following: Lignin is not the only component that dissolves in the organosolv process, hemicellulose does that as well; The lignin could be contaminated with hemicellulose on the other hand.[68] However, while the concentration of glucose only got reduced to about 70% of the initial concentration, the peaks for glucose, which can be seen in the hot-water extraction samples in *Appendix 8, 10 & 12*, no longer remain in the NMR spectra in *Figure 13*. This could be due to some other, less tightly bound polymers containing glucose being extracted, for example starch, galactoglucomannan and stilbene glucosides, while the cellulose remained unaffected by the extraction. However, the organosolv extraction was performed on the 20M samples after the hot-water extraction. Stilbene Glucosides and cellulose might therefore be major sources of glucose.

The FTIR spectra in *Figure 14* together with *Table 4* display signals of the organosolv residue. These signals that come from lignin sub-structures exhibit a decreased intensity compared to corresponding peaks that occur in the hot-water extractions. This implies, of course, that there is some lignin left in the residue although in lower relative concentrations, which is in line with what can be seen in the carbohydrate analysis, see *Figure 9*, where about 24% of the initial lignin amount was shown to remain in the residue after organosolv extraction. Other well defined peaks belonging to cellulose and hemicellulose are present in the spectra. This confirms that cellulose is a major component in the residue.

6.4 PAA Delignification

The carbohydrate analysis of the residue from the delignification shows that most lignin has been removed. According to *Figure 10* only about 12% of the initial lignin amount remains and the residue now consists mostly of glucose. From the FTIR analysis shown in *Figure 14* it can also be seen that many of the characteristic peaks for lignin are no longer present in the delignified sample. These results are anticipated since the purpose of delignification is the removal of lignin and the isolation of pure cellulose. Although, it can be seen that the sample is not fully purified since it still contains some mannose, xylose and a significant amount of other, unknown components which were not characterized in this experiment. Just like for the soxhlet extraction, this is probably due to the large size of the bark sample which prevents high yields of extractions.

6.5 Upscaling

The question still remains; Is it possible to upscale the lignin extraction from bark to an industrial scale? In theory, yes it is, since it would be possible to upscale the whole extraction processes using relevant equipment. However, one may have to sacrifice some of the environmentally friendly factors of the method, and also consider the economical aspect. The

yield of lignin through the organosolv extraction and the delignification is still relatively low and it would need to be increased for the upscale to be profitable. It would also be required to make use of all the extracted components, to make a potential upscale profitable. However, that would most likely increase the costs significantly in a way that would make it difficult to go through with the upscale.

The conclusion of the viability of the upscaling with these methods seems similar to prior research.[69] In addition, it would perhaps be possible to upscale the extraction of lignin through other methods. It could be possible to extract the lignin without extraction of pectins and hemicelluloses beforehand in order to increase the yield, as discussed previously. This would increase the lignin yield, making the process more profitable, but also remove potential revenue streams as it would then be harder to achieve good yields of pectin and hemicellulose.

Potential alternative methods that can be used for extraction of lignin is through a longer dioxane/water treatment which has been reported to give yields of 37% [70] or through an initial steam explosion followed by DES, deep eutectic solvent, treatment which resulted in yields of up to 65%.[71] Another method is to only isolate Klason lignin, also called acid insoluble lignin, which can be done through treatment with 72% sulfuric acid. This method would give a better yield of removed lignin, but ruin the structure and lower the quality of it. It would also be possible to isolate Willstätter lignin, with concentrated hydrochloric acid, from the bark.[72] However, further research on extraction and isolation methods are needed.

6.6 Errors

One of the filters broke during the carbohydrate analysis, and had to be replaced. However, it is likely that a small amount of the sample was lost in the process and could therefore affect the result. But since an average of two analyses were used for that results, the possible error should have been minimized.

7. Conclusions

From an analysis of all the achieved results in this experiment, one can draw the conclusion that a smaller sample size leads to an extraction process with higher efficiency than an extraction with a sample of a larger size. The FTIR and NMR analysis aligned with previous studies and the yield of lignin from spruce bark were lower than expected. The extraction process of this study could be upscaled but it may not be an economically viable strategy due to the low lignin yield in these experiments. Further research would be necessary in order to succeed with the process of upscaling the extraction method of lignin.

