

THESIS FOR THE DEGREE OF LICENTIATE OF PHILOSOPHY

Viscoelastic properties and plasticization potential
of native, residual, and technical lignin

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Abstract

Lignin in biomass, pulp, and isolated during pulping all have great potential as renewable and inexpensive thermoplastic components and materials, but widespread utilization is hindered by high glass transition temperatures (T_g), brittleness, and poor flow properties. The aim of this thesis is to elucidate what molecular structures govern lignin thermoplasticity and how these can be efficiently manipulated. To accomplish this, we first isolated lignins of high yield and purity from wheat straw, Norway spruce, and softwood kraft pulp, and fractionated softwood kraft lignin. The T_g of these lignins, as well as their relative dynamic moduli, were determined using a novel dynamic mechanical analysis (DMA) approach for non-self-supporting materials. Application of the Flory-Fox equation for modelling the T_g revealed that residual lignin in pulp had an apparent free volume more similar to that of kraft lignin than isolated native lignin. However, the chain length had a larger influence on the flow properties than degree of condensation. To elucidate the role of lignin structure in plasticization, we blended four different lignins with three distinct plasticizers (protic, aprotic, and aromatic) selected based on the hypothesis that various lignin structures would be susceptible to different types of plasticizing mechanisms. The viscoelastic properties of the resulting blends were studied using the DMA setup. Contrary to the hypothesis, the plasticizers exhibited a remarkably consistent T_g -depression and mechanical damping profiles across all four lignins. This would imply that the similarities in lignin structure (aromatic backbone, OH groups) were more important than the differences (alcohol/phenol content, lignol unit and linkages between lignols) for achieving efficient plasticization. However, one notable difference was observed upon plasticization of the diverse lignins: the condensed lignins exhibited a more pronounced reduction in T_g compared to the native lignins. This phenomenon could be attributed to the greater benefit of increasing the intermolecular distances within the rigid, condensed structures present in these lignins. Additionally, the impact of external plasticization was not limited to reducing T_g ; it also led to a narrower transition, suggesting a more homogeneous ordering of the material.

“The more I paint, the more I like everything”

John Ruskin

List of Publications and Presentations

This thesis is based on the following appended manuscripts:

I. Viscoelastic properties of lignin isolated with high yield from softwood kraft pulp, Norway spruce and wheat straw

Åke Henrik-Klemens, Fabio Caputo, Roujin Ghaffari, Gunnar Westman, Ulrica Edlund, Lisbeth Olsson, Anette Larsson

Submitted

II. On the structure dependence of lignin plasticization

Åke Henrik-Klemens, Ulrica Edlund, Anette Larsson

Manuscript

Contribution Report

The author of this thesis has made the following contributions to the publications included:

- I. **First author.** Concept and experimental design were developed in collaboration with all co-authors. Performed most experimental work, except enzymatic hydrolysis performed by FC and fractionation of kraft lignin performed by RG. Analyzed and interpreted results in collaboration with all co-authors. Wrote the first draft of the paper. Revision was done in collaboration with all co-authors.

- II. **First author.** Concept and experimental design were developed in collaboration with all co-authors. Performed all experimental work. Wrote the first draft of the paper. Revision was done in collaboration with co-authors.

Abbreviations and Symbols

ATR-FTIR	Attuned total reflectance Fourier transform infrared spectroscopy
DEP	Diethyl phthalate
DMA	Dynamic mechanical analysis
DSC	Differential scanning calorimetry
DMSO	Dimethyl sulfoxide
E'	Storage modulus
E''	Loss modulus
EMAL	Enzymatic mild acidolysis lignin
G	Guaiacyl
GLY	Glycerol
GPC	Gel permeation chromatography
H	4-hydroxyphenyl
LCC	Lignin carbohydrate complexes
M _n	Number average molecular weight
M _w	Weight average molecular weight
MWL	Milled wood lignin
NMR	Nuclear magnetic resonance
p-CA	p-coumaric acid
PMMA	Poly(methyl methacrylate)
S	Syringyl
TA	Triacetin
T _g	Glass transition temperature

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1. Introduction

A significant challenge in our global efforts to reduce greenhouse gas emissions is transitioning from using fossil-based materials to renewable alternatives (Ragauskas et al., 2006). A biopolymer with the potential to replace many fossil-based sources and materials is lignin; however, lignin is a double-sided macromolecule in that it also has many inherent obstacles to utilization.

Lignin is a high-glass transition temperature (T_g) polymer (>200 °C at high molecular weights), primarily due to its aromatic structure and strong secondary interactions (Mishra and Ekielski, 2019; Back and Salmén, 1982). The high T_g is a challenge in lignin and lignocellulose processing since both lignin and carbohydrates tend to degrade at high temperatures (Back and Salmén, 1982). From an industrial perspective, there are three kinds of lignin: native lignin in biomass, residual lignin in pulped or processed biomass and technical lignin that has been isolated during the processing of biomass. As the pulping process depolymerizes and solvates lignin, the molecular structure and size between these three types of lignin are different. Consequently, the thermal and viscoelastic properties of these materials must also change.

The viscoelastic properties of lignin are important in most of its processing and application, from pulping and fibre molding, to developing plastics and coatings from technical lignin. To increase the usage of lignin, both in the more environmentally friendly high-yield pulp and in lignin recovered from pulping, a better understanding of how its viscoelastic properties relate to structure is needed. It is also important to progress the understanding of how these properties can be efficiently modified for the development of new and improved lignin-containing materials.

There are three major obstacles to studying the viscoelastic properties of lignin. 1) lignin in wood and paper have transitions in the same temperature region as hemicellulose and unordered cellulose, which hinders *in situ* measurements (Ashaduzzaman et al., 2020). 2) Isolation of lignin often only isolates part of the lignin or modifies it so that it is no longer representative. 3) Most isolated lignins cannot be cast or molded into continuous shapes, and therefore not subject to the common methods of viscoelastic characterization of solid materials.

In this thesis, isolated lignins are studied to circumvent obstacle 1 and allow for analysis of structure and molecular weight; however, obstacle 2 still remains. The multilayered, multicomponent structure of wood makes it difficult to extract lignin without chemically or physically degrading the wood structure, and in doing so, degrading lignin. Isolating lignin is often a question of balancing extraction yield and lignin degradation. In the last two decades, new analytical isolation procedures that appear to isolate lignin at high yield while retaining much of the native structure have been developed (Giummarella et al., 2019; Wu and Argyropoulos, 2003; Giummarella et al., 2016; Du et al., 2013). But to the best of our knowledge, lignins isolated with these high yields, which would be better representative of the *in situ* material, have never been subjected to viscoelastic analysis.

Obstacle 3, that lignin cannot be cast in to self-standing samples, have in some studies been solved by conducting dynamic mechanical analysis (DMA) in a parallel plate setup, where the sample in form of a powder is pressed between the two plates (Shrestha et al., 2017; Karaaslan et al., 2021; Li and McDonald, 2014; Sun et al., 2016; Sevastyanova et al., 2014). This allows for determination of thermal transitions as well as relative moduli, but large sample sizes are often needed (~500 mg). In the last decade, powder sample holders have been developed for use in DMA (Mahlin et al., 2009; Jones et al., 2012; Ou et al., 2015; de Paula et al., 2019). Their primary use has been to determine the T_g of pharmaceutical materials, that are often not self-supporting, but the technique is ideal for isolated lignins, as it requires small sample sizes (25-100 mg).

