Organosolv lignins isolation from different biomasses and their characterization

Bachelor thesis

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Organosolv ligniinide isoleerimine erinevatest biomassidest ja nende iseloomustamine

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Declaration

Hereby I declare that I have compiled the thesis independently and all works, important standpoints and data by other authors have been properly referenced and the same thesis has not previously been presented for grading.

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Table of Contents

List of Abbreviations

BHT	butylated hydroxytoluene
DLATGS	deuterated L-alanine doped triglycene sulphate
DO(L)	dioxane Organosolv (lignin)
DRI	differential refractive index
EO(L)	ethanol Organosolv (lignin)
EtOH	ethanol
FTIR	Fourier-transform infrared spectroscopy
G	guaiacyl
GPC	gel-permeation chromatography
H	*p*-hydroxyphenyl
HCl	hydrochloric acid
IR	infrared
KBr	potassium bromide
M_n	number average molecular weight
M_w	weight average molecular weight
MW(D)	molecular weight (distribution)
M_z/M_{z+1}	higher average molecular weight
NMR	nuclear magnetic resonance
PI	polydispersity index
PS-DVB	polystyrene-divinylbenzene
%RSD	relative standard deviation
S	syringyl
sd	standard deviation
SEC	size-exclusion chromatography
THF	tetrahydrofuran
UV	ultraviolet
UV-Vis	ultraviolet-visible

Introduction

Sustainable production has become increasingly important in the recent decades. Large industries, such as timber industry, produce vast quantities of production residues, which oftentimes are not further used effectively. One such residue of the cellulose processing industry is lignin, which is usually burned for energy purposes or landfilled, leading to inefficient environment-resource consumption. Due to its structure, lignin has a high potential for production of value-added chemicals and biofuels. However, for efficient valorization, the structure of lignin needs to be evaluated, which is hard to achieve due to its complexity and heterogeneity.

The objectives of this work are the following:

1. To develop and optimize analytical methods for the determination of lignin molecular weight distribution and the presence of functional groups.
2. To characterize the lignin from different types of biomass: hardwoods, softwoods, and grasses.
3. To describe the effect of different solvents used for lignin extraction on its properties and structure.

Several lignin characterization methods are in use, including chromatographic, spectroscopic, gravimetric etc. In this work, two methods have been implemented to characterize lignin structure. Size-exclusion chromatography (SEC) is a chromatographic method, that allows for the determination of lignin molecular weight distribution to make assumptions about its chemical and physical properties. Using the infrared light to cause molecular vibrations, the second applied method, Fourier-transform infrared spectroscopy (FTIR), can give information about functional groups and the presence of main structural units within lignin.

The first section of this thesis, the literature review, covers lignocellulosic biomass and explains the lignin structure; the importance of lignin valorization; the overview of used lignin extraction method (Organosolv) and its advantages; overview of main lignin characterization methods (size-exclusion chromatography, and FTIR). Secondly, experimental part, which describes the procedures for each method used and lists the reagents and equipment needed to repeat these experiments. Thirdly, the results section gives information on how the different biomass and extraction solvent usage effects extracted lignin yield and color, molecular weight distribution, and functional groups and structural units presence. The final, discussion part, compares obtained results with publications, analyzes the experimental data collected, and makes conclusions about the study results. The thesis contains 14 figures, 2 tables, 2 extras, and references 45 sources.

As the result of this study, methods for lignin molecular weight distribution and structural units determination were optimized and implemented. The main conclusion is that lignin properties and structure are strongly dependent on the type of biomass source and the method of extraction, including different solvents usage.

1 Literature Review

1.1 Lignocellulosic biomass and lignin

Lignocellulosic biomass is a complex structural material found in the cell wall of woody plants [1]. It is mainly composed of cellulose (30-50%), hemicellulose (20-35%), and lignin (15-30%) (**Figure 1**) [2]. The proportions of each component are dependent on the plant species and origin [3]. Sugar monomers of cellulose and hemicellulose are covalently bound to lignin [4]. Proteins, resins, and inorganic substances are also present in low concentrations [1]. One of the main uses of lignocellulose are as a feedstock for the pulp and paper industry [4], and the production of bioethanol through the conversion of cellulose and hemicellulose [5].

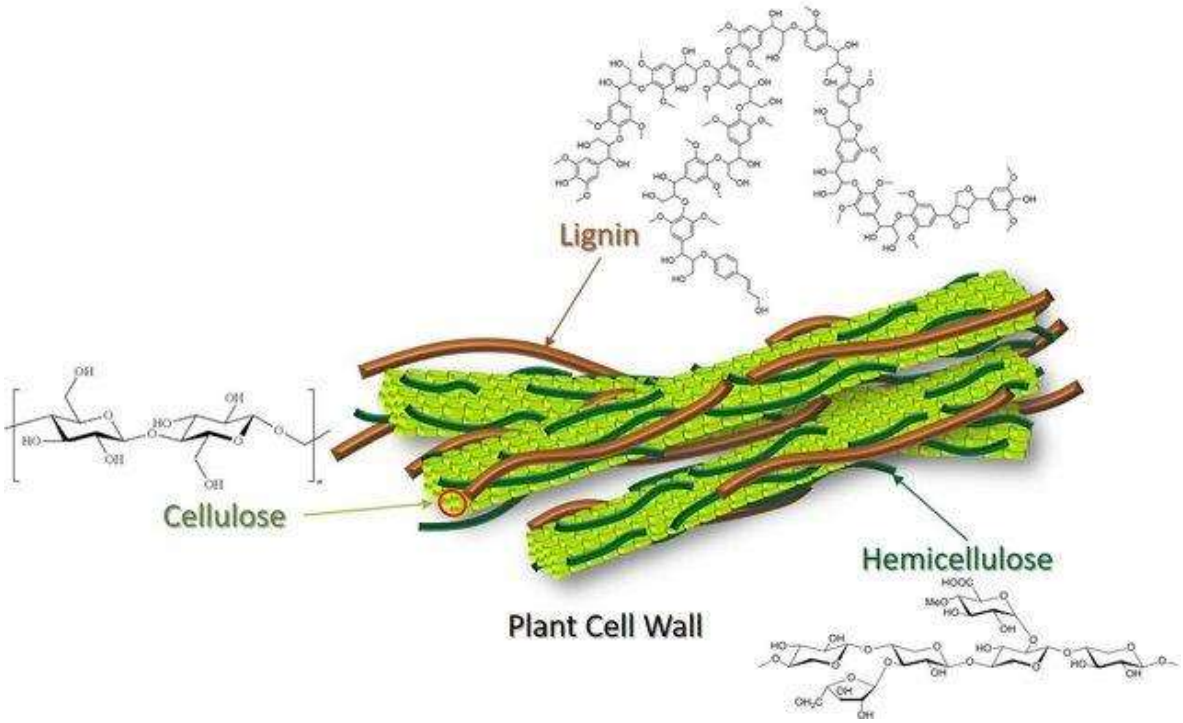

Figure 1. Structure of lignocellulosic biomass [1].

Lignin is a heterogenic phenylpropanoid biopolymer found in most terrestrial plants, which gives the plant its mechanical strength [4-6]. It is the most abundant natural aromatic polymer, accounting for roughly 30% of organic carbon in the biosphere [3], and acts as a cellular glue material to interconnect cellulose and hemicellulose [7]. The lignin polymer consists of three different cross-linked monolignol units: *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. The *p*-hydroxyphenyl unit (H), guaiacyl unit (G), and syringyl unit (S) are the building blocks based on these monolignols when integrated into the lignin macromolecule (**Figure *2***). There is a difference in the distribution of monolignol units depending on the type of plant biomass. [8] Additionally, lignins contain small amounts of monolignols that are incomplete or modified [9].