8. References

(1) Ducatez-Boyer, L.; Majourau, P. The Multiple Functions of Tree Bark. **2017**, 7.
(2) Howard, E. T. Bark Structure of the Southern Pines. *Wood Sci. Vol 33 136-148* **1971**.
(3) Ministry of Forests, Mines and Lands - Province of British Columbia - Tree Book - What are the Parts of a Tree? https://www.for.gov.bc.ca/hfd/library/documents/treebook/parts.htm (accessed May 19, 2021).
(4) Gibson, L. J. The Hierarchical Structure and Mechanics of Plant Materials. *J. R. Soc. Interface* **2012**, *9* (76), 2749–2766. https://doi.org/10.1098/rsif.2012.0341.
(5) B. A., B.; A. S., N. Cell Wall Structure and Function https://www.thoughtco.com/cell-wall-373613 (accessed May 19, 2021).
(6) Plant Cells, Chloroplasts, Cell Walls | Learn Science at Scitable https://www.nature.com/scitable/topicpage/plant-cells-chloroplasts-and-cell-walls-14053956/ (accessed May 10, 2021).
(7) Räisänen, T.; Athanassiadis, D. BASIC CHEMICAL COMPOSITION OF THE BIOMASS COMPONENTS OF PINE, SPRUCE AND BIRCH. **2013,** http://biofuelregion.se/wp-content/uploads/2017/01/1_2_IS_2013-01-31_Basic_chemical_composition.pdf (accessed May 19, 2021)
(8) Wang, L.; Dibdiaková, J. Characterization of Ashes from Different Wood Parts of Norway Spruce Tree. *Mater. Sci. Environ. Sci.* **2014**, *37*, 37–42.
(9) Increasing the value of bark as fuel https://www.andritz.com/newsroom-en/success-stories/increasing-the-value-of-bark-as-fuel (accessed Feb 22, 2021).
(10) *Wood and Cellulosic Chemistry*, 2nd ed., rev.expanded.; Hon, D. N.-S., Shiraishi, N., Eds.; Marcel Dekker: New York, 2001.
(11) Krogell, J.; Holmbom, B.; Pranovich, A.; Hemming, J.; Willför, S. Extraction and Chemical Characterization of Norway Spruce Inner and Outer Bark. *Nord. Pulp Pap. Res. J.* **2012**, *27*, 6–17. https://doi.org/10.3183/NPPRJ-2012-27-01-p006-017.
(12) Barbini, S.; Sriranganadane, D.; España Orozco, S.; Kabrelian, A.; Karlström, K.; Rosenau, T.; Potthast, A. Tools for Bark Biorefineries: Studies toward Improved Characterization of Lipophilic Lignocellulosic Extractives by Combining Supercritical Fluid and Gas Chromatography. *ACS Sustain. Chem. Eng.* **2021**, *9* (3), 1323–1332. https://doi.org/10.1021/acssuschemeng.0c07914.
(13) Li, S.-H.; Niu, X.-M.; Zahn, S.; Gershenzon, J.; Weston, J.; Schneider, B. Diastereomeric Stilbene Glucoside Dimers from the Bark of Norway Spruce (Picea Abies). *Phytochemistry* **2008**, *69* (3), 772–782. https://doi.org/10.1016/j.phytochem.2007.08.033.
(14) Rencoret, J.; Neiva, D.; Marques, G.; Gutiérrez, A.; Kim, H.; Gominho, J.; Pereira, H.; Ralph, J.; Río, J. C. del. Hydroxystilbene Glucosides Are Incorporated into Norway Spruce Bark Lignin. *Plant Physiol.* **2019**, *180* (3), 1310–1321. https://doi.org/10.1104/pp.19.00344.
(15) Jyske, T.; Laakso, T.; Latva-Mäenpää, H.; Tapanila, T.; Saranpää, P. Yield of Stilbene Glucosides from the Bark of Young and Old Norway Spruce Stems. *Biomass Bioenergy* **2014**, *71*, 216–227. https://doi.org/10.1016/j.biombioe.2014.10.005.
(16) Raitanen, J.-E.; Järvenpää, E.; Korpinen, R.; Mäkinen, S.; Hellström, J.; Kilpeläinen, P.; Liimatainen, J.; Ora, A.; Tupasela, T.; Jyske, T. Tannins of Conifer Bark as Nordic Piquancy—Sustainable Preservative and Aroma? *Molecules* **2020**, *25* (3). https://doi.org/10.3390/molecules25030567.
(17) Vane, C. H.; Drage, T. C.; Snape, C. E. Bark Decay by the White-Rot Fungus Lentinula Edodes: Polysaccharide Loss, Lignin Resistance and the Unmasking of Suberin. *Int. Biodeterior. Biodegrad.* **2006**, *57* (1), 14–23. https://doi.org/10.1016/j.ibiod.2005.10.004.
(18) Pizzi, A. Tannins: Prospectives and Actual Industrial Applications. *Biomolecules* **2019**, *9* (8). https://doi.org/10.3390/biom9080344.
(19) Adamczyk, B.; Salminen, J.-P.; Smolander, A.; Kitunen, V. Precipitation of Proteins by Tannins: Effects of Concentration, Protein/Tannin Ratio and PH. *Int. J. Food Sci. Technol.* **2012**, *47* (4), 875–878. https://doi.org/10.1111/j.1365-2621.2011.02911.x.
(20) HEMICELLULOSE | Definition of HEMICELLULOSE by Oxford Dictionary on Lexico.com also meaning of HEMICELLULOSE https://www.lexico.com/definition/hemicellulose (accessed May 19, 2021).
(21) Scheller, H. V.; Ulvskov, P. Hemicelluloses. *Annu. Rev. Plant Biol.* **2010**, *61* (1), 263–289. https://doi.org/10.1146/annurev-arplant-042809-112315.
(22) Donev, E.; Gandla, M. L.; Jönsson, L. J.; Mellerowicz, E. J. Engineering Non-Cellulosic Polysaccharides of Wood for the Biorefinery. *Front. Plant Sci.* **2018**, *9*. https://doi.org/10.3389/fpls.2018.01537.
(23) Vanitha, T.; Khan, M. Role of Pectin in Food Processing and Food Packaging. *Intechopen* **2019**.
(24) Britannica, T. Editors of Encyclopaedia. Pectin | biochemistry https://www.britannica.com/science/pectin