In a general sense, the parameters influencing viscoelastic properties of lignin are known: molecular weight, type of monomer, aromatic structure, hydrogen bonding, etc.; however, how these properties influence each other has not been established. Likewise, how different lignin structures respond to different types of modification is another understudied subject. The overarching aim of this thesis is to bridge these gaps in knowledge and seek to increase the utilization potential of lignin and lignin-containing materials.

The thesis has the following objectives with accompanying hypotheses:

- Establish structure-viscoelastic property relationships for lignins of different botanical and industrial origins.
 - Hypothesis: differences in chemical structure of lignins will lead to polymers with different correlations between molecular weight and T_g .
- Elucidate how the origin and molecular structures of lignin influence external and internal plasticization.
 - Hypothesis: different lignin structures will require different modifications to be efficiently plasticized (T_g reduction).

The thesis comprises two appended papers that address the objectives by studying lignin isolated in high yield (50-70 wt%) from Norway spruce, wheat straw, and softwood kraft pulp using the enzymatic mild acidolysis lignin (EMAL) protocol (Wu and Argyropoulos, 2003), and softwood kraft lignin fractionated into groups with different molecular weights. Paper I covers the isolation procedure and characterization of the lignins and the use of a powder sample holder for DMA to study the viscoelastic properties of the isolated lignins. In Paper II, the lignins were subjected to external plasticization with additives with different functionalities (protic, aprotic, aromatic) and internal plasticization via acetylation, and their thermal properties were determined using DMA and DSC.

2. Background

2.1. Native lignin

Lignin is an aromatic macromolecule that imparts cohesiveness, hydrophobicity, and protection against microbiological attack on plant cell walls (Hägglund, 1951; Sjöström, 1981). It makes up a large part of the dry mass of most woods (approximately 25-35 %) and monocot plants (5-25 %) (Iiyama and Wallis, 1990; Rowell, 2013). In plant fibres, lignin is unevenly distributed in the cell wall. The middle lamella, the layer that holds adjoining fibres together, is mainly constituted of lignin, whereas lignin inside the fibre wall fills out the space between microfibrillar aggregates, occupying nanometer-size volumes (Rowell, 2013).

Lignin is an amorphous polymer composed of several different monomeric units of phenyl propane (**figure 1**). Softwood lignin consists of mainly guaiacyl (G) units, whereas hardwood and monocots contain different amounts of G, 4-hydroxyphenyl (H) and syringyl (S) units (Tian et al., 2017; Pereira et al., 2017; Faleva et al., 2021). In native lignin, these monomers are polymerized through enzyme-initiated, but not enzyme-controlled, radical reactions (Ralph et al., 2019). This biosynthesis route creates irregular chains, but with some linking units being more common than others. The radical polymerization also lead to bonds forming between lignin and carbohydrates, in so called lignin carbohydrate complexes (LCC).

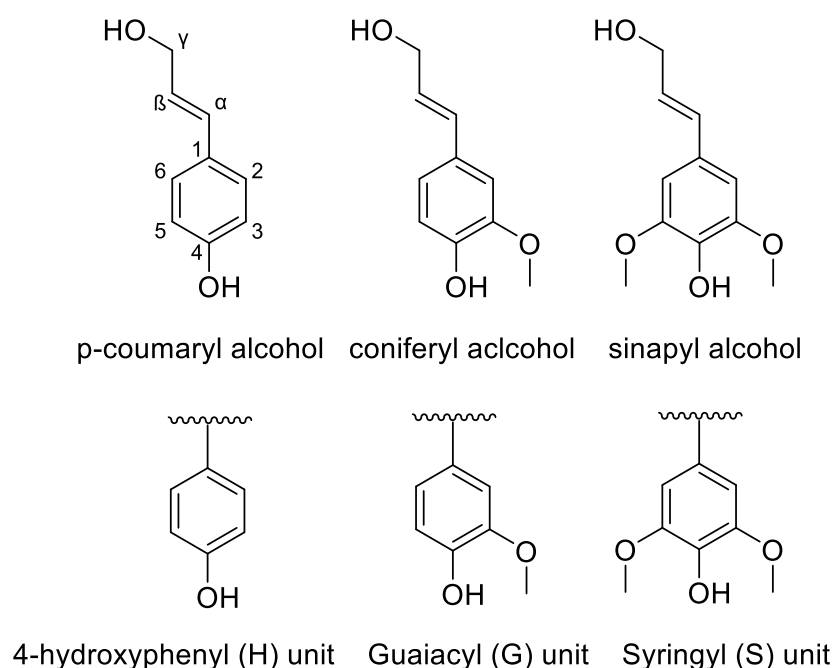


Figure 1. Structure of lignin phenyl propane monomers (top) and respective lignol unit (bottom). p-coumaryl alcohol has been labeled with the conventional numbering and lettering of lignol carbons.

The most common linkage in lignins from all types of plants is β -O-4, found in approximately 80/100 aromatic units in monocot lignin, and 50/100 and 60/100 in hardwood and softwood, respectively (Zhang et al., 2022). β -O-4 and other bonding motifs, such as 4-O-5 and β -5₁, can be seen in the model structure in **figure 2**, as well as the distribution of H, G and S lignols. The models depict monocot and softwood lignin, as they are the main focus of this thesis. The models are not quantitative, as the frequency of the linkages is not entirely representative, but serve the purpose of providing an approximate visualization of native lignin macromolecules.

Monocot lignin is more structurally complex than softwood lignin. Apart from the difference in H, G and S units, monocot lignin typically also contains the flavonoid tricetin and p-coumaric acid (p-CA). The flavonoid is the starting point of the polymerization whereas p-CA is less likely to undergo radical coupling and therefore remains as a side chain (Ralph et al., 2019).