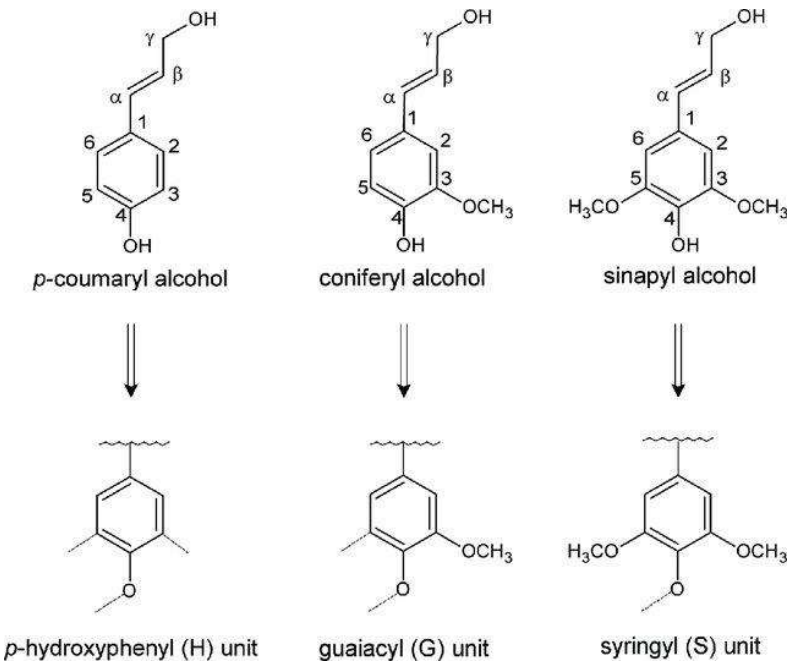

Figure 2. Chemical structure of monolignols and the corresponding building blocks in lignin [8].

Globally increasing efforts have been made to develop a circular economy, which will increasingly require large industries, including the timber industry, to provide appropriate and sustainable use of bio-resources [10]. As the most underrated component of lignocellulose and due to its high calorific value, lignin is usually used either for energy purposes in the paper and pulp industry [7] or disposed off in landfills, endangering the environment in a serious way [11]. Though lignin has a great potential for the production of fuels and aromatic compounds, its valorization is hampered by the complexity of the lignin structure and its strong dependence on the type of plant biomass, and the polymer extraction/isolation method [12].

1.2 Lignin isolation by Organosolv method

There are a variety of methods to separate lignin from other biomass components. Kraft pulping process, the most dominant and long-established method, uses a water solution of sodium hydroxide and sodium sulfide at elevated pressure and temperature to dissolve lignin and separate the fiber components [13, 14]. Another method, called soda pulping, involves the use of an anthraquinone-catalyzed solution of sodium hydroxide in cooking liquor to break up lignin into smaller fragments [15]. The choice of method determines the structure, composition, solubility, and reactivity of lignin [12].

The pretreatment of lignocellulosic biomass with an organic solvent (Organosolv method) has several advantages over other processes. It allows to isolate high-quality lignin with inherent structure, which is crucial for its further valorization. This method can also thrive with low input since the solvent is easily recovered by distillation. [16]

Several organic solvents can be used for Organosolv pretreatment. However, low molecular weight alcohols such as ethanol and methanol are preferred due to their lower boiling point [17], lower cost,

and ease of recovery. To increase the rate of lignin separation and to decrease the temperature of the process, acidic catalysts are used to cleave acid-labile bonds and stabilize lignin fragments. Mineral and organic acids are used. [16]

The Organosolv method takes advantage of the difference in solubility of lignocellulosic biomass components in organic solvents and water. As lignocellulosic biomass is treated with an organic solvent at high temperatures and pressures for a specified time, bonds between the components are cleaved, and the solvent is able to dissolve degraded hemicellulose and lignin, but not the cellulose. Further, cellulose can be easily separated by filtration, while lignin can be precipitated in water. Lignin can be purified from hydrolyzed hemicellulose (sugars) and other chemicals by washing with water. [16] A schematic representation of the Organosolv process is shown in **Figure 3**.

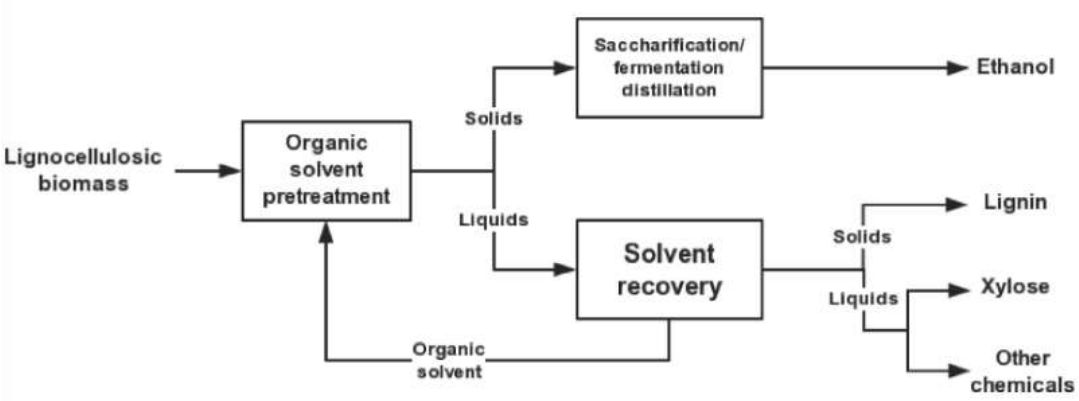

Figure 3. Scheme of Organosolv lignocellulosic biomass pretreatment [16].

1.3 Methods for lignin characterization

To valorize lignin, it is important to know its chemical and physical properties. Several methods are used to characterize lignin structure and its properties, including spectroscopic, chromatographic, gravimetric etc. analysis.

Wet chemistry (Klason method [18], Soxhlet extraction), gas chromatography, liquid chromatography and mass spectrometry can give information about lignin, monolignols and wood extractives content in biomass. The molecular weight distribution of lignin can be determined by size-exclusion chromatography [19]. Since lignin is aromatic in its structure, it can be easily detected with a UV detector that is used in chromatography [20]. Another significant property of lignin is the presence of different functional groups, which can provide information about the proportions of monolignols, and can be determined using infrared spectroscopy [21] and NMR spectroscopy [22]. For sugar content analysis, capillary electrophoresis and chromatography are used [23]. A scanning electron microscope can be used to investigate the ultrastructure of lignin. Mineral content determination is done using atomic absorption spectroscopy or inductively coupled plasma mass spectrometry.

1.3.1 Size-exclusion chromatography

Size-exclusion chromatography (SEC), also known as gel permeation chromatography (GPC), is a chromatographic method in which molecules are separated based on their size (hydrodynamic volume) [24]. The main purpose of the SEC technique is to obtain a molecular weight distribution of a particular polymeric material [20].

With this method, the separation efficiency is determined only by the stationary phase [25]. The SEC column is packed with an inert, porous material, with controlled pore size. Smaller solutes are able to permeate the pores, resulting in longer travelpath through the column, whereas larger solutes remain in the interstitial space, eluting sooner (**Figure 4**). [24]

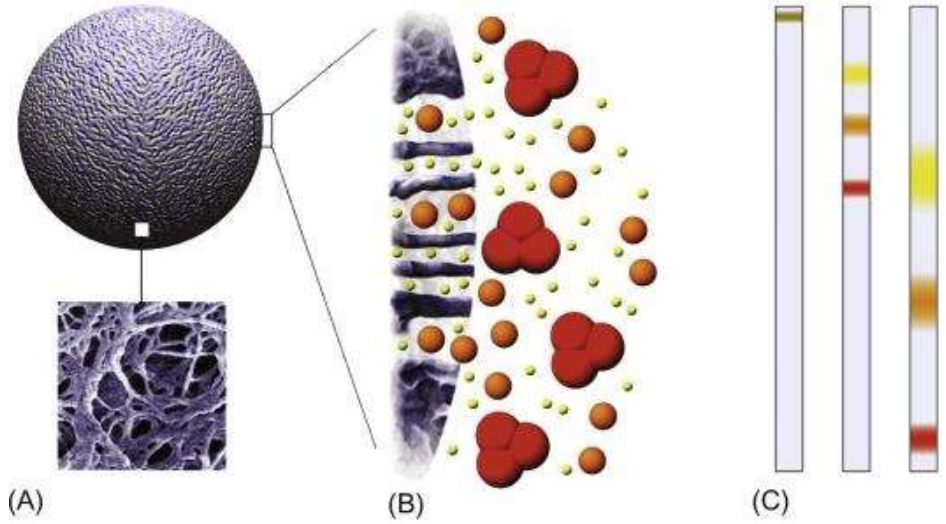

Figure 4. SEC principle, (A) – chromatography resin bead, (B) – sample molecules diffusing into or being excluded from the bead pores, (C) – graphical description of separation inside the column. Adapted from [26].