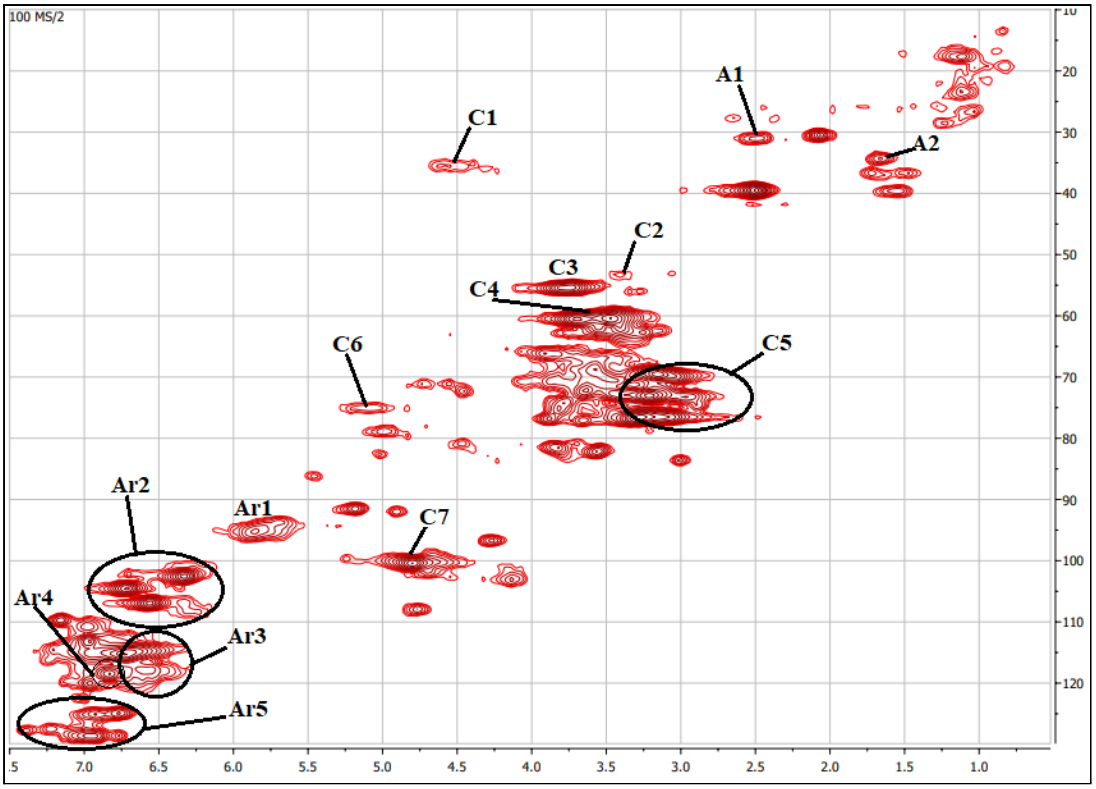
(accessed May 19, 2021).
(25) Ebringerová, A.; Hromádková, Z.; Heinze, T. Hemicellulose. In *Polysaccharides I: Structure, Characterization and Use*; Heinze, T., Ed.; Advances in Polymer Science; Springer: Berlin, Heidelberg, 2005; pp 1–67. https://doi.org/10.1007/b136816.
(26) Flutto, L. PECTIN | Food Use. In *Encyclopedia of Food Sciences and Nutrition (Second Edition)*; Caballero, B., Ed.; Academic Press: Oxford, 2003; pp 4449–4456. https://doi.org/10.1016/B0-12-227055-X/00902-0.
(27) Sriamornsak, P. Chemistry of Pectin and Its Pharmaceutical Uses : A Review /paper/Chemistry-of-Pectin-and-Its-Pharmaceutical-Uses-%3A-A-Sriamornsak/922fee3d5152252bcc21f1320855cb7b4838c20a (accessed May 19, 2021).
(28) Luis Serrano; Rafael Luque; Bert F. Sels. *Lignin Chemistry*; Topics in current chemistry collections; 2020.
(29) Francisco G. Calvo‐Flores; José A. Dobado; Joaquín Isac‐García; Francisco J. Martín‐MartíNez. *Lignin and Lignans as Renewable Raw Materials*, 1st ed.; John Wiley & Sons, Ltd, 2015. https://doi.org/10.1002/9781118682784.
(30) Fernandes, A. N.; Thomas, L. H.; Altaner, C. M.; Callow, P.; Forsyth, V. T.; Apperley, D. C.; Kennedy, C. J.; Jarvis, M. C. Nanostructure of Cellulose Microfibrils in Spruce Wood. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (47), E1195-1203. https://doi.org/10.1073/pnas.1108942108.
(31) Olsson, C.; Westman, G. *Direct Dissolution of Cellulose: Background, Means and Applications*; IntechOpen, 2013. https://doi.org/10.5772/52144.
(32) Granström, M. Cellulose Derivatives: Synthesis, Properties and Applications, University of Helsinki, 2009.
(33) A. Marais, "Tailoring fibre and paper properties using physical adsorption of polyelectrolytes", Royal Institute of Technology (KTH), Stockholm, 2012.
(34) Fig. 1. (A) Applications of cellulose; (B) Structure of cellulose... https://www.researchgate.net/figure/A-Applications-of-cellulose-B-Structure-of-cellulose-adopted-from-Keller-2019_fig1_336674452 (accessed May 19, 2021).
(35) Phanthong, P.; Reubroycharoen, P.; Hao, X.; Xu, G.; Abudula, A.; Guan, G. Nanocellulose: Extraction and Application. *Carbon Resour. Convers.* **2018**, *1* (1), 32–43. https://doi.org/10.1016/j.crcon.2018.05.004.
(36) Hassan, I.; Ai-Jawhari, H. Nanocellulose for Sustainable Future Applications; 2020; pp 1–12. https://doi.org/10.1007/978-3-030-11155-7_16-1.
(37) Scion - Bark: A building block for a circular bioeconomy https://www.scionresearch.com/about-us/about-scion/corporate-publications/scion-connections/past-issues-list/scion-connections-issue-32,-june-2019/bark-a-building-block-for-a-circular-bioeconomy (accessed May 19, 2021).
(38) Scion - Towards a bark biorefinery https://www.scionresearch.com/about-us/news-and-events/news/2018-news-and-media-releases/towards-a-bark-biorefinery (accessed May 19, 2021).
(39) Rietzler, B.; Ek, M. Adding Value to Spruce Bark by the Isolation of Nanocellulose in a Biorefinery Concept. *ACS Sustain. Chem. Eng.* **2021**, *9* (3), 1398–1405. https://doi.org/10.1021/acssuschemeng.0c08429.
(40) A Zygler; M Siominska; J Namiesnik. 2.04 – Soxhlet Extraction and New Developments Such as Soxtec | Elsevier Enhanced Reader https://reader.elsevier.com/reader/sd/pii/B9780123813732000375?token=1400A204AA07BA9DA7CABE035BCFD8F7C1EF511F628847378E72AD0F740C7E24A249A5E3B50490A9F41DF71F5FFC22D8 (accessed Feb 23, 2021). https://doi.org/10.1016/B978-0-12-381373-2.00037-5.
(41) Teo, C. C.; Tan, S. N.; Yong, J. W. H.; Hew, C. S.; Ong, E. S. Pressurized Hot Water Extraction (PHWE). *J. Chromatogr. A* **2010**, *1217* (16), 2484–2494. https://doi.org/10.1016/j.chroma.2009.12.050.
(42) López-Bascón, M. A.; Luque de Castro, M. D. Chapter 11 - Soxhlet Extraction. In *Liquid-Phase Extraction*; Poole, C. F., Ed.; Handbooks in Separation Science; Elsevier, 2020; pp 327–354. https://doi.org/10.1016/B978-0-12-816911-7.00011-6.
(43) Schutyser, W.; Renders, T.; Bosch, S. V. den; Koelewijn, S.-F.; Beckham, G. T.; Sels, B. F. Chemicals from Lignin: An Interplay of Lignocellulose Fractionation, Depolymerisation, and Upgrading. *Chem. Soc. Rev.* **2018**, *47* (3), 852–908. https://doi.org/10.1039/C7CS00566K.
(44) Liu, L.-Y.; Patankar, S. C.; Chandra, R. P.; Sathitsuksanoh, N.; Saddler, J. N.; Renneckar, S. Valorization of Bark Using Ethanol–Water Organosolv Treatment: Isolation and Characterization of Crude Lignin. *ACS Sustain. Chem. Eng.* **2020**, *8* (12), 4745–4754. https://doi.org/10.1021/acssuschemeng.9b06692.
(45) Tampieri, A.; Szabó, M.; Medina, F.; Gulyás, H. A Brief Introduction to the Basics of NMR Spectroscopy and Selected Examples of Its Applications to Materials Characterization. *Phys. Sci. Rev.* **2021**, *6* (1). https://doi.org/10.1515/psr-2019-0086.