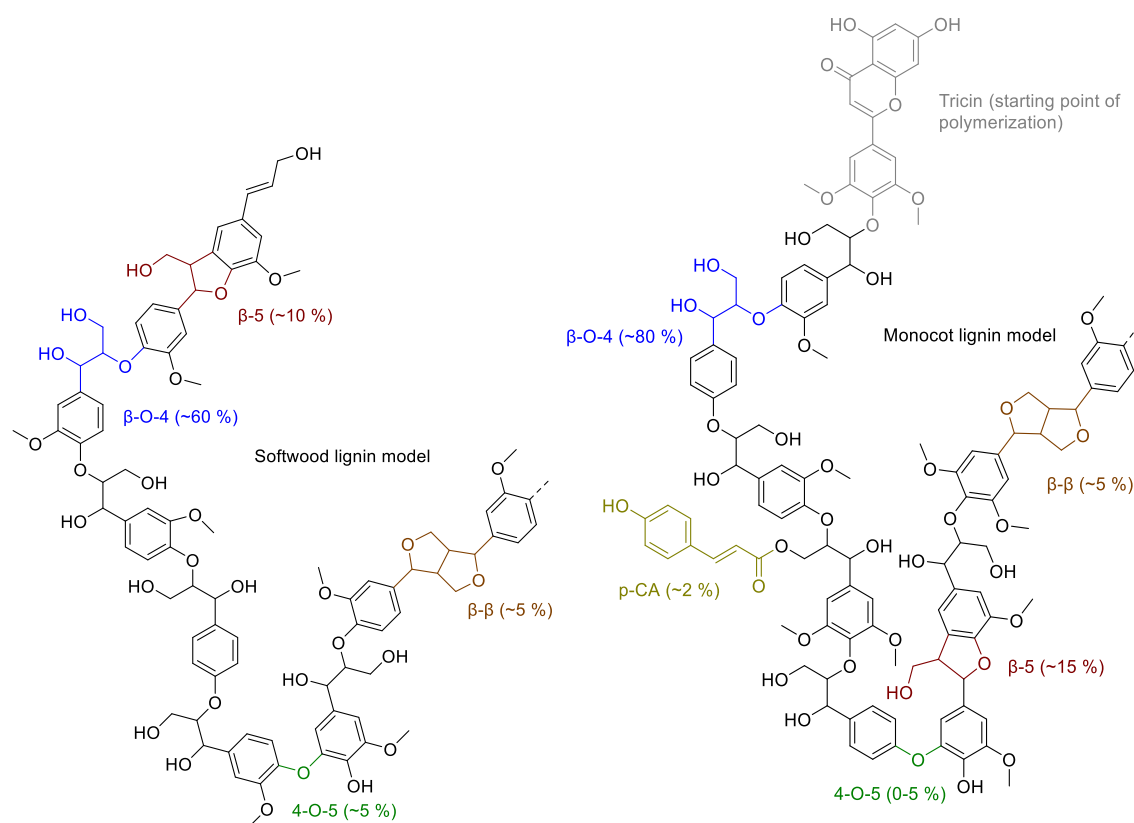


Figure 2. Softwood (left) and monocot lignin models (right), both adapted from (Ralph et al., 2019). In parentheses are the approximate content per 100 aromatic unit. The models are not to be seen as quantitative and the dotted line denotes a continuation of the polymer.

When lignin was first discovered, isolated by Anselme Payen in 1838, it was believed to form one single crystal within trees; however, with the development of polymer science, lignin was soon understood to be a macromolecule (McCarthy and Islam, 1999). Due to the difficulty in isolating intact lignin from the cell wall, the absolute molecular weight of native lignin in plants is still not known today. A speculative estimation, based on the fact that the polymerization is uncontrolled, and that many present compounds can terminate the reaction (Ralph et al., 2019), would be that it has a degree of polymerization of a few hundred, but with a very high dispersity.

2.2. Technical lignin

Technical lignins are those that are removed from biomass during pulping or other delignification processes, like lignosulfonates, kraft lignin or organosolv lignin. They differ greatly among themselves, but they are all characterized by chemical changes from the native material. They are typically depolymerized as a part of solvation, either under basic or acidic conditions, with or without an additional nucleophile. It is the cleaving of the abundant β -O-4 bond that is the main reaction of most pulping processes (Espinoza-Acosta et al., 2014; Sixta, 2006). Kraft lignin, the focus of this dissertation, is solvated under alkaline conditions with sulfide. A recent study on gel permeation chromatography (GPC) coupled with multi-angle laser scattering detector has determined the absolute number average molecular weight (M_n) of softwood kraft lignin to 3400 g/mol, which equals approximately 20 lignol units, which is much lower than what is typically found for native lignin (Zinovyev et al., 2018).

During kraft pulping, coupling reactions also occur. Nucleophilic attacks by lignin phenols or lignin and carbohydrate alcohols on the quinone methide (a quinone formed under alkaline conditions) are possible; however, only the latter of these, carbohydrate-ethers, have been found in significant amounts (Crestini et al., 2017). Another polymerization pathway is the reaction of phenoxy radicals, of either mono- or oligomers, which can result in 5-5' or 4-O-5 bonds. The reaction mechanisms for many of these polymerization or coupling reactions are not fully elucidated, and the structure of kraft lignin is not completely known, but in general these condensation reactions lead to short atom distances between the aromatic rings (Crestini et al., 2017; Balakshin et al., 2003; Gellerstedt and Zhang, 2001). A model structure of softwood kraft lignin is found in **figure 3**.

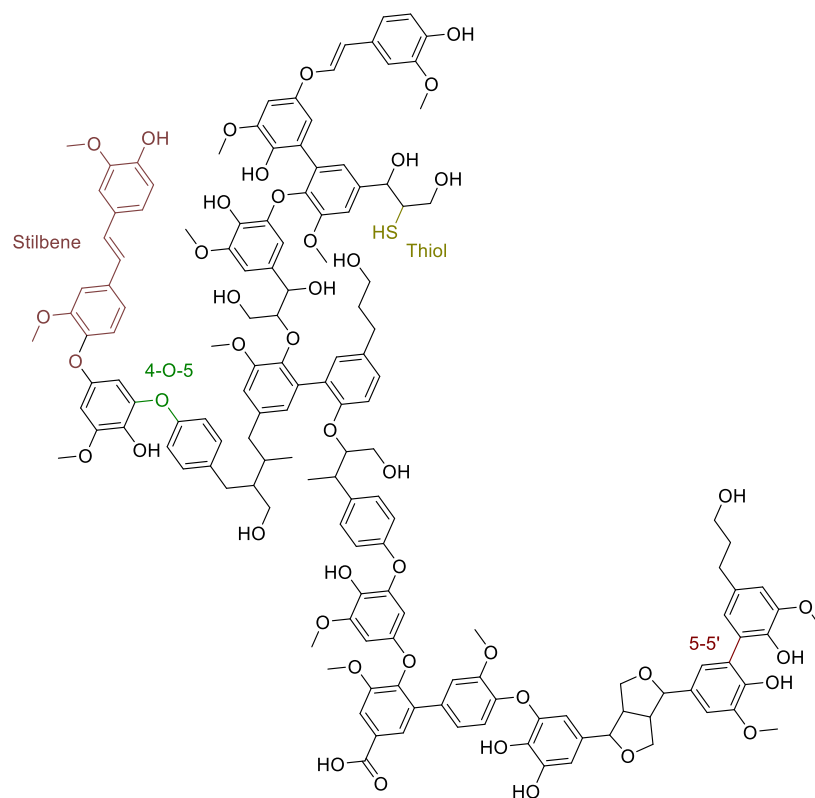


Figure 3. A model structure of softwood kraft lignin adapted from (Crestini et al., 2017). The relative abundance of the different groups and bonding motifs are not expressed as they vary greatly, but 5-5' and 4-O-5 are often the most abundant.

The hydroxyl group composition of kraft lignin is different from native lignin as the cleavage of β -O-4 results in the formation of phenolic OH. The amount of aliphatic OH is also much reduced during kraft pulping, mainly due to the retroaldol reaction, in which aliphatic side chains are lost (Crestini et al., 2017).

2.3. Residual lignin

Residual lignin has been much less characterized than native and technical lignin. Residual kraft lignin is often described as being in-between native and kraft lignin in structure, as it has an intermediate content of alcohol and phenol groups as well as β -O-4 and carbon-carbon linkages (Argyropoulos et al., 2002; Balakshin et al., 2003; Jääskeläinen et al., 2003; Froass et al., 1996); however, the molecular weight has not been determined in many of these studies, which hinders comparison. Whether it is the original structure of the residual lignin that makes it resistant to pulping

reactions, or if there are condensation reactions during pulping that hinder its depolymerization and solvation remains an open question.

2.4. Viscoelastic properties of lignin

Lignins are both amorphous and viscoelastic. Amorphous means that it is a non-crystalline solid and viscoelastic that the material can act both as a solid (elastic) and a liquid (viscous) upon stress. If stress is applied rapidly, the material is deformed elastically, as there is too little time for stress release by chain movements, and it springs back into shape after the stress is released. If the stress is applied slowly, molecules have time to rearrange, and a viscous deformation occurs (Ward and Sweeney, 2004).

The glass transition is an important property of many amorphous viscoelastic materials. Below the glass transition temperature (T_g), large scale movements, involving long chain segments, are not possible. In this glassy state, materials are stiff and elastic, but when heated above the T_g , and segmental movements become possible, materials soften and become more viscous.