V_0 is the total interstitial volume in the chromatographic system and point in the chromatogram before which no polymer molecule can elute. V_t is the total permeation volume, which is the sum of interstitial and the total pore volume (V_p). The smallest molecules in the sample mixture would elute at V_t. The separation takes place in the so-called selective permeation range, between V_0 and V_t (**Figure 5**). [20]

The elution time (or retention volume) can be correlated with molecular weight (**Figure 5**) [24]. To determine this correlation, the SEC system must be calibrated for the polymer of interest. The most common method is calibration with linear narrow MWD polymer standards of known molecular weight, such as polystyrene. A calibration curve can be used directly to determine average molecular weights and MWD for unknown samples. [27]

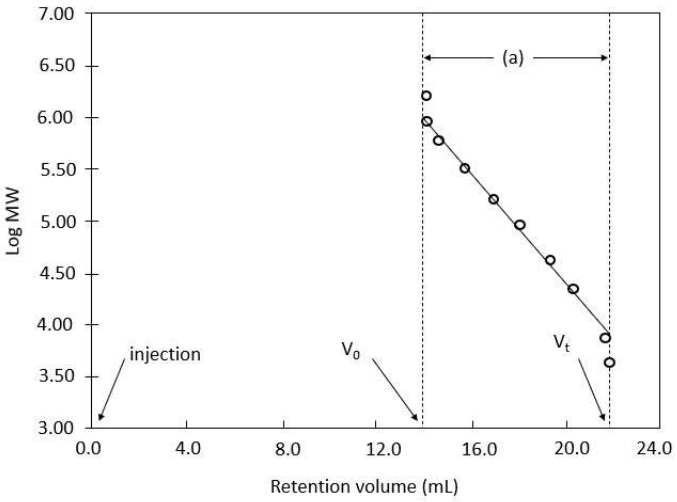

Figure 5. Representation of typical SEC calibration curve, (a) – selective permeation range [20].

Size-exclusion chromatography equipment and experimental conditions

SEC system (**Figure 6**) starts with a reservoir, filled with the mobile phase, which is degassed ultrasonically or with helium to prevent air bubbles from entering the system. Using a high-pressure pump, the mobile phase is forced through a line filter and pulse dampener to reach the sample injector, where a sample of diluted polymer solution is introduced either manually or with an autosampler. The sample is then carried through the column(s) where the separation takes place. The separation typically lasts for 20 minutes to one hour. Columns are located at the thermostat oven. Despite SEC being temperature independent, the temperature has an indirect effect on separation through its influence on the viscosity of the sample. The discriminated sample elutes from the column(s) and passes through a detector, where an electrical signal is collected. The signal is transmitted to a computer for further processing by the corresponding software. [20]

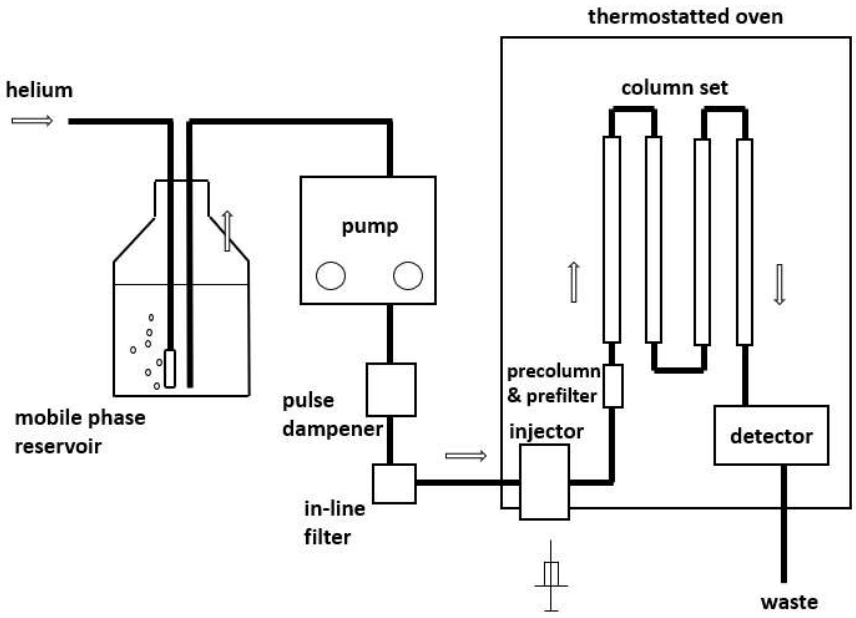

Figure 6. Schematic representation of a common size-exclusion chromatograph [20].

The mobile phase should fully dissolve the polymer sample, have low viscosity, and prevent the sample molecules from interacting energetically with the stationary phase [20]. Mobile phase flow rate must be selected according to the size of the solutes; smaller solutes require higher flow rates, while larger molecules require lower flow rates [26]. In SEC, isocratic flow rate is used [20].

Sample concentration should be within the recommended limits, since high viscosity of concentrated sample can lead to peak distortion, including asymmetric peaks and various tailing and fingerprint phenomena [26]. The recommended SEC sample concentration is 0.1–10 g/L, with an injection volume of 1-100 µL [28]. The higher the sample molecular weight is, the lower concentration should be used [29].

The stationary phase should not interact chemically with the sample, be stable at the required operating temperature, and have sufficient pore volume and size range to effectively separate the solutes. The most common column packing material for organic mobile phases is polystyrene-divinylbenzene (PS-DVB) and trimethylsilane-derivatized silica. Typical pore size for high-performance SEC ranges from 5 to 10 µm. [20]

The most widely used detectors in SEC are differential refractive index (DRI), ultraviolet (UV), and infrared (IR) detectors [20]. DRI response is a function of the difference in refractive indexes of the pure mobile phase and the eluate [30]. DRI is able to respond to all solutes by proper mobile phase choice [27]. However, it is highly temperature sensitive, and its effectiveness breaks down at low molecular weights. UV and IR detectors respond to a variety of chemical groups. UV is commonly used for aromatic rings or regular backbone unsaturation detection, while IR has been used to characterize polyolefins. [20]

Molecular weight averages and polydispersity index

The molecular weight of the polymer is a distribution rather than a specific value since the polymerization takes place in such a way that different chain lengths are formed [31]. Many of the physical properties of polymers are dictated by the molecular weight and molecular weight distribution of the polymer. These include tactility, tensile strength, melt flow, and solution properties. Weight average molecular weight (M_w), number average molecular weight (M_n), and z-average (higher-average) molecular weights (M_z and M_{z+1}) are statistical parameters used to define the molecular weight distribution of polymers. [32] The M_n is defined as the total weight of polymer divided by the total number of molecules. The M_w is defined as the weight fraction of a particular molecular species divided by the total weight of polymer. The higher average molecular weights represents the frequency of the polymer lengths. For a polydisperse polymer, $M_{z+1}>M_z>M_w>M_n$ (**Figure 7**) [24].

The M_n, M_w, M_z, and M_{z+1} are defined by the following formulas:

$$M_n = \frac{\sum N_i M_i}{\sum N_i} \qquad M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} \qquad M_z = \frac{\sum N_i M_i^3}{\sum N_i M_i^2} \qquad M_{z+1} = \frac{\sum N_i M_i^4}{\sum N_i M_i^3}$$

M_i – the molecular weight of species, N_i – the number of molecules of a particular molecular species.

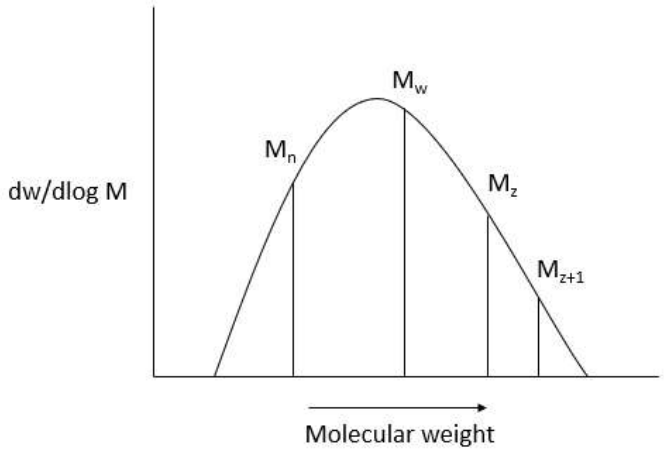

Figure 7. The representative molecular weight distribution of a polymer, M_n and M_w – number- and weight-average molecular weights, M_z and M_{z+1} – higher average molecular weights. Adapted from [32].