(46) NMR: Introduction https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Spectroscopy/Magnetic_Resonance_Spectroscopies/Nuclear_Magnetic_Resonance/Nuclear_Magnetic_Resonance_II (accessed May 19, 2021).
(47) NMR - Theory https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Spectroscopy/Magnetic_Resonance_Spectroscopies/Nuclear_Magnetic_Resonance/NMR_-_Theory (accessed May 19, 2021).
(48) Corradini, C.; Cavazza, A.; Bignardi, C. High-Performance Anion-Exchange Chromatography Coupled with Pulsed Electrochemical Detection as a Powerful Tool to Evaluate Carbohydrates of Food Interest: Principles and Applications https://www.hindawi.com/journals/ijcc/2012/487564/ (accessed Feb 24, 2021). https://doi.org/10.1155/2012/487564.
(49) FTIR Analysis | RTI Laboratories, 2016.
(50) How Does FTIR Work? *Innovatech Labs*, 2015.
(51) FTIR Spectroscopy https://www.sigmaaldrich.com/technical-documents/articles/biology/ftir-spectroscopy.html (accessed May 19, 2021).
(52) Giummarella, N.; Lawoko, M. Structural Insights on Recalcitrance during Hydrothermal Hemicellulose Extraction from Wood. *ACS Sustain. Chem. Eng.* **2017**, *5* (6), 5156–5165. https://doi.org/10.1021/acssuschemeng.7b00511.
(53) Karlsson, M.; Giummarella, N.; Lindén, P. A.; Lawoko, M. Toward a Consolidated Lignin Biorefinery: Preserving the Lignin Structure through Additive‐Free Protection Strategies. *ChemSusChem* **2020**, *13* (17), 4666–4677. https://doi.org/10.1002/cssc.202000974.
(54) Zikeli, F.; Vinciguerra, V.; D'Annibale, A.; Capitani, D.; Romagnoli, M.; Scarascia Mugnozza, G. Preparation of Lignin Nanoparticles from Wood Waste for Wood Surface Treatment. *Nanomaterials* **2019**, *9* (2), 281. https://doi.org/10.3390/nano9020281.
(55) Rencoret, J.; Marques, G.; Gutiérrez, A.; Nieto, L.; Santos, J. I.; Jiménez-Barbero, J.; Martínez, Á. T.; Río, J. C. del. HSQC-NMR Analysis of Lignin in Woody (Eucalyptus Globulus and Picea Abies) and Non-Woody (Agave Sisalana) Ball-Milled Plant Materials at the Gel State 10th EWLP, Stockholm, Sweden, August 25–28, 2008. **2009**, *63* (6), 691–698. https://doi.org/10.1515/HF.2009.070.
(56) Lancefield, C. S.; Wienk, H. L. J.; Boelens, R.; Weckhuysen, B. M.; Bruijnincx, P. C. A. Identification of a Diagnostic Structural Motif Reveals a New Reaction Intermediate and Condensation Pathway in Kraft Lignin Formation. *Chem. Sci.* **2018**, *9* (30), 6348–6360. https://doi.org/10.1039/C8SC02000K.
(57) Pandey, K. K. A Study of Chemical Structure of Soft and Hardwood and Wood Polymers by FTIR Spectroscopy. *J. Appl. Polym. Sci.* **1999**, *71* (12), 1969–1975. https://doi.org/10.1002/(SICI)1097-4628(19990321)71:12<1969::AID-APP6>3.0.CO;2-D.
(58) Durmaz, S.; Özgenç, Ö.; Boyacı, İ. H.; Yıldız, Ü. C.; Erişir, E. Examination of the Chemical Changes in Spruce Wood Degraded by Brown-Rot Fungi Using FT-IR and FT-Raman Spectroscopy. *Vib. Spectrosc.* **2016**, *85*, 202–207. https://doi.org/10.1016/j.vibspec.2016.04.020.
(59) Long, W.; Lai; Rahim, N.; Hashim, E.; cob, Z.; Idris, A.; Akhtar, J. STUDY ON COMPOSITION, STRUCTURAL AND PROPERTY CHANGES OF OIL PALM FROND BIOMASS UNDER DIFFERENT PRETREATMENTS. **2016**, *50*, 9–10.
(60) Özgenç, Ö.; Durmaz, S.; Kuştaş, S. Chemical Analysis of Tree Barks Using ATR-FTIR Spectroscopy and Conventional Techniques. *Bioresources* **2017**, *12*, 9143–9151. https://doi.org/10.15376/biores.12.4.9143-9151.
(61) Popa, V. CONTRIBUTION TO THE MODIFICATION AND CHARACTERIZATION OF DIFFERENT TYPES OF LIGNINS. *Cellul. Chem. Technol.* **2009**, *43*, 409–418.
(62) Le Normand, M.; Moriana, R.; Ek, M. Isolation and Characterization of Cellulose Nanocrystals from Spruce Bark in a Biorefinery Perspective. *Carbohydr. Polym.* **2014**, *111*, 979–987. https://doi.org/10.1016/j.carbpol.2014.04.092.
(63) Marles, M. A. S.; Coulman, B. E.; Bett, K. E. Interference of Condensed Tannin in Lignin Analyses of Dry Bean and Forage Crops. *J. Agric. Food Chem.* **2008**, *56* (21), 9797–9802. https://doi.org/10.1021/jf800888r.
(64) Wojtasz-Mucha, J.; Hasani, M.; Theliander, H. Dissolution of Wood Components during Hot Water Extraction of Birch. *Wood Sci. Technol.* **2021**. https://doi.org/10.1007/s00226-021-01283-9.
(65) Yadav, P.; Athanassiadis, D.; Antonopoulou, I.; Rova, U.; Christakopoulos, P.; Tysklind, M.; Matsakas, L. Environmental Impact and Cost Assessment of a Novel Lignin Production Method. *J. Clean. Prod.* **2021**, *279*, 123515. https://doi.org/10.1016/j.jclepro.2020.123515.
(66) Nitsos, C.; Stoklosa, R.; Karnaouri, A.; Vörös, D.; Lange, H.; Hodge, D.; Crestini, C.; Rova, U.; Christakopoulos, P. Isolation and Characterization of Organosolv and Alkaline Lignins from Hardwood