The glass transition is a theoretically difficult phenomenon to explain. It does not have the properties of a 1st order thermodynamic transition – a specific transition temperature at a given pressure – as the heating rate or the rate of an applied stress will affect the T_g . However, the transition has thermodynamic consequences, as the degrees of freedom are significantly increased over the glass transition temperature, as can be seen with differential scanning calorimetry (DSC) (Biroli and Garrahan, 2013; Cavagna, 2009).

One theory that explains these changes in properties upon heating is the theory of free volume. The theory states that polymers occupy a certain volume, and in between the polymer chains, there is free unoccupied space. The only possible movement for the polymer chains is to move into these unoccupied spaces. The amount of free volume in the system is thus a determinant of molecular mobility. At higher temperatures, the free volume expands because the increased molecular

vibrations result in increased distances between molecules. At temperatures below the T_g , the free volume is too small to accommodate large-chain movements, but above the T_g , segmental rearrangements are possible (Cavagna, 2009; Biroli and Garrahan, 2013).

The free-volume theory will be used in this thesis, as it is often good for predicting polymer behavior. For example, the influence of molecular weight on the T_g can often be successfully modelled with the Fox-Flory equation (equation 1).

$$T_g = T_{g,\infty} - \frac{K}{M_n} \quad [1]$$

where $T_{g,\infty}$ is the T_g at infinitely high M_n , and K is a constant related to the free volume in the sample and that relates M_n to T_g . Chain ends have more free volume than the main chain segments, and M_n , a measure on the concentration of chain ends in the material, is therefore inversely correlated to T_g (Fox and Flory, 1954; Fox and Flory, 1950).

As was stated in the introduction, lignin is a high- T_g polymer. The two main reasons for this are the aromatic rings in the backbone and the strong intra- and intermolecular interactions, as the large and inflexible benzene unit and lignin-lignin hydrogen bonds hinder chain movements.

In the 1960s, 70s, and 80s, there was a significant interest in exploring the viscoelastic properties of lignin. Since then, the interest in investigating the intricate relationship between lignin's structure and its properties has diminished, at least when it comes to residual and native lignin. A short exposé of this research period is offered in the introduction in Paper I. One of the problems that many of the early studies had was isolating pure lignin of high yield that was representative of the native *in situ* material. The glass transitions of these isolated lignins were typically between 125 and 175 °C (Goring, 1963; Back and Salmén, 1982), whereas *in situ* lignin in wood and in pulp was found to be over 200 °C (Salmén, 1982; Startsev et al., 2017). The discrepancy is likely due to the low yield of these isolated fractions (typically 10-30 wt%). These

isolates can, in practice, be seen as fractionation, where only the low molecular weight lignins were extracted. The issue of isolating representative lignin is further discussed in the next section.

The viscoelastic properties of technical lignins have been extensively studied as they are readily available and the focus of a lot of the material development. The T_g typically range between 100-160 °C with kraft and soda lignin in the higher range, and organosolv lignins in the lower (Wang et al., 2016). The reason for their low T_g is chain scissioning during pulping. Technical lignins are often very disperse in structure and molecular weight, and are therefore often fractionated before further processing (Gioia et al., 2018; Karaaslan et al., 2021; Ebrahimi Majdar et al., 2019). For molecular weight fractions, T_g is often seen to rise dramatically with molecular weight, following the Flory-Fox behavior (Ebrahimi Majdar et al., 2020; Yoshida et al., 1987).

2.5. Lignin isolation

As mentioned in the introduction, the isolation of lignin is one of the main problems in lignin science. If the chemical structure and molecular weight of lignin are to be determined, isolation would ideally be quantitative and non-degrading; however, the layered and hierarchical structure of wood requires some kind of physical or chemical degradation of the material to access the lignin.

In an early attempt at isolating non-degraded lignin, wood was milled to millimeter size and extracted with ethanol. This procedure managed to extract about 10 % of the lignin content (Brauns, 1939). By ball milling wood into a submillimeter powder and using dioxane with small additions of water as solvent, Björkman (1956) was able to isolate about 20-30 % of lignin. This latter method, isolation from ball milled wood with dioxane, known as Björkman's lignin or milled wood lignin (MWL), has become the standard method for isolating plant lignin as it is believed to largely retain the native molecular structure. The method is known to have limitations, e.g. it only isolates up to a third of the lignin, and ball milling depolymerizes the wood

polymers to some extent (Sapouna and Lawoko, 2021), but few other lignins have been as frequently and thoroughly characterized as this one, and from so many sources (Lupoi et al., 2015).

As milling depolymerizes polymeric materials, other techniques have been used to aid lignin isolation. Holocellulose-degrading fungi have been used to liberate lignin from the cell wall, and around 20 % of the lignin was successfully isolated (Browning, 1967). Later, more refined enzymatic treatments were developed with a somewhat higher yield (~30 %) (Polčín and Bezúch, 1978). The main drawback of these techniques is the low yield and high carbohydrate and protein contamination.

A factor that greatly affects the yield of lignin isolation is the solubility of lignin. Pure lignins, even of high molecular weight, are typically soluble in organic solvents such as dioxane and dimethyl sulfoxide (DMSO) (Sameni et al., 2017); however, lignin bonded to carbohydrates, LCCs, are more difficult to dissolve (Balakshin et al., 2014). These compounds have been close to quantitatively isolated by successive dissolutions in different solvents (Giummarella et al., 2019). The different fractions are of interest as they can be analyzed for structural information, but their bulk properties are not representative, as they are no longer mixed, like in the cell wall.

At the turn of the last century, a combination of ball milling, enzyme hydrolysis of holocellulose and acidic hydrolysis was suggested to isolate lignins of high yield (~70 %) but with a chemical structure very close to that of MWL (Wu and Argyropoulos, 2003). The obvious downside of this technique is that it still utilizes ball milling and that naturally occurring lignin-carbohydrate bonds are cut. However, several lignins have been isolated with this technique, and it is believed that in many aspects, it is a good representation of bulk properties (Argyropoulos et al., 2002; Jääskeläinen et al., 2003; Wu and Argyropoulos, 2003; Guerra et al., 2006; Guerra et al., 2007; Asikkala et al., 2012). There is to date one type of characterization that EMAL isolation of native lignins have not gone through – a viscoelastic analysis.

2.6. Plasticization of lignin

The first study on external plasticization of lignin with small organic molecules investigated their effect on softwood thiol (heavily condensed lignin) and dioxane lignin (isolated native lignin) (Sakata and Senju, 1975). The effect of phthalic, phosphoric, and aliphatic esters with different aliphatic chain lengths was studied. The phthalates were found to be most efficient in lowering the T_g , and for all types of plasticizers, shorter side chains, lead to a greater reduction in T_g . At long enough side chains, 7-8 carbons, the molecules no longer acted as plasticizers, likely due to phase separation. The plasticization of both kinds of lignins showed that they followed the same trend with regard to side chain length; however, the study did not disclose if the two lignins respond differently to phthalates, phosphates, and aliphatic esters.

Many different compounds have been found to lower the T_g of technical lignin, provided they have some polarity (Ayoub et al., 2021; Banu et al., 2006; Milotskyi et al., 2019; Sakata and Senju, 1975). A study by Bouajila et al. (2006) shed light on the critical requirement of saturating the OH groups in lignin to effectively reduce its T_g . They found that water could serve as a plasticizer until all OH groups were fully engaged and substituting lignin OH with acetyl groups produced a similar effect to adding an equivalent amount of water, underscoring the significance of disrupting internal hydrogen bonds in the process of lowering lignin's glass transition temperature. A moisture content dependence of the T_g of lignin has also been found in molecular dynamic simulations (Vural et al., 2018).