M_n influences samples' colligative properties, refractive index, density, and specific heat capacity. It is highly sensitive to the presence of low molecular weight species. M_w affects properties such as melt and solution viscosity. Viscoelastic properties are dependent on M_z and M_{z+1} values. [32]

The width of distribution, defined as a polydispersity index (PI), is the ratio of M_n and M_w [32]:

$$PI = M_w/M_n$$

A monodisperse or single molecular weight polymer gives PI = 1, while a condensation polymer gives PI = 2. PI is close to 1 for polymers made by anionic polymerization, and greater than 2 for polymers made by radical polymerization. [32]

1.3.2 Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) is a widely used technique that uses the beam of infrared radiations to identify the functional groups in the gaseous, liquid, and solid materials [21]. The IR region is at a higher wavelength than the UV-Vis light, and a shorter wavelength than microwave radiations, 0.75 to 1000 µm [33]. It is divided into near-IR (14 000 – 4000 cm^{-1}), mid-IR (4000 – 400 cm^{-1}), and far-IR (400 – 40 cm^{-1}) [21]

To determinate functional groups in a molecule with FTIR, it must be IR active. That means it should have a dipole moment. IR radiations cause molecular motions in the molecule that create a net dipole moment. [21] The number of vibrational modes of a molecule depends on its structure, a molecule with N number of atoms will have 3N degrees of freedom. A non-linear molecule will exhibit 3N-6 vibrational modes, while a linear will have 3N-5 vibrational modes since it is unable to rotate upon its axis. There are two fundamental types of molecular vibration: bond stretching and bending. Asymmetric and symmetric stretching affect the bond length, while bending vibrations consist of bond angle change through twisting, rocking, wagging, and scissoring (**Figure 8**). [34] Stretching vibrations usually occur at higher energy, compared to bending vibrations [21].

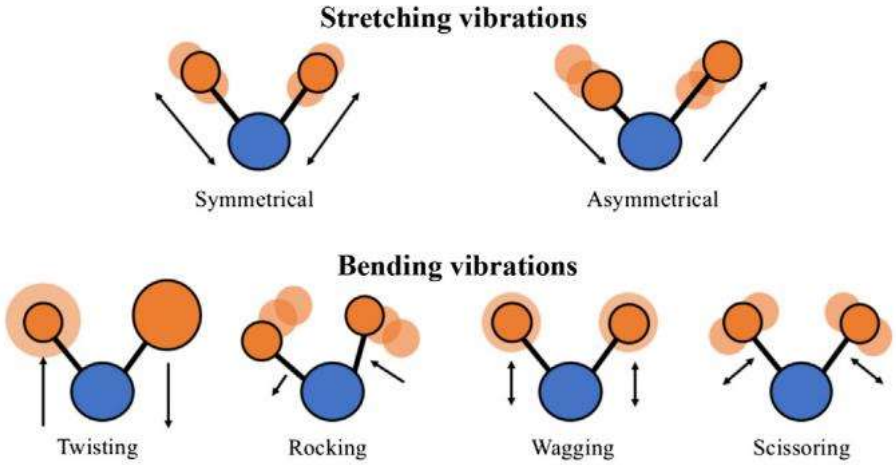

Figure 8. Common vibrational modes of chemical bonds [34].

The infrared spectrum is the plot of measured IR light intensity on the y-axis, and its property, such as energy range on the x-axis. FTIR spectrum can be recorded in absorbance or transmittance mode. [21]

The absorbance measures the amount of light absorbed by the sample and it is calculated based on the following equation [21]:

$$A = \log\left(\frac{I_0}{I}\right)$$

A – absorbance, I_0 – intensity in the background spectrum, I – intensity in the spectrum of sample.

The transmittance measures the percentage of light transmitted by the compound and can be calculated as follows [21]:

$$\%T = 100 \times (I_0/I)$$

$\%T$ – percent transmittance, I_0 – intensity in the background spectrum, I – intensity in the spectrum of sample.

FTIR spectrometers (**Figure 9**) typically contain an IR light source, interferometer, sample compartment, detector, amplifier, and computer. The IR light source generates the radiation, striking the sample and passing through the interferometer, which consists of a beam splitter, a fixed and moveable mirror. The interferometer collects the signal and converts it to an interferogram of a sample. Then, the Fourier Transformation is performed by the computer to display the spectrum. [21]

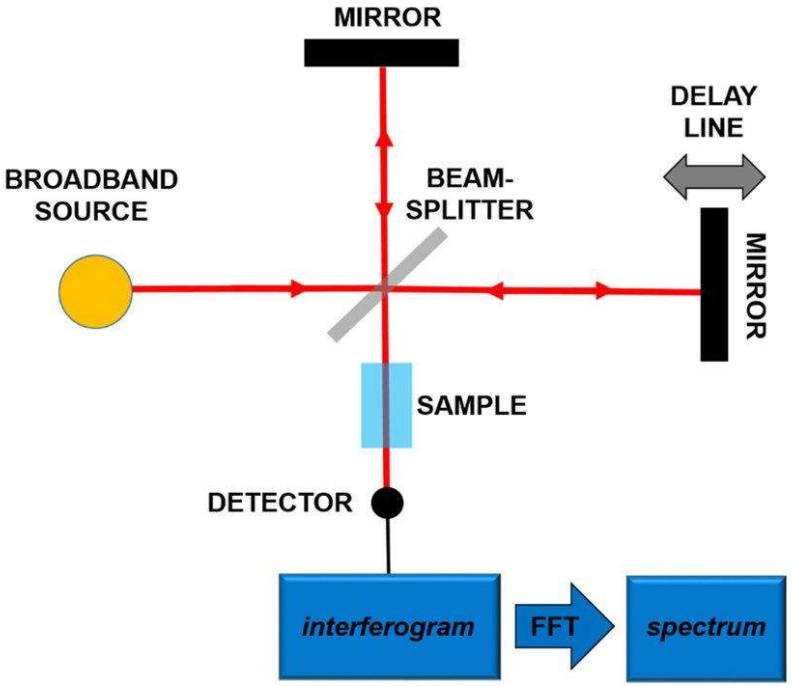

Figure 9. Working principle of FTIR spectrophotometer [35].

As an infrared radiation source in FTIR, different types of lamps are used for different ranges. The silicon carbide heated to 1200 K is used for mid-infrared radiation, tungsten-halogen lamp for the near-infrared, and mercury discharge lamp for far-infrared range. [21]

There are three methods available for solid sample preparation. In the first method, the sample is finely ground with dry KBr salt, at a ratio of about 1% [36], and pressed to form a transparent pellet. In the second method, the sample is ground with mineral oil (so-called Nujol oil) to form a thick suspension which is placed between the salt plates. The third method requires sample dissolution in an organic solvent, usually chloroform. A disadvantage of the latter two methods is the appearance of peaks, characteristic of Nujol oil or the organic solvent used. [21]

2 Aims of the Study

The study is based on the hypothesis that the properties and structure of lignin depend on the biomass source from which it is extracted, as well as the extraction method and solvent used. In order to confirm or reject this hypothesis, the following aims were formulated:

1. To develop a size exclusion chromatography method for the determination of molecular weight distribution of lignins and to provide a statistical quality control of the obtained results;
2. To set up an FTIR method for measurement of infrared spectra of lignins and compare absorbance intensities at specific wavenumbers for semi-quantitative analysis of lignin structure;
3. To carry out Organosolv extraction of lignin from different biomasses (aspen, pine and barley straw) by using two solvents – ethanol and dioxane;
4. To evaluate the structure and properties of the obtained lignins by SEC and FTIR methods.

3 Experimental

Lignin was extracted by an Organosolv method from three different biomass types: aspen wood (hardwood), pine wood (softwood) and barley straw (grass). For all three biomasses, two different solvents were used for extraction, which were ethanol and dioxane. Subsequently, extracted lignins were analyzed and characterized by size-exclusion chromatography and by Fourier-transform infrared spectroscopy methods.

3.1 Lignin extraction

Chemicals and equipment

For lignin extraction from lignocellulosic biomass, the following chemicals were used: 1,4-dioxane (≥99.8%, Fisher Scientific), ethanol (96.6%, Merck), HCl (puriss, Fluka), MilliQ ultrapure water (Millipore, USA).