and Softwood Biomass | ACS Sustainable Chemistry & Engineering https://pubs.acs.org/doi/pdf/10.1021/acssuschemeng.6b01205 (accessed May 19, 2021).
(67) Tarasov, D.; Leitch, M.; Fatehi, P. Lignin–Carbohydrate Complexes: Properties, Applications, Analyses, and Methods of Extraction: A Review. *Biotechnol. Biofuels* **2018**, *11* (1), 269. https://doi.org/10.1186/s13068-018-1262-1.
(68) Shrotri, A.; Kobayashi, H.; Fukuoka, A. Chapter Two - Catalytic Conversion of Structural Carbohydrates and Lignin to Chemicals. In *Advances in Catalysis*; Song, C., Ed.; Academic Press, 2017; Vol. 60, pp 59–123. https://doi.org/10.1016/bs.acat.2017.09.002.
(69) Kemppainen, K.; Siika-aho, M.; Pattathil, S.; Giovando, S.; Kruus, K. Spruce Bark as an Industrial Source of Condensed Tannins and Non-Cellulosic Sugars. *Ind. Crops Prod.* **2014**, *52*, 158–168. https://doi.org/10.1016/j.indcrop.2013.10.009.
(70) Neiva, D. M.; Rencoret, J.; Marques, G.; Gutiérrez, A.; Gominho, J.; Pereira, H.; Del Río, J. C. Lignin from Tree Barks: Chemical Structure and Valorization. *ChemSusChem* **2020**, *13* (17), 4537–4547. https://doi.org/10.1002/cssc.202000431.
(71) Tian, D.; Chandra, R. P.; Lee, J.-S.; Lu, C.; Saddler, J. N. A Comparison of Various Lignin-Extraction Methods to Enhance the Accessibility and Ease of Enzymatic Hydrolysis of the Cellulosic Component of Steam-Pretreated Poplar. *Biotechnol. Biofuels* **2017**, *10* (1), 157. https://doi.org/10.1186/s13068-017-0846-5.
(72) Jingjing, L. **2011** ISOLATION OF LIGNIN FROM WOOD, Bachelor's thesis, Saimaa University of Applied Sciences, Imatra
(73) Zhang, L.; Gellerstedt, G. 2D Heteronuclear (1H–13C) Single Quantum Correlation (HSQC) NMR Analysis of Norway Spruce Bark Components. In *Characterization of Lignocellulosic Materials*; Hu, T. Q., Ed.; Blackwell Publishing Ltd.: Oxford, UK, 2008; pp 1–16. https://doi.org/10.1002/9781444305425.ch1.
(74) Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities. *J. Org. Chem.* **1997**, *62* (21), 7512–7515. https://doi.org/10.1021/jo971176v.
(75) Makarova, E. N.; Shakhmatov, E. G.; Udoratina, E. V.; Kutchin, A. V. Structural and Chemical Charactertistics of Pectins, Arabinogalactans, and Arabinogalactan Proteins from Conifers. *Russ. Chem. Bull.* **2015**, *64* (6), 1302–1318. https://doi.org/10.1007/s11172-015-1011-6.

Appendix

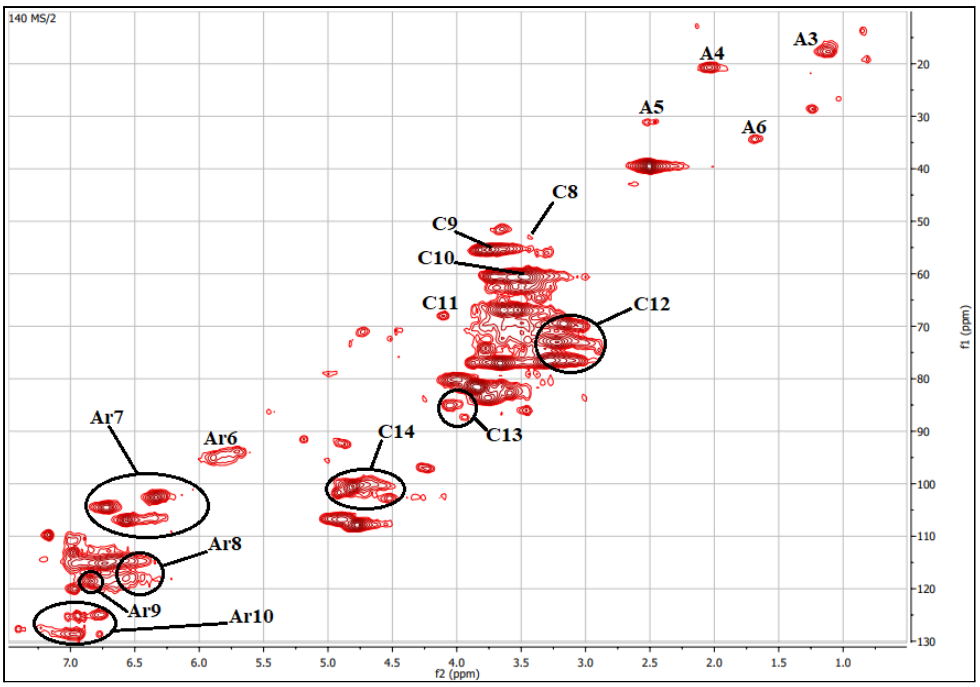
Appendix 1. *Powdered bark with size of 0.8 mm in diameter, 20M (left) and matchstick-sized bark, MS (right).*



Appendix 2. 2D HSQC-NMR spectra from Hot-water extraction of MS at 100 $^{\circ}C$. The assignments are explained in *Appendix 3.* Abbreviations are the following: "A" = Aliphatics, "C" = Carbohydrates and "Ar" = Aromatics.

Appendix 3. Assignment of peaks on the MS sample from 100 $^{\circ}C$Hot-water extraction