There has been some interest in looking at how different plasticizers affect the viscoelastic properties of *in situ* lignin (Sadoh, 1981; Miyoshi et al., 2018; Chowdhury and Frazier, 2013; Salmén, 1984; Salmén et al., 1984). Generally, water, ethylene glycol, formamide and glycerol are good plasticizers for *in situ* lignin in wood, but DMSO, dimethyl formamide and N-Methyl-2-pyrrolidone have been found to reduce the glass transition temperature even further. The specific interactions have not been fully elucidated, but the aprotic nature appears important.

3. Methodology

3.1. Lignin isolation, fractionation, and characterization

Lignin from Norway spruce, wheat straw and softwood kraft pulp were isolated in this study according to the EMAL protocol (Wu and Argyropoulos, 2003; Argyropoulos et al., 2002). The process involves ball milling of the biomass, then enzyme hydrolysis of holocellulose and lastly, isolation of lignin in a mildly acidic dioxane solution (figure 4). A detailed description can be found in Paper I.

Fractionation of softwood kraft lignin was performed according to (Duval et al., 2016): successive dissolution in small amounts of organic solvents: ethyl acetate, ethanol, methanol, and acetone. The method isolates five fractions with different molecular weights. For details see Paper I and Ghaffari et al. (2023).

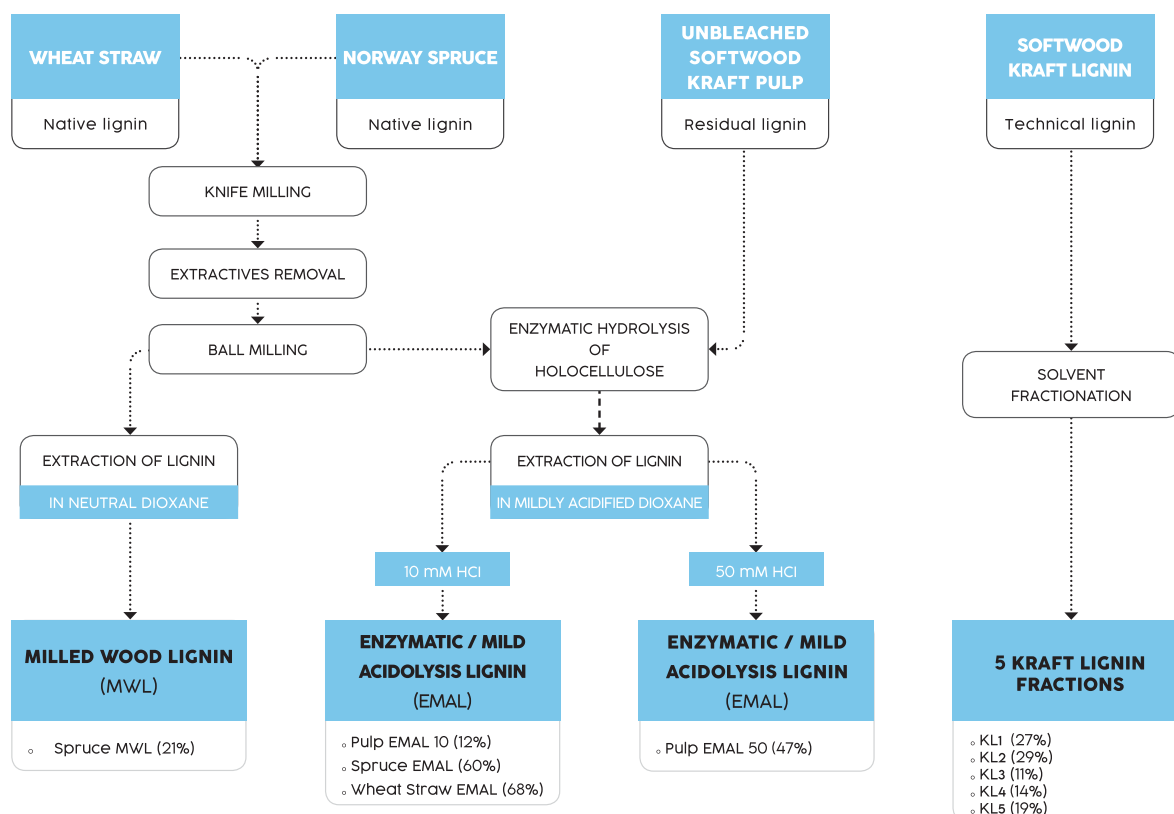


Figure 4. Flow chart of isolation. Values in parentheses are the yield of isolation (wt/wt, see details in table 1).

Lignins in the study were analyzed according to the list below. For more details see Paper I.

- Pullulan-calibrated GPC in DMSO with 10 mM LiBr to determine molecular weight distribution. Pullulan in DMSO underestimates the size of lignin, but this underestimation is linear, so that comparisons can be made within the same study (Zinovyev et al., 2018). See **table 1** for results.
- ³¹P NMR of phosphorylated lignin, which allows for the quantification of OH groups. Analyses were carried out according to (Balakshin and Capanema, 2015), see **table 2** for results.
- Purity of the lignin were determined by measuring the ash (thermogravimetric analysis), protein (quantification of N through pyrolysis-mass spectroscopy) and Klason lignin and acid soluble lignin content, see **table 1** for results.

Table 1. Yield, molecular weight, and purity of lignins.

Lignin	Yield	M_n (kDa)	M_w (kDa)	Purity^c
Spruce MWL	21% ^a	2.8	7.2	95.2% (0.4)
Spruce EMAL	60% ^a	3.8	27.5	92.6% (0.2)
Wheat straw EMAL	68% ^a	3.1	15.7	93.2% (0.4)
Pulp EMAL 50	47% ^a	5.8	77.3	93.7% (0.1)
Pulp EMAL 10	12% ^a	4.7	37.0	90.3% (0.5)
Kraft lignin	-	1.6	12.2	93.5% (0.6)
KL1	27% ^b	0.7	1.8	-
KL2	29% ^b	1.9	5.4	-
KL3	11% ^b	2.6	6.1	-
KL4	14% ^b	6.8	13.4	-
KL5	19% ^b	14.8	42.5	-

^aYield = mass of extract/mass of Klason and acid soluble lignin of biomass.

^bYield = mass of extract/sum of the mass of all fractions.

^cPurity = (Klason and acid soluble lignin - ash content - protein contamination)/mass of sample. Values in parenthesis are the pooled standard deviations. See Paper I for details.

Table 2. Quantification of OH groups by ³¹P NMR.

Sample	Aliphatic (mmol/g)	Phenolic			COOH (mmol/g)
		5-subst (mmol/g)	G _{noncond.} (mmol/g)	H (mmol/g)	
Spruce MWL	7.7	0.5	1.5	0.0	0.2
Spruce EMAL	7.7	0.5	1.1	0.0	0.0
Wheat straw EMAL	8.7	0.3	0.9	0.7	0.2
Pulp EMAL 50	4.8	1.6	1.4	0.1	0.4
Pulp EMAL 10	5.7	1.6	1.5	0.1	0.3
Kraft lignin	2.4	2.6	2.6	0.2	0.4
KL1	0.9	1.9	2.5	0.2	0.7
KL2	2.1	2.1	1.8	0.2	0.6
KL3	2.2	2.2	1.5	0.1	0.5
KL4	2.1	2.1	1.3	0.2	0.4
KL5	2.5	1.5	0.9	0.1	0.3

3.2. Lignin modification

3.2.1. External plasticization

Lignins were plasticized to the desired concentration in glass vials by adding methanol solutions (20 wt%) of the additives (**figure 5**). The solvent swollen, wetted powders were then mixed with a glass rod and left with a lid for 24 h, after which they were allowed to dry by evaporation at room temperature. The samples were then put in a vacuum oven at 40 °C for 1 h to remove any remaining solvent.