The equipment used in lignin extraction procedure was the following: 2L round bottom flask, 1L volumetric flask, 2L beaker, 1L plastic tubes, condenser, electrical and analytical balance, electric jacket and electric stirrer, rotary evaporator, magnetic stirrer, centrifuge, and convection oven.

Experiment procedure

For lignin extraction by Organosolv method, ethanol and dioxane were used as a solvent. As an acid catalyst, hydrochloric acid (HCl) was used. Method conditions were developed in earlier studies.

The lignin extraction process was carried out in a 2L one-neck round bottom glass flask connected with a condenser and heated by an electric jacket under atmospheric pressure. Fifty grams of biomass were packed into the flask followed by the addition of 1L EtOH/1,4-dioxane 0.28 M HCl. The system was heated up to the solvents boiling point and stirred with an electrical stirrer for 6 hours. After delignification, the mixture was filtered under pressure, the filtrate was collected, and the solvent was evaporated with a rotary evaporator. The concentrate was washed with 50 mL of acetone and precipitated with 2L of ultrapure water, being stirred with a magnetic stirrer for 60 minutes. The precipitated lignin was centrifuged in 1L tubes at 4200 rpm for 10 minutes. The liquid was then poured off the precipitate and washed three times with ultrapure water, repeating the centrifuging each time. The washed lignin was dried in a convection oven at 30°C for 24 hours. The extraction efficiency was determined gravimetrically, and the results are represented in

Table 1. The yield of lignin extraction was calculated as follows:

$$\%Y = \frac{L}{B - MC} \times 100\%$$

$\%Y$ – the yield of lignin extraction [%], L – the mass of extracted lignin [g], B – the mass of biomass used for extraction [g], MC – the moisture content of biomass.

3.2 Lignin molecular weight distribution determination by size-exclusion chromatography

Chemicals and equipment

Chemicals used for SEC analysis were the following: tetrahydrofuran (≥99.9%, Honeywell), butylated hydroxytoluene (>99%, Fluka Chemika), EasiVial GPC/SEC Polystyrene Calibration Standards 2mL (Agilent Technologies, USA).

The SEC experiment was implemented by a Shimadzu Prominence LC-20A Modular HPLC system (Japan) with diode-array detector. Agilent Technologies (USA) GPC/SEC MesoPore PLgel columns were used (guard column and two main columns). The parameters of columns are the following: particle size 3 µm, length 300 mm, inner diameter 7.5 mm.

To prepare lignin sample solution, an analytical balance, 1.5 mL plastic tubes, 1000 µL and 200 µL calibrated automatic pipettes were used.

Experiment procedure

For lignin polymer sample composition separation, a guard column and two main columns were used. The column packing material (stationary phase) was polystyrene, cross-linked with divinylbenzene (PS-DVB).

As mobile phase, tetrahydrofuran (THF) was used. THF was stabilized with 250 ppm of butylated hydroxytoluene (BHT) to suppress the organic peroxides formation process from THF. The chemical structures of THF and BHT are represented in **Figure 10**.

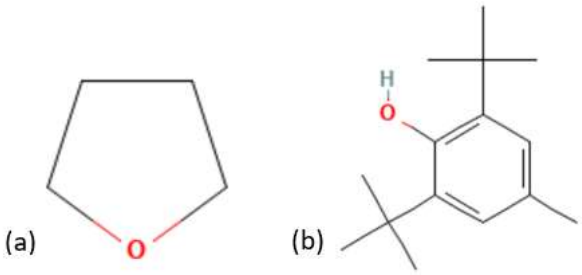

Figure 10. Chemical structure of SEC experiment mobile phase components, (a) – tetrahydrofuran, (b) – butylated hydroxytoluene [37, 38].

Lignin was dissolved in the mobile phase at a concentration of 1 mg/mL to prepare a sample for SEC analysis. 20 µL of the sample were injected manually into the system. The separation process lasted for 24 minutes and took place under 40°C and 80 bar, with a 1 mL/min flow rate. The signal was detected in the UV range at 254 nm. The received data was processed using LabSolutions software (Shimadzu).

The system was calibrated with polystyrene standards with molecular weight range of 162 – 46 380 g/mol. Three experiment parallels were conducted for each standard, and polynomial 3rd order calibration curve was constructed using LabSolutions software (Shimadzu) (**Extra 1**).

3.3 Lignin FTIR spectra measurement

Chemicals and equipment

Chemicals used for FTIR analysis: KBr (≥99%, Sigma-Aldrich).

The lignin IR spectrum was taken with Shimadzu (Japan) IRTracer-100 Fourier transform infrared spectrophotometer with high-energy ceramic as a light source, DLATGS detector, and germanium-coated KBr beam splitter. For sample preparation, an analytical balance, a mortar, a pestle, a special pellet forming kit, and a hydraulic press were used.

Experiment procedure

To prepare a sample for FTIR analysis, lignin and KBr were mixed and grinded with mortar and pestle in a ratio of 1:100 (1 mg of lignin and 100 mg of KBr). Further, the mixture was transferred into special pellet forming kit and pressed into a transparent pellet using hydraulic press and pressure around 9 tons.

Spectrum was taken in absorbance mode in the range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}. Blank KBr pellet was used for a background scanning. The averaged spectra of 32 scans per sample was analyzed using the LabSolutions software (Shimadzu).

4 Results

4.1 Lignin extraction

The usage of different biomasses and extraction solvents resulted in the variation in obtained lignin yields (

Table 1). Lignin extraction by an Organosolv method, using dioxane as the solvent, resulted in 2 to 3 times higher lignin yield, compared to ethanol. The average extracted lignin yield was 6% and 15% for EO and DO, respectively. The highest lignin yield was achieved by dioxane extraction from pine wood (20.4%). The lowest result was obtained with EO from aspen wood (4.6%).

Table 1. Lignin extraction efficiency, E – ethanol, D – 1,4-dioxane.

Source material	Extraction solvent	Lignin yield [%]
Aspen wood	E	4.6
	D	13.6
Pine wood	E	7.0
	D	20.4
Barley straw	E	5.9
	D	11.7

Additionally, the extraction with different solvents resulted in the variation in the color of lignin (**Figure 11**). DOL had dark brown color for all three biomasses. However, the EOLs were lighter in color. In contrast to the aspen and barley EOLs, pine EOL exhibited a pinkish hue.

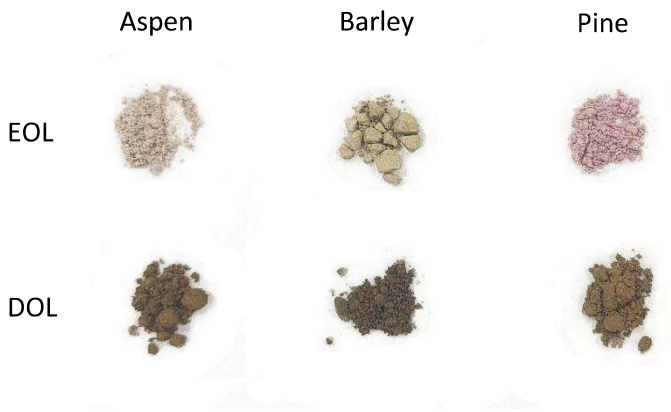

Figure 11. Lignin extracted using different biomasses and solvents.

4.2 Size-exclusion chromatography analysis

Compared with DOL, EOL showed about 20% higher MW for M_n (number average molecular weight) and about 25% for M_w (weight average molecular weight). Pine DOL, exceptionally, produced a higher value for M_w. The highest and lowest values belong to aspen EOL and barley DOL, respectively. The PI value ranged from 1.37 to 1.76, no correlation was found between PI and biomass or solvent used.

Table 2. Lignin molecular weight distribution results, E–ethanol, D–1,4-dioxane.

Source biomass	Extraction solvent	M_n [g/mol] ± sd (n=3)	M_w [g/mol] ± sd (n=3)	PI
Aspen wood	E	2601±27	3743±43	1.44
	D	1925±6	2670±32	1.38
Pine wood	E	2343±27	3391±28	1.45
	D	1959±24	3450±5	1.76
Barley straw	E	2187±15	3211±29	1.47
	D	1809±16	2469±23	1.37

A similar pattern appeared in the lignin SEC chromatograms (**Figure 12**). All samples resulted a wide main lignin fraction peak, followed by depolymerization products and monomers peaks. Two systematic peaks were also observed at the end of the chromatogram. Since these peaks are out of the calibration ranges, their MW could not be determined. The first, appearing around 1150 s, has a correlative intensity with the corresponsive main lignin peak. A systematic peak around 1300 s has the same intensity on each chromatogram. Conspicuously, the pine EOL chromatogram has a comparatively low intensity lignin peak, followed by a high-intensity peak.