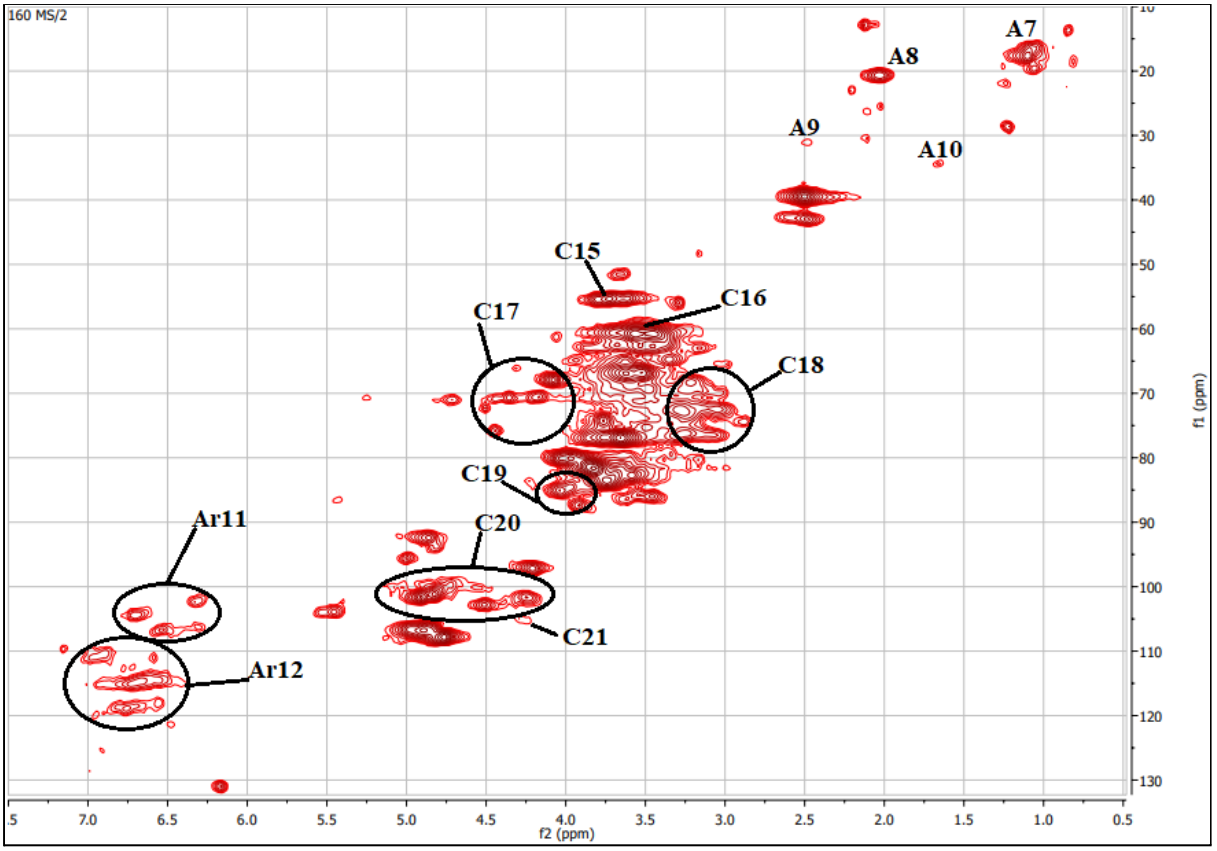
Symbol	1H	13C	Assignment	References
A1	2.5	32	Condensed tannin	73
A2	1.7	34.5	Condensed tannin	73
C1	4.3-4.6	35.5	Condensed tannin	73
REF	2.5	39	DMSO	74
C2	3.4	54	Phenylcoumaran	52
C3	3.5-4	56	-OMe in guaiacyl	52
C4	3.3-4	62	Mannose	52
C5	2.75-3.5	69-77	Glucose	73
C6	4.9-5.2	75	Condensed tannin	73
Ar1	5.6-5.9	94-96	Condensed tannin	73
C7	4.5-5	100	Glucose	52,73
Ar2	6.2-6.9	102-108	Stilbene	14,73
Ar3	6.4-6.6	114-122	Condensed tannin	73
Ar4	6.7	118	Stilbene	73
Ar5	6.75-7.4	125-130	Stilbene	73



Appendix 4. 2D HSQC-NMR spectra from Hot-water extraction of MS at 140 $^{\circ}C$. The assignments are explained in *Appendix 5*. Abbreviations are the following: "A" = Aliphatics, "C" = Carbohydrates and "Ar" = Aromatics.

Appendix 5. Assignment of peaks on the MS sample from 140 $^{\circ}C$Hot-water extraction

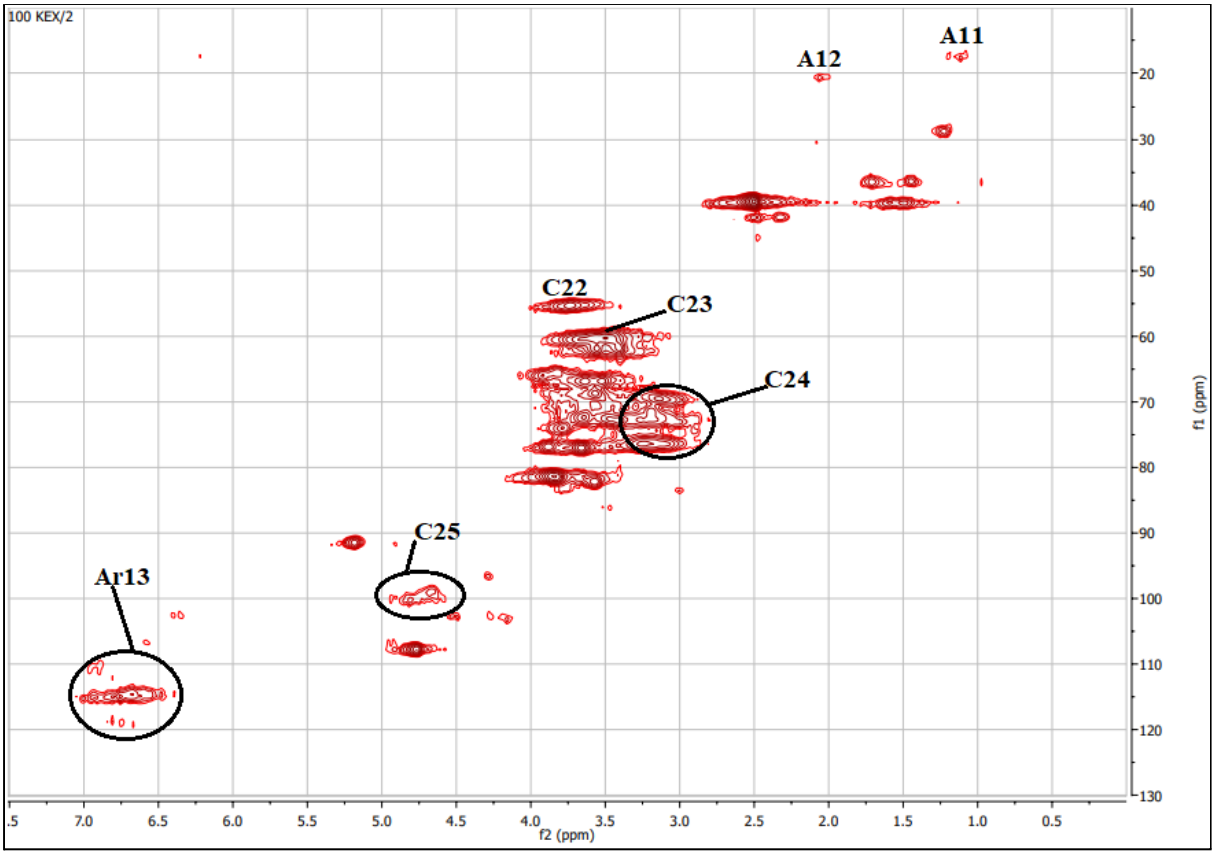
Symbol	1H	13C	Assignment	References
A3	1.1-1.2	16-18	Rhamnose	75
A4	2.1	21	O-Ac RhG	75
A5	2.5	31	Condensed tannin	73
A6	1.7	34	Condensed tannin	73
REF	2.5	39	DMSO	74
C8	3.4	54	Phenylcoumaran	52
C9	3.5-3.8	56	-OMe in guaiacyl	52
C10	3.2-3.8	62	Mannose	52
C11	4.1	68	Galactose	75
C12	2.8-3.4	69-77	Glucose	73
C13	4-4.1	75	Arabinose	52,75
Ar6	5.7-5.9	94-96	Condensed tannin	73
C14	4.5-4.9	99-103	Glucose, Xylose, Mannose	52,73
Ar7	6.25-6.6	102-108	Stilbene	14,73
Ar8	6.4-6.7	114-120	Catechol, guaiacyl	55,73
Ar9	6.7	118	Stilbene	73
Ar10	6.75-7.4	125-130	Stilbene	73



Appendix 6 2D HSQC-NMR spectra from Hot water extraction of MS at 160°*C*. The assignments are explained in *Appendix 7*. Abbreviations are the following: "A" = Aliphatics, "C" = Carbohydrates and "Ar" = Aromatics.