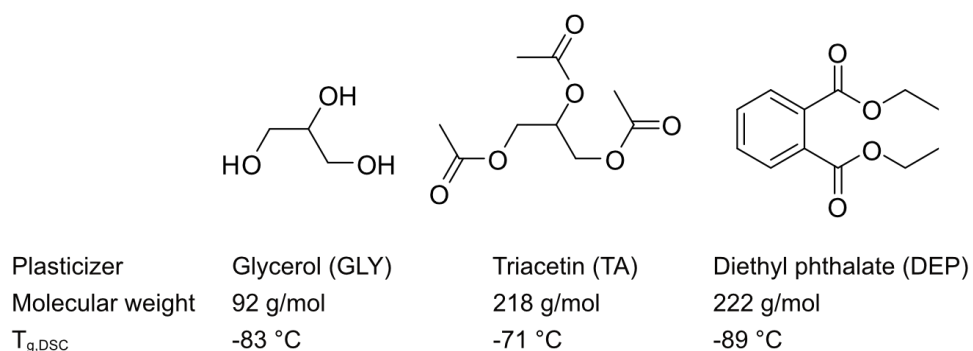


Figure 5. Molecular structure, weight and T_g of plasticizers. The T_g of the plasticizers was determined with DSC (Paper II).

3.2.2. Internal plasticization by acetylation

A quantitative acetylation method was used to acetylate the isolated lignins and kraft lignin (Månsson, 1983). Lignin was dissolved in a solution of equal amounts of pyridine and acetic anhydride. The reaction was carried out at room temperature for 24 h and was terminated with the addition of methanol. Several repeated rotor-evaporation and dissolutions (acetone and water) were performed to remove excess pyridine and acetic acid. Attuned total reflectance Fourier-transform IR (ATR-FTIR) spectra were acquired for the final products and the acetylation appears to be close to complete as the OH stretching had reduced by a factor of about 10 (**figure 6**). The carbonyl stretching of phenyl and alkyl acetates have been marked in the spectra (Faix et al., 1994).

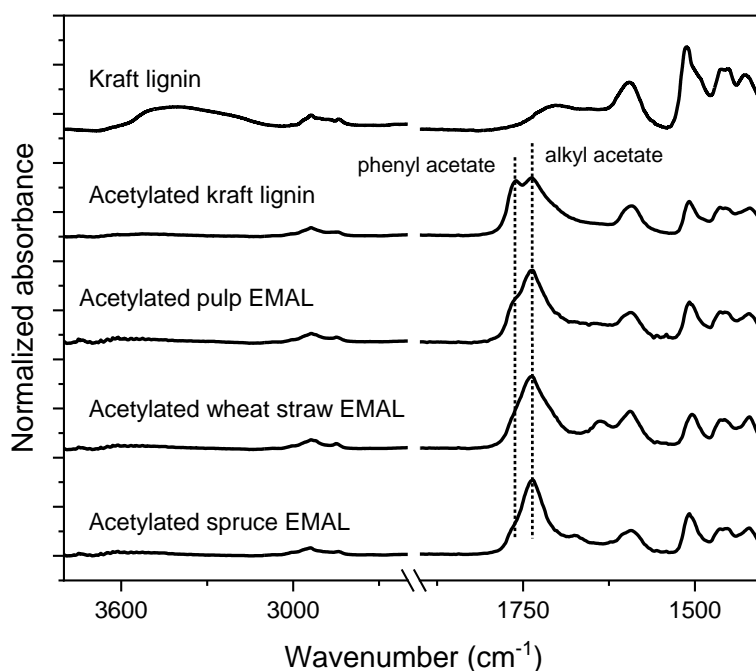


Figure 6. ATR-FTIR spectra of acetylated lignin normalized to the aromatic stretch peak at 1509 cm^{-1} . Kraft lignin is included as a reference. The peaks corresponding to the carbonyl stretch of phenyl acetate and alkyl acetate are marked (Faix et al., 1994).

3.3. Dynamic mechanical analysis (DMA)

In dynamic mechanical analysis, a sample is subjected to oscillating strain. The phase lag (δ) between the applied stress and the following strain is measured. A perfectly elastic material will deform directly upon applied stress, whereas viscous deformation will lead to a lag.

The modulus of dynamic deformation consists of two components. The storage modulus (E') measures the energy stored in the material upon deformation, whereas the loss modulus (E'') measures energy loss due to viscous deformations. The tangent of the phase lag, the $\tan \delta$ or $\tan \delta$, was the only output from older instruments, and is often cited in the literature (Menard, 1999). This value corresponds to the dissipation of energy in the system due to molecular movements.

A DMA thermogram for an amorphous polymer is illustrated in **figure 7**. E' is reduced radically over the glass transition, often by 3 magnitudes. The loss modulus first increases as segmental movements start to be possible and friction increases,

but then also declines as chain movements become less hindered. Tan delta reaches a maximum when the quota between the viscous and elastic components is the highest (Menard, 1999).

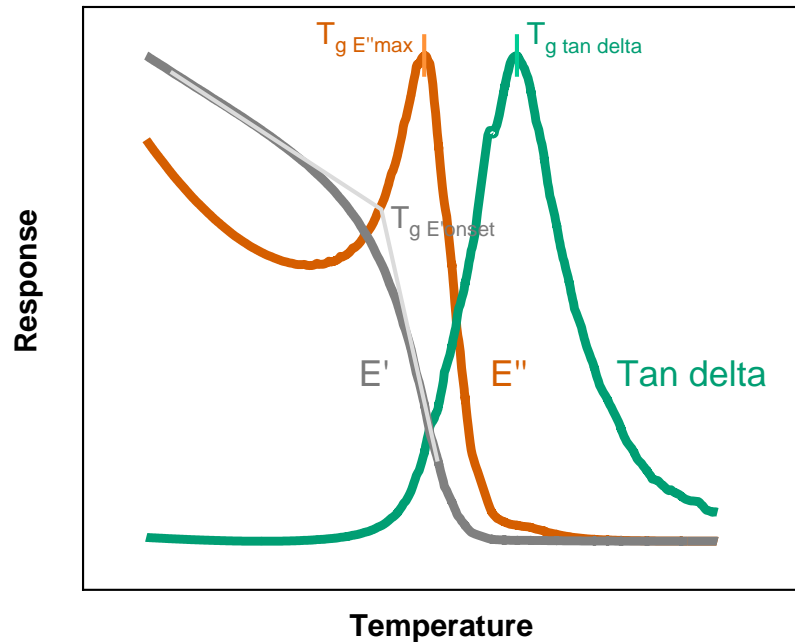


Figure 7. DMA thermogram illustrating the glass transition of an amorphous polymer.

In this thesis, a powder sample holder, or powder pocket, was used for analysis (**figure 8**). These holders were developed for amorphous materials from which self-standing shapes could not be cast or molded. They require small samples sizes (25-100 mg) and simple sample preparation. However, they do not give absolute moduli values, as the pocket itself is strained, and the geometry factor of a powder is not known (Jones et al., 2012).