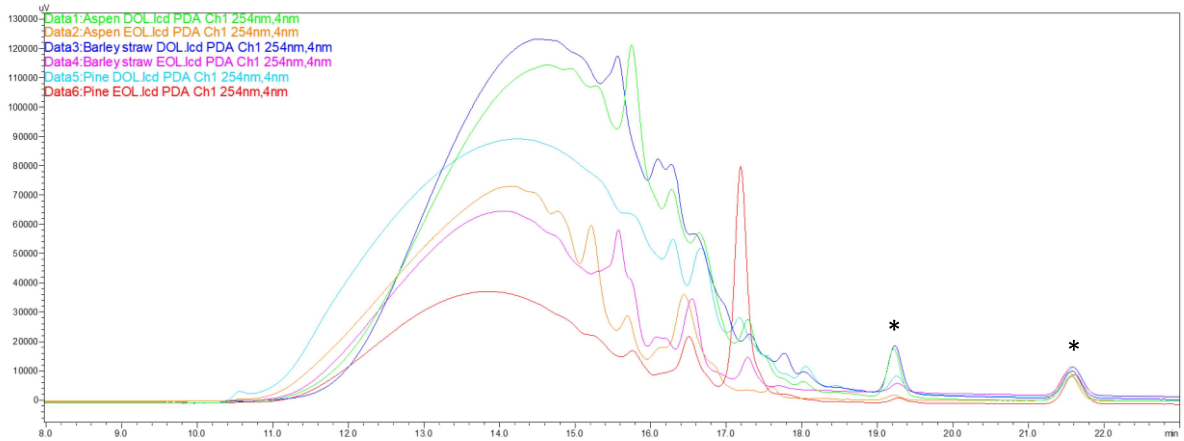

Figure 12. Investigated lignins SEC chromatograms overlay, * – systematic peaks.

4.2.1 Quality control of SEC experiments

Several quality control tests were conducted to ensure the accuracy of the SEC analysis results. For repeatability[i] tests, ten experiments with each standard were performed in one day. The relative standard deviation (%RSD) was calculated to represent the results. Polystyrene standards have shown the repeatability <1.10% for M_n and <1.22% for M_w. Three parallel measurements were conducted for each lignin, and the %RSD results can be found in

Table 2. For M_n and M_w, experiments showed repeatability <1.14% and <1.19%, respectively.

The within-laboratory reproducibility[ii] of the experiment was checked with an X-chart before each test session. The standards' molecular weight of (1920 g/mol) was chosen since it is the closest to the molecular weight of the lignin analyzed. Based on the first ten values, the warning and action limits (±2sd and ±3sd) were fixed [39]. If the subsequent test results were out of stability area, the system check was performed, and the cause of the

inaccuracy was eliminated. **Figure 13** demonstrates the importance of using quality control tools. The clogging of the column (a) caused high pressure in the column system, where as a result, the value of the standard was outside of the stability range and further tests would have shown incorrect results for lignin samples. After cleaning column (b), the pressure dropped considerably, and the test results could not still be reliable. Thus, the instrument was recalibrated with a new pressure rate, restoring the stability of the standard value (c).

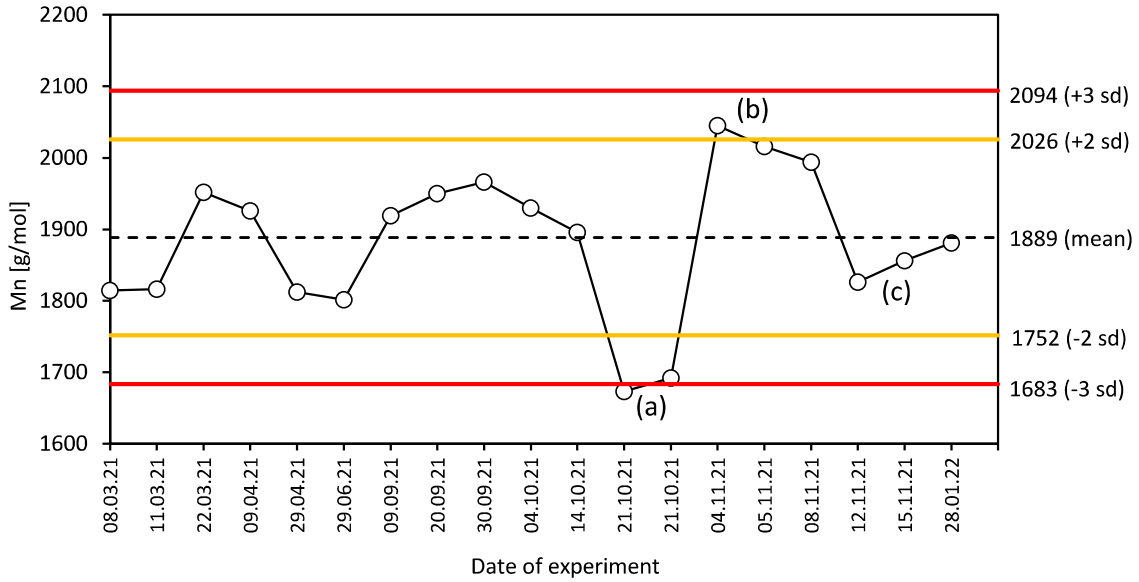

Figure 13. X-control chart of the yellow standard, (a) – the column cloggage, (b) – after removing the column cloggage, (c) – new calibration.

[i] Repeatability – represents variety of sample values analyzed several times in short time, by one person in one laboratory, and with the same instrument [39].

[ii] Within-laboratory reproducibility - represents variety of sample values analyzed several times at different times in one laboratory using the same analytical procedure [39].

4.3 Fourier-transform infrared spectroscopy analysis

The absorption bands corresponding to bond vibrations in FTIR spectra were assigned based on previously reported data [40, 41], and the analyzed lignin spectra are shown overlayed on **Figure 14**. The spectral profiles and the relative intensities of the absorption bands of vibration are similar in all lignins, which indicates that the core of the structure of lignin did not change significantly by changing the organic solvent. The signal at 3429 cm^{-1} corresponds to phenolic and aliphatic (O–H) group stretching in lignin. Two bands in 3000–2850 cm^{-1} region are assigned to C-H stretching vibrations in the methyl and methylene groups. These bands, mainly attributed to methoxy groups, are substantially higher for ethanol lignins when compared to dioxane lignin. Signal at 1711 cm^{-1} indicates the presence of unconjugated carbonyl groups. The bands occurring at 1595 cm^{-1}, 1516 cm^{-1}, 1424 cm^{-1} and 1369 cm^{-1} are assigned to aromatic skeletal vibrations. An absorption at 1464 cm^{-1} is related to C–H deformations in $–CH_2–$ and $–CH_3$. The spectral region below 1400 cm^{-1} contains vibrations, that are specific to different lignin structural units, and allows for the determination of monolignols presence in the sample. The bands referring to the presence of S-unit are found at 1331 cm^{-1} and 1123 cm^{-1}. Signals, characteristic to G-unit appeared at 1271 cm^{-1}, 1219 cm^{-1}, and 1031 cm^{-1}. A weak intensity peaks at around 830 cm^{-1} refer to aromatic C–H out-of-plane deformation in G, S, and H units.

According to the FTIR spectra, aspen wood EOL lignins have high amounts of S units, and half the abundance of G units when compared to the S units. Aspen DOL lignin has less intensive S unit bands. Pine lignin has almost equal S and G units intensity, both for EOL and DOL. In barley straw lignin spectra, the S-unit band at 1123 cm^{-1} is negligible, and G units intensity is lower, compared to other biomasses. For all ethanol extracted lignins, the more intensive methyl and methylene groups bands were observed, compared to dioxane extracted lignins.