Appendix 7. Assignment of peaks on the MS sample from 160°*C*Hot-water extraction

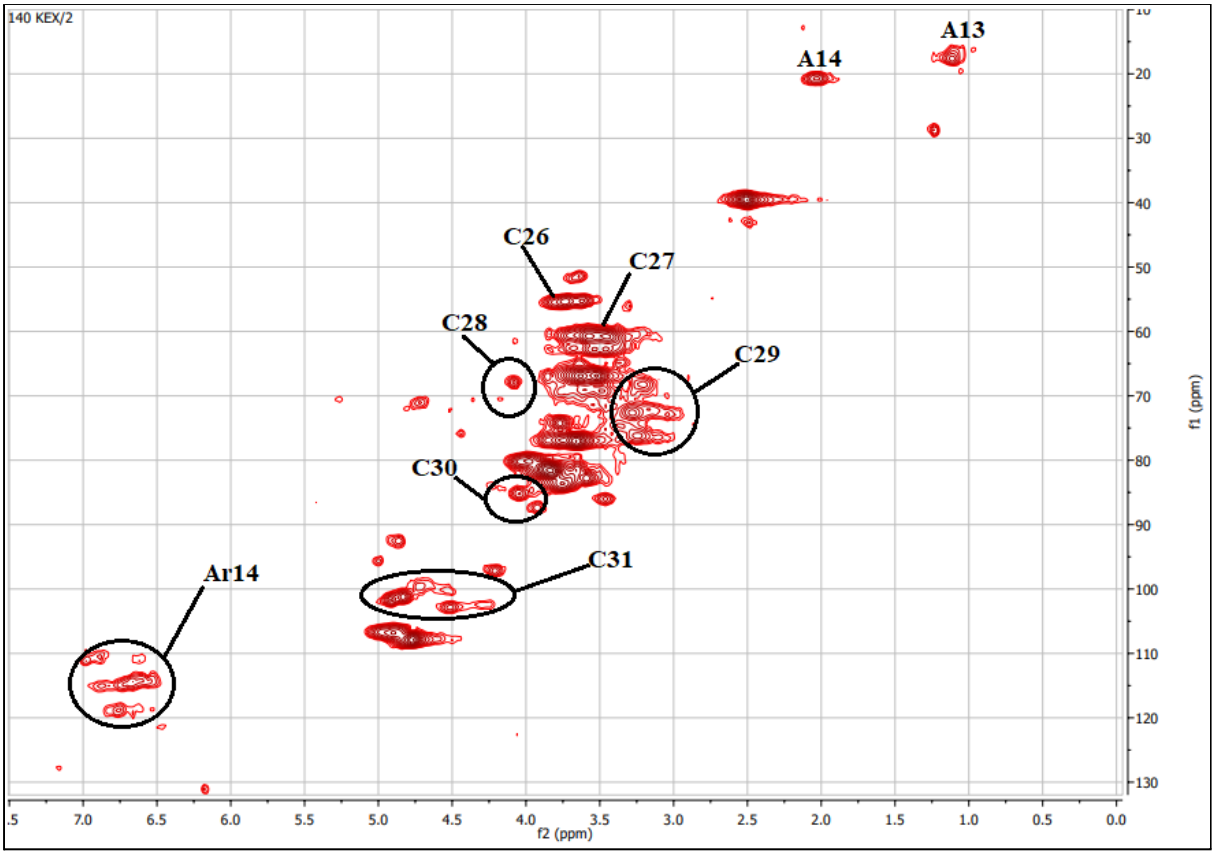
Symbol	1H	13C	Assignment	References
A7	1-1.25	16-20	Rhamnose	75
A8	2.1	21	O-Ac RhG	75
A9	2.5	31	Condensed tannin	73
A10	1.7	34	Condensed tannin	73
REF	2.5	39	DMSO	74
C15	3.5-3.9	56	-OMe in guaiacyl	52
C16	3.2-3.9	62	Mannose	52
C17	4-4.5	68-75	Galactose	75
C18	2.8-3.3	68-77	Glucose	73
C19	4-4.1	85	Arabinose	52,75
C20	4.2-5	99-103	Mannose, xylose, glucose	52,73
Ar11	6.3-6.7	102-108	Stilbene	14,73
C21	4.2	105	Galactose	75
Ar12	6.5-7	110-120	Guaiacyl	55



Appendix 8. 2D HSQC-NMR spectra from Hot-water extraction of 20M at $100^{\circ}C$. The assignments are explained in *Appendix 9.* Abbreviations are the following: "A" = Aliphatics, "C" = Carbohydrates and "Ar" = Aromatics.

Appendix 9. Assignment of peaks on the 20M sample from $100^{\circ}C$Hot-water extraction