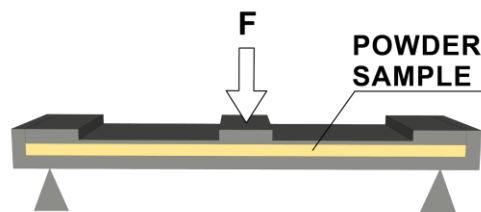


Figure 8. Illustration of powder sample holder for DMA.

In a previous study, a high clamping force has been shown to reduce the broadening of the transition due to inter-particle friction, as the clamping hindered particle movements (Mahlin et al., 2009). We adopted their running conditions, and validation experiments are presented in Appendix 1.

The DMA measurements were performed on a Q800 (TA instruments) with the powder sample holder (TA instruments) in the dual cantilever set-up. Cooling was done with liquid nitrogen. The standard measurement was performed from 25 °C to 50 °C above the $T_{g, E''_{max}}$ with a heating rate of 3 °C/min and with a 5 μm strain that oscillates with a frequency of 1 Hz. Unmodified lignin samples were run with an annealing of 5 min at 120 °C to remove moisture. Plasticized and acetylated samples could not be annealed, as many of the samples started to flow out of the holder at higher temperatures, which reduced the force needed for deformation. However, samples ran with and without annealing had very similar transition temperatures, so the moisture content (typically around 3 wt%) did not appear to affect the materials very much.

4. Results and discussion

4.1 Chemical structure, molecular weight and representativity of isolated lignins.

As the objective of this thesis is to establish structure-property relationships, lignins of varying structures were isolated. Native lignins were isolated from plants with two of the most varied types of lignins, softwood and monocot, represented by Norway spruce and wheat straw. Lignins were also isolated from softwood kraft pulp, which often have high molecular weight and somewhat condensed structure. Lastly, a softwood kraft lignin is included, as it represents a heavily degraded lignin with few of its original linkages. The kraft lignin was also fractionated into molecular weight groups, to obtain lignin that spans over a large molecular weight range. The term *chemical structure* or *lignin structure* is used plentifully in this thesis and refers to the monomer content (H, G, S, p-CA, triclin, etc.), the linkages between monomers or lignols, the side chains and the functional groups.

The lignins were isolated according to a protocol, from which Norway spruce, wheat straw and kraft pulp lignin have previously been isolated and thoroughly characterized. To corroborate our results with the previous studies, ³¹P NMR was conducted. As this method assesses both the degree of depolymerization, by detecting free phenols, the loss of aliphatic side chains, by the loss of aliphatic OH, and the following polymerization reactions by the formation of 5-substituted phenolics, it is an excellent method to determine the structural changes brought on by kraft pulping. As is seen in **figure 9**, the isolated native lignins have high aliphatic and low phenolic OH content, whereas the opposite is true for kraft lignin. Pulp EMAL is intermediate. Both pulp and kraft lignin have high C5-substitution, which indicates that both insoluble and solvated lignin are subjected to condensation reactions. These results agree with previous characterization of EMAL lignins from

similar sources (Argyropoulos et al., 2002; Jääskeläinen et al., 2003; Wu and Argyropoulos, 2003; Guerra et al., 2006; del Río et al., 2012; Crestini et al., 2017).

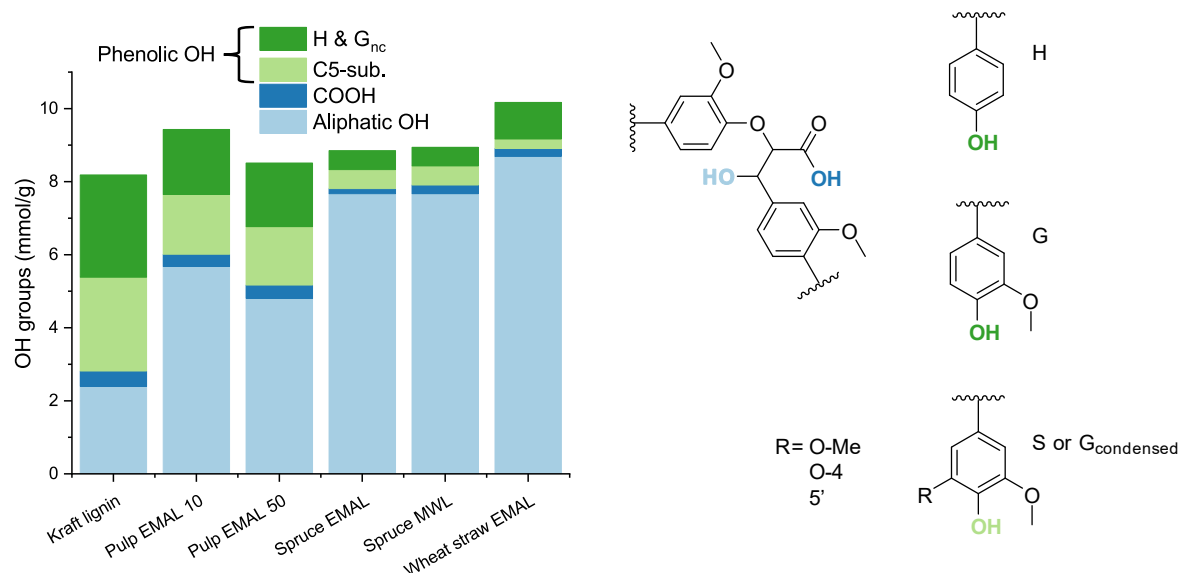


Figure 9. The OH groups, as determined by ^{31}P NMR (left), and their corresponding structures (right). R represents methoxy (S unit) or substitutions ($G_{\text{condensed}}$), such as 4-O-5 and 5-5'. See Paper I for full analysis.

In **figure 10**, the lignins have been arranged according to their molecular weight and on a scale from native to condensed. I use the term condensed here, as is common in the lignin literature (Balakshin et al., 2003; Crestini et al., 2017), to summarize both depolymerization and polymerization reactions. There is no absolute scale for the degree of condensation, and the figure only illustrates an intra-relative order.