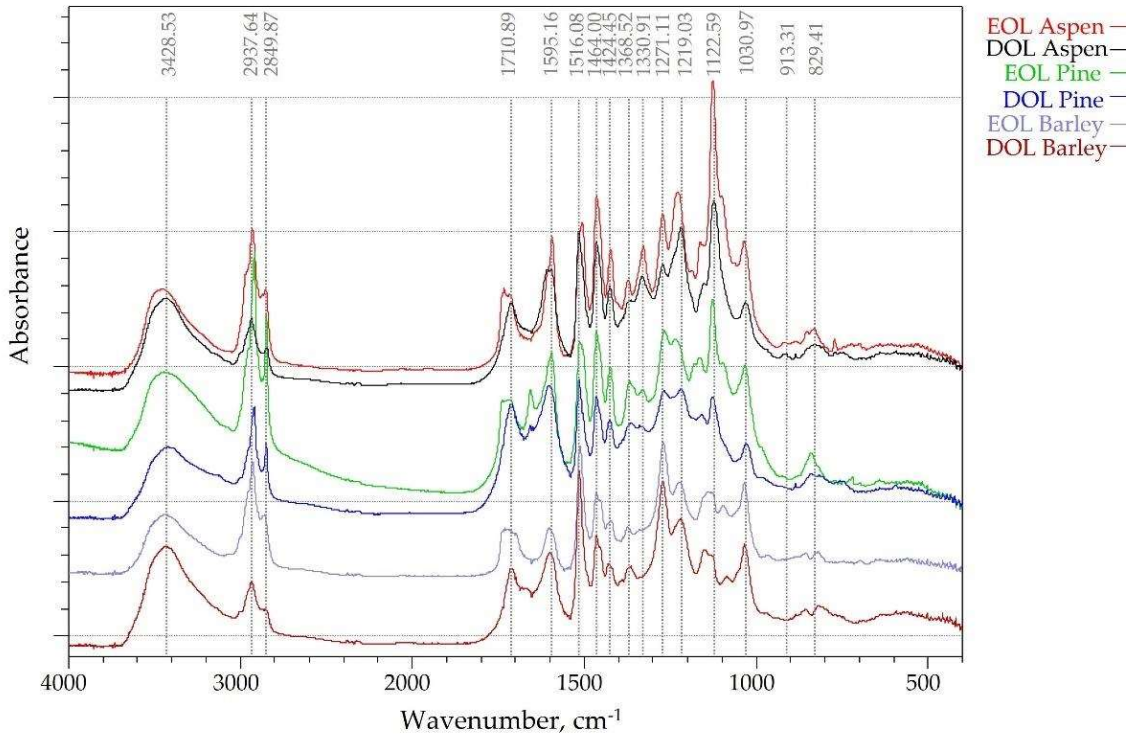

Figure 14. Analyzed lignin spectra overlay (compiled by P. Jõul).

5 Discussion

The variation in lignin color could be caused by the appearance of different chromophores during the extraction process. Quinoids, catechols, aromatic ketones, stilbenes, conjugated carbonyls with phenolics and metal complexes contained in lignin are able to alter its coloration. [42]

In a previous study carried out in our group, the contents of lignin in aspen, pine, and barley straw biomasses were estimated to be 27%, 33%, and 21% , respectively [43]. The obtained lignin yield made up ca 20% of aforementioned lignin content for ethanol, and ca 55% for dioxane extraction. Lower ethanol extractions yields could be caused by the formation of colloidal suspensions of lignin in the water washes, as well as the lower extraction temperature (~80-85°C for ethanol and ~95-100°C for dioxane extraction).

As described in the results section, the ethanol extraction lignins showed slightly higher M_n and M_w, compared to the lignin isolated using dioxane. However, the polydispersity index of EOL and DOL were similarly narrow (1.37–1.76), indicating homogeneity of the polymeric material. No correlation between the PI and used biomass/solvent was observed. The high-intensity peak appeared around 1030 s on pine EOL chromatogram, with molecular weight around 200 g/mol, could be a result of ethyl-esterified compounds formation, caused by a high ethanol concentration [44]. The earlier appearing systematic peak is presumably a part of the lignin samples, since it did not appear on polystyrene standards chromatograms (**Extra 2**). The second systematic peak is the BHT, used to stabilize the THF.

FTIR results showed that aspen wood (hardwood) contains mostly S and G units, in an estimated ratio of around 2:1 for EOL and around 1:1 for DOL. Pine wood (softwood) showed S and G ratios of around 1:1 for EOL and DOL. The barley straw (grass) contains S and G units in an estimated ratio of around 1:2, where the result was similar for both, EOL and DOL . The obtained results for EOLs is in good correlation with the previously mentioned study [45].

6 Conclusions

In present study the properties and structure of Organosolv lignins extracted from different biomass sources were evaluated by SEC and FTIR methods and the following conclusions were made:

1. Lignin properties are strongly dependent on the feedstock biomass, extraction method, and extraction solvent;
2. The highest lignin extraction yield was achieved when using dioxane as a solvent in Organosolv method;
3. The appearance of different chromophores during the extraction process has an effect on the lignin color;
4. Organosolv extraction with ethanol as a solvent causes ethyl-esterified compounds formation and thus decreases the main lignin fraction yield;
5. The biomass source and extraction solvent influences lignin molecular weight distribution: EOLs have slightly higher number (M_n) and weight (M_w) average molecular weights, comparing to DOLs.
6. The polydispersity index is similar for all obtained lignins;
7. FTIR experiments showed that investigated lignins have minor structural differences, caused by the biomass source and solvent used for extraction, though the spectrum profile is similar in all samples.
8. S and G units dominate in ethanol Organosolv hardwood lignin, where the ratio is about 2:1, respectively. However, in the case of hardwood DOL, the S unit quantity decreases.
9. Softwood lignins show slight difference in S/G ratio, S is prevailing, both for EOL and DOL.
10. Grass lignin has the lowest presence of S unit. S unit is outnumbered by a G unit in a half.

Abstract

The development of new strategies for lignocellulose valorization goes in line with the demand of implementing circular economy principles by large industries, such as the timber industry. Lignin, which for a long time has been considered as a is a waste residue of pulp and paper industry, is generally burned or landfilled in substantial quantities. While contributing to environmental pollution, such handling of lignin is also an ineffective usage of a potentially high-valued polymer.

One of the main barriers for lignin valorization stems from its complicated and heterogenic structure. A number of methods for lignin extraction and characterization have been developed over the course of decades. Using the Organosolv method, it is possible to preserve lignin's structure close to its native form as it exists in the plant cell wall through the extraction with organic solvents. Size-exclusion chromatography (SEC) is presently the most effective and optimal method for lignins molecular weight distribution determination. Fourier transform infrared spectroscopy (FTIR) is a technique that is useful for the determination of functional groups and structural units (monolignols) present in lignin.

The purpose of this study was to optimize and implement analytical procedures for lignin analysis by size-exclusion chromatography and Fourier transform infrared spectroscopy, as well as investigate the effect of the different solvent usage in Organosolv extraction method on lignin properties and structure. A study was conducted on lignin extracted from three different biomass types: hardwood (aspen wood), softwood (pine wood), and grassy biomas (barley straw). From each biomass, the lignin was extracted with usage of ethanol and 1,4-dioxane as the solvent.

The usage of different solvents in the extraction showed variability in the yield and of the color of the lignin. Dioxane extraction resulted in 2-3 times higher yield and darker color of lignin. Lignins extracted with ethanol were of lighter color, where pine wood lignin had a pinkish hue. In the case of pine wood, an formation of ethoxylated compounds was observed during extraction with ethanol.

The results from size-exclusion chromatography showed, that lignin extracted with ethanol has slightly higher molecular weight (avg. M_n≈2300 g/mol, M_w≈3400 g/mol), compared to lignin extracted from the same biomass using dioxane as the extraction solvent (avg. M_n≈1900 g/mol, M_w≈2800 g/mol). The highest and lowest molecular weights belonged to the aspen ethanol lignin and barley straw dioxane lignin, respectively. The polydispersity indices ranged from 1.37 to 1.76. No correlation was observed between the polydispersity index and the biomass/extraction solvent used. The method showed high repeatability results (<1.2%).

The FTIR results showed that aspen wood lignin is rich in S and G units (∼2:1 with ethanol and ∼1:1 with dioxane extraction). The presence of S and G units in pine wood did not show significant difference for ethanol and dioxane extracted lignin, the ratio was ∼1:2. The barley straw lignins monolignol ratio was not affected by extraction solvent, where the S and G unit ratio was ∼1:2.

Based on current work, it can be concluded that the choice of the biomass feedstock and extraction solvent influence molecular weight distribution of lignins, as well as on the content of monolignols. All raised objectives were achieved, and the initial hypothesis of this study was confirmed.