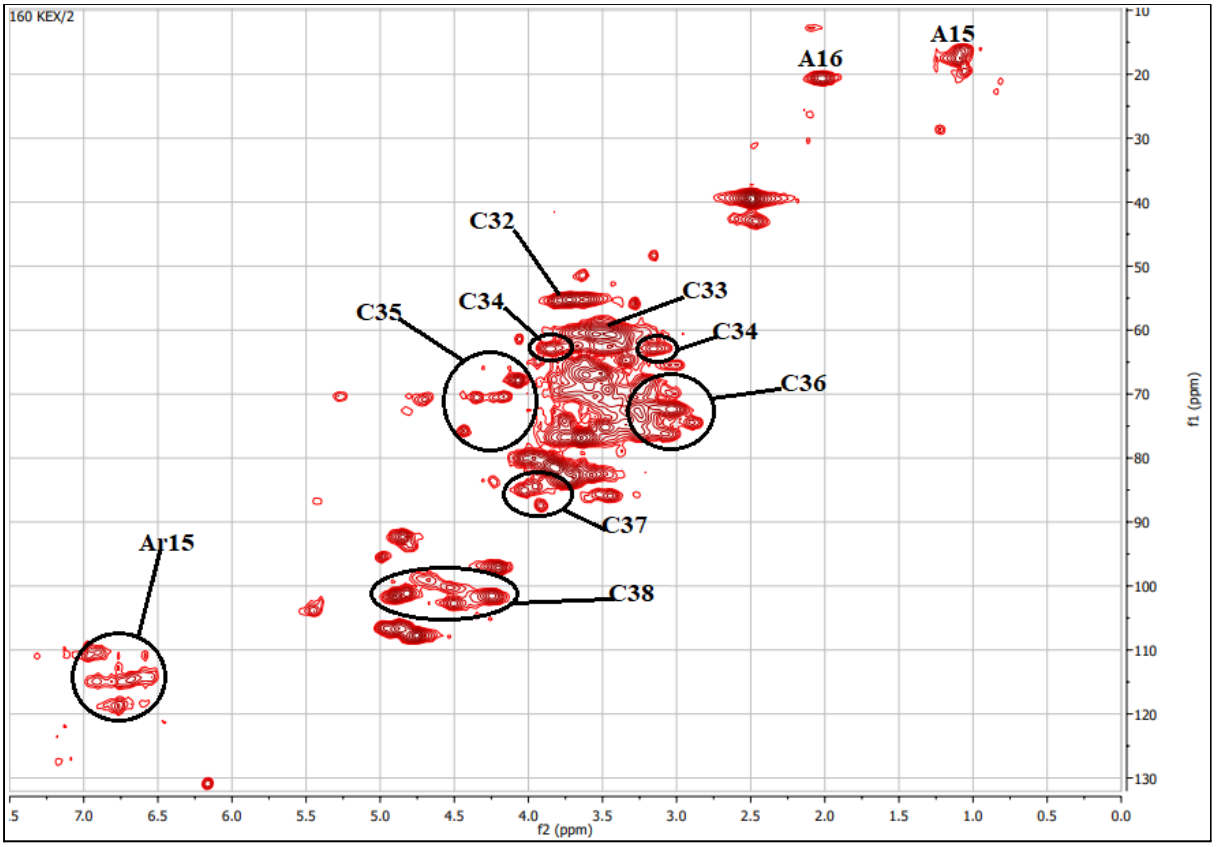
Symbol	1H	13C	Assignment	References
A11	1.2	18	Rhamnose	75
A12	2.1	21	O-Ac RhG	75
REF	2.5	39	DMSO	74
C22	3.5-3.9	56	-OMe in guaiacyl	52
C23	3.2-3.9	62	Mannose	52
C24	2.8-3.4	68-77	Glucose	73
C25	4.6-4.9	99-101	Glucose	52,73
Ar13	6.5-7	115	Guaiacyl	55



Appendix 10. 2D HSQC-NMR spectra from Hot-water extraction of 20M at $140^{\circ}C$. The assignments are explained in *Appendix 11*. Abbreviations are the following: "A" = Aliphatics, "C" = Carbohydrates and "Ar" = Aromatics.

Appendix 11. Assignment of peaks on the 20M sample from $140^{\circ}C$Hot-water extraction

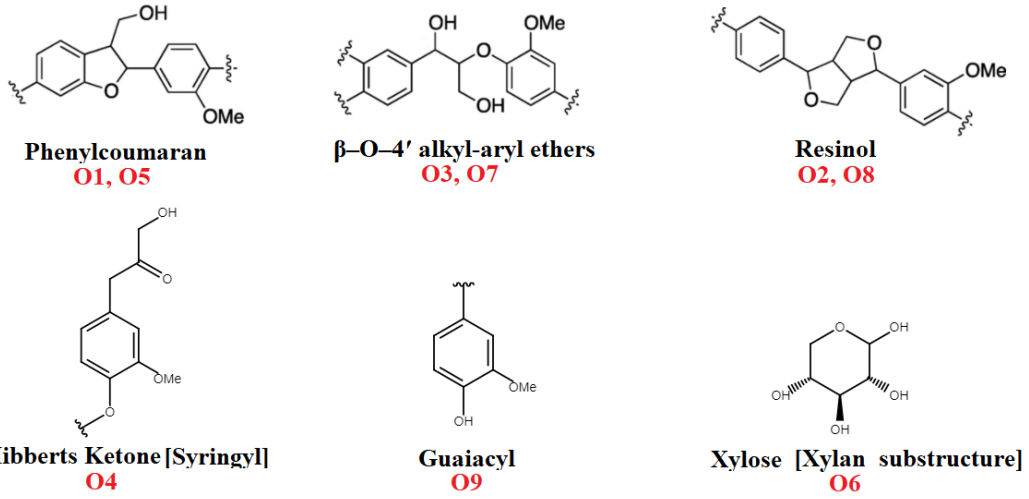
Symbol	1H	13C	Assignment	References
A13	1.2	18	Rhamnose	75
A14	2.1	21	O-Ac RhG	75
REF	2.5	39	DMSO	74
C26	3.5-3.9	56	-OMe in guaiacyl	52
C27	3.2-3.9	62	Mannose	52
C28	4.1	68	Galactose	75
C29	3-3.4	68-77	Glucose	73
C30	4.1	85	Arabinose	52,75
C31	4.25-5	99-104	Mannose, xylose, glucose	52
Ar14	6.5-7	110-120	Guaiacyl	55



Appendix 12. 2D HSQC-NMR spectra from Hot-water extraction of 20M at 160 $^{\circ}C$. The assignments are explained in *Appendix 13*. Abbreviations are the following: "A" = Aliphatics, "C" = Carbohydrates and "Ar" = Aromatics.

Appendix 13. Assignment of peaks on the 20M sample from 160 $^{\circ}C$Hot-water extraction

Symbol	1H	13C	Assignment	References
A15	1-1.5	15-20	Rhamnose	75
A16	1.9-2.1	21	O-Ac RhG	75
REF	2.5	39	DMSO	74
C32	3.5-3.9	56	-OMe in guaiacyl	52
C33	3.1-3.9	62	Mannose	52
C34	3.2, 3.8	64	Xylose	52
C35	4-4.4	68-75	Galactose	75
C36	3-3.4	68-77	Glucose	73
C37	4.1	85	Arabinose	52,75
C38	4.2-5	97-104	Mannose, xylose, glucose	52
Ar15	6.5-7	110-120	Guaiacyl	55



Appendix 14. Displayed structures of the peaks observed in *Table 3*, also, which peak respective structures were assigned to. Lignin- and xylan-substructure is shown.