Kokkuvõte

Uute tehnoloogiate arendus lignotselluloosse biomassi väärindamiseks on oluline seoses ringmajanduse põhimõtete rakendamise nõudlusega suuremates tööstusharudes, nagu näiteks puidutööstuses. Tselluloosi- ja paberitehastes suurtes kogustes tööstusjäägina tekkivat ligniini on tavaliselt põletatud või ladestatud prügimägedele, põhjustades keskkonnareostust ning potentsiaalselt kõrge väärtusega polümeeri ebaefektiivset kasutamist.

Ligniini väärindamise takistuseks on tihti selle keerukas ja heterogeenne struktuur. Aastakümnete jooksul on välja töötatud mitmeid meetodeid ligniini isoleerimiseks ja iseloomustamiseks. Organosolv meetodit kasutades on orgaaniliste solventide abil ekstraheerides võimalik säilitada ligniini struktuur, mis on ligilähedane ligniini algupärasele struktuurile taimeraku seinas. Eksklusioonkromatograafia (*SEC*) on hetkel efektiivseim ja optimaalseim meetod ligniini molekulmassijaotuse määramiseks. Fourier teisendusega infrapunaspektroskoopia (*FTIR*) on meetod, mis võimaldab määrata funktsionaalrühmade ja struktuuriühikute (monolignoolide) esinemist ligniini polümeeris.

Töö eesmärgiks oli optimeerida ja rakendada analüütilisi meetodeid ligniini analüüsiks eksklusioonkromatograafiaga ja Fourier teisendusega infrapunaspektroskoopiaga ning samuti uurida erinevate Organosolv ekstraktsioonil kasutatavate lahustite mõju ligniini omadustele ja struktuurile. Uuring viidi läbi ligniinidega, mis olid ekstraheeritud kolmest erinevast biomassitüübist: lehtpuu (haavapuidust), okaspuu (männipuidust) ja kõrreline (odrapõhust). Igast biomassist ekstraheeriti ligniini, kasutades lahustina etanooli ja 1,4-dioksaani.

Erinevate lahustite kasutamise tulemusena varieerus nii ligniini saagis kui ka värvus. Dioksaaniga ekstraheerimisel saadi 2-3 korda kõrgem saagis ja tumedamat värvi ligniin. Etanooliga ektraheeritud ligniinid olid heledama värvusega, sh männi ligniin, mis oli roosakat tooni. Samuti täheldati etanooliga ekstraheerimisel männipuidu puhul etoksüleeritud ühendite moodustumist.

Eksklusioonkromatograafia tulemustest ilmneb, et etanooliga ekstraheeritud ligniinide molekulmassid on veidi kõrgemad (kesk. M_n≈2300 g/mol, M_w≈3400 g/mol) võrreldes samast biomassist dioksaaniga ekstraheeritud ligniiniga (kesk. M_n≈1900 g/mol, M_w≈2800 g/mol). Kõrgeim ja madalaim molekulmass kuulusid vastavalt haavapuieu etanooli- ja odrapõhu dioksaani ligniinile. Polüdisperssuse indeks jäi vahemikku 1,37–1,76. Polüdisperssuse indeksi ja kasutatud biomassi/ekstraktsioonilahusti vahel korrelatsiooni ei ole täheldatud. Meetod näitas kõrget korratavuse tulemust (<1.2%).

FTIR tulemused näitasid, et haavapuidu ligniin on rikas S- ja G-ühikute poolest (∼2:1 etanooliga ning ∼1:1 dioksaaniga ekstraheerimisel). S- ja G-ühikute esinemine männi ligniinis ei näidanud olulist erinevust eri solventide vahel, suhe oli vastavalt ∼1:2. Ekstraheerimislahusti ei mõjutanud ka põhuligniinide monolignoolide esinemist, S- ja G-ühikute esinemise suhe oli ∼1:2.

Antud töö tulemuste põhjal võib järeldada, et lähtebiomassi ja ekstraheerimislahusti valik mõjutab ligniini molekulmassijaotust ning ka monolignoolide jaotust . Kõik töö alguses seatud eesmärgid olid täidetud, ja esialgne püstitatud hüpotees leidis kinnitust.

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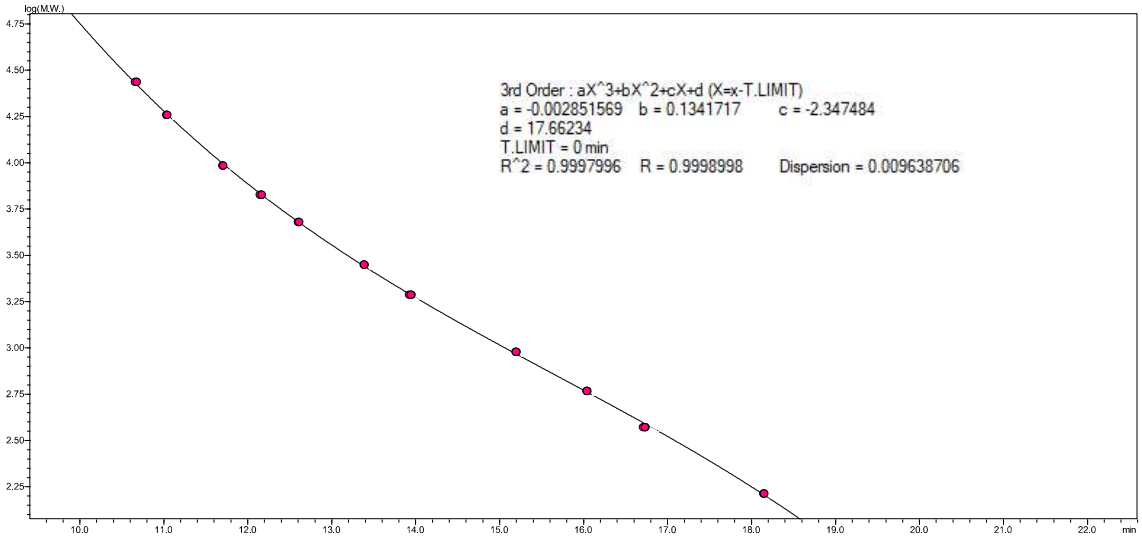
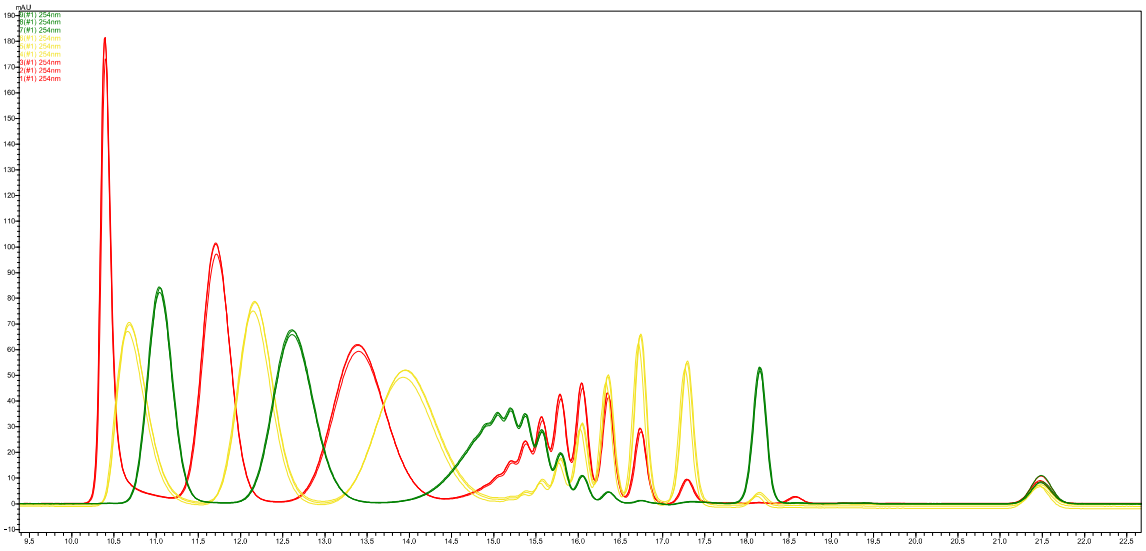
Extras

Extra 1 SEC calibration curve



Extra 2 Chromatogram overlay of polystyrene calibration standards



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