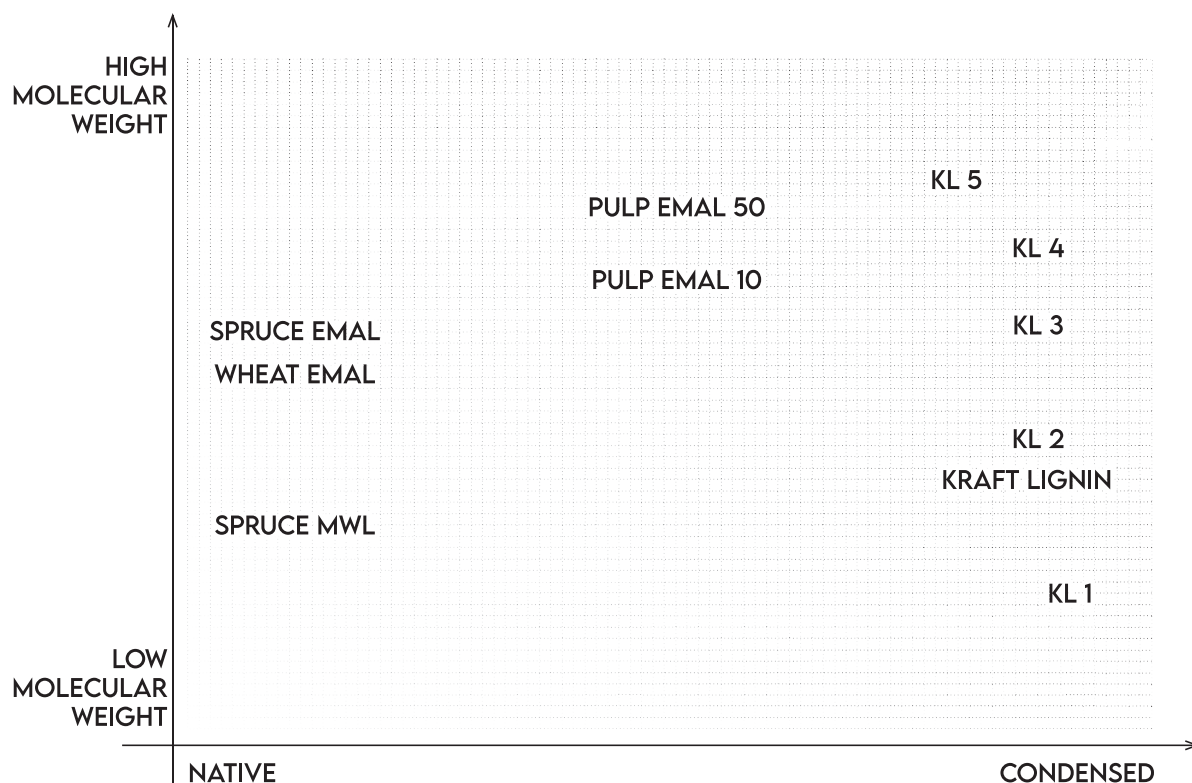


Figure 10. Visualization of the variability in molecular weight and degree of condensation. There are no absolute values for degree of condensation but based on lignin source and on ^{31}P NMR analysis, relative assignments have been made.

For the results of this thesis to be relevant, the isolated lignins have to be representative of their *in situ* counterparts. The higher yield and molecular weight of EMAL compared to MWL, paired with an OH composition that indicates no substantial condensation or hydrolysis reactions, would suggest that EMAL is a better representative of lignin in spruce.

The chemical similarity between pulp EMAL 10 and 50 and the large difference in yield, suggests that the lignin has been little changed by the increase in acid content, but that the higher concentration was needed to cleave LCC and solubilize the lignin. This is also corroborated by the fact that pulp EMAL 10, had a carbohydrate contamination that was five times larger than for pulp EMAL 50 (see Paper I table S1).

4.2 Viscoelastic analysis of isolated and fractionated lignin

This thesis set out to establish property-structure relationships for lignins of different origin. Molecular weight is a strong determinant for T_g , but different polymers have different relationships between T_g and M_n , as expressed by the K constant of Flory-Fox equation (equation 1). This empirical relationship can be interpreted as the difference in free volume between different polymers, but it can also be understood in a more general sense, that the T_g at infinite molecular weight is related to the stiffness of the polymer structure.

In **figure 11**, the glass transition temperatures of the different lignins have been plotted against their number average molecular weight. The first thing to note is that the KL fractions span the whole range, and in the reciprocal plot, fitted with a line, they follow the Flory-Fox equation relatively well ($R^2=0.970$). Lignins above this line, will, assuming that all lignins have a similar Flory-Fox behavior, have a larger K constant than kraft lignin, and vice versa if they are below the line. All the isolated native lignins are positioned below the line, which would mean that they have a larger free volume than kraft lignin. On the contrary, the two Pulp EMAL are above the line, which suggests that they have a smaller free volume.

Based on the ^{31}P NMR analysis, the C5-substituted OH content of kraft lignin and pulp EMAL were 2.6 and 1.6 mmol/g respectively. Assuming an average lignol unit of 180 g/mol, these concentrations corresponds to 46% and 29% of the lignols being linked at the C5-position with a free phenol. The corresponding value in spruce EMAL and MWL is 9%. These linkages are constituted of mainly 5-5', 4-O-5 or β_5 (Granata and Argyropoulos, 1995; Gioia et al., 2018). These linkages are likely stiffer than β -O-4, based on the close range between the aromatic rings (Bicerano, 2002). The difference in K, suggested by the T_g values, between the condensed lignins and the native, is likely due to the decline in β -O-4 and the increase in short, aryl-aryl bonds.

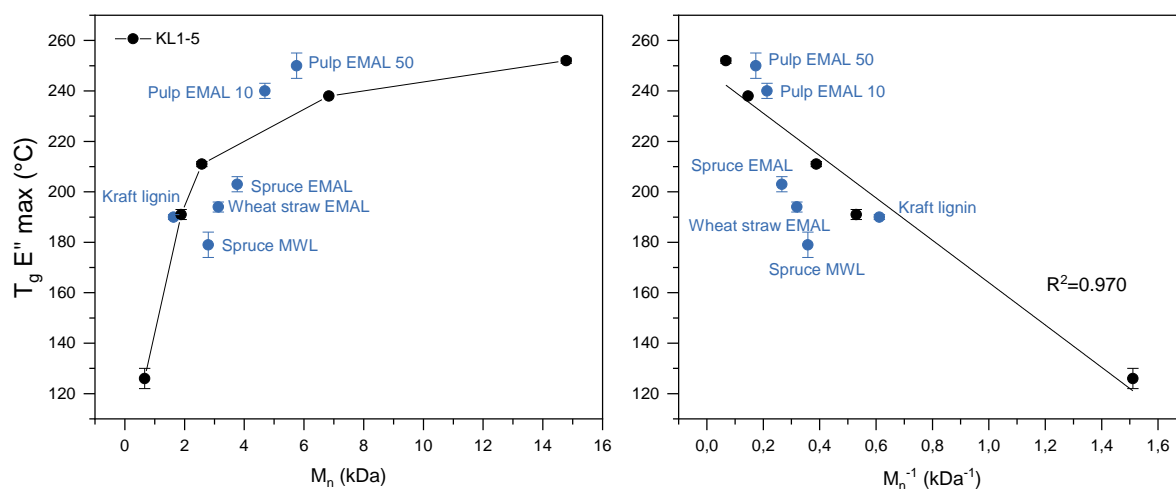


Figure 11. $T_{g,E''\max}$ plotted against the number average molecular weight (M_n , left) and the reciprocal M_n (right). The kraft lignin fractions (KL1-5) in the right plot are fitted with a line.

Another important aspect of the thermal processing of plastics, apart from the T_g , is the degree of thermal softening of the material. There are some reports on the glass transition of *in situ* pulp and wood lignin in the literature, but there have been no attempts to quantify the magnitude of their thermomechanical properties. In **figure 12**, the max-normalized E' , are displayed as a function of temperature. As the geometry factor, the amount of sample, and the packing conditions were kept constant, the differences seen in relative E' are related to differences in friction and movement in the material. The powders are clamped in the pocket, to minimize particle movements and the dissipation of energy due to friction between particles; thus, the observed differences should primarily be related to molecular movements.

In the rubbery state, the length of the polymer greatly impacts the strength of the material and its ability to withstand stress (Ward and Sweeney, 2004). This can be seen in **figure 12**, as the drop in E' is greater the smaller the molecular weight. This is true for the kraft lignin fractions as well as for the isolated lignins; thus, the chemical structure appears to be less important for the thermal softening than molecular weight. There is only one exception to this: KL5 repeatedly has a larger drop in E' than KL4, even though its M_n is more than double in size. The reason for

this could be due to the lower degree of condensation of KL5 as revealed by the ^{31}P NMR analysis (table 2, lower phenol/alcohol ratio and C5-substitution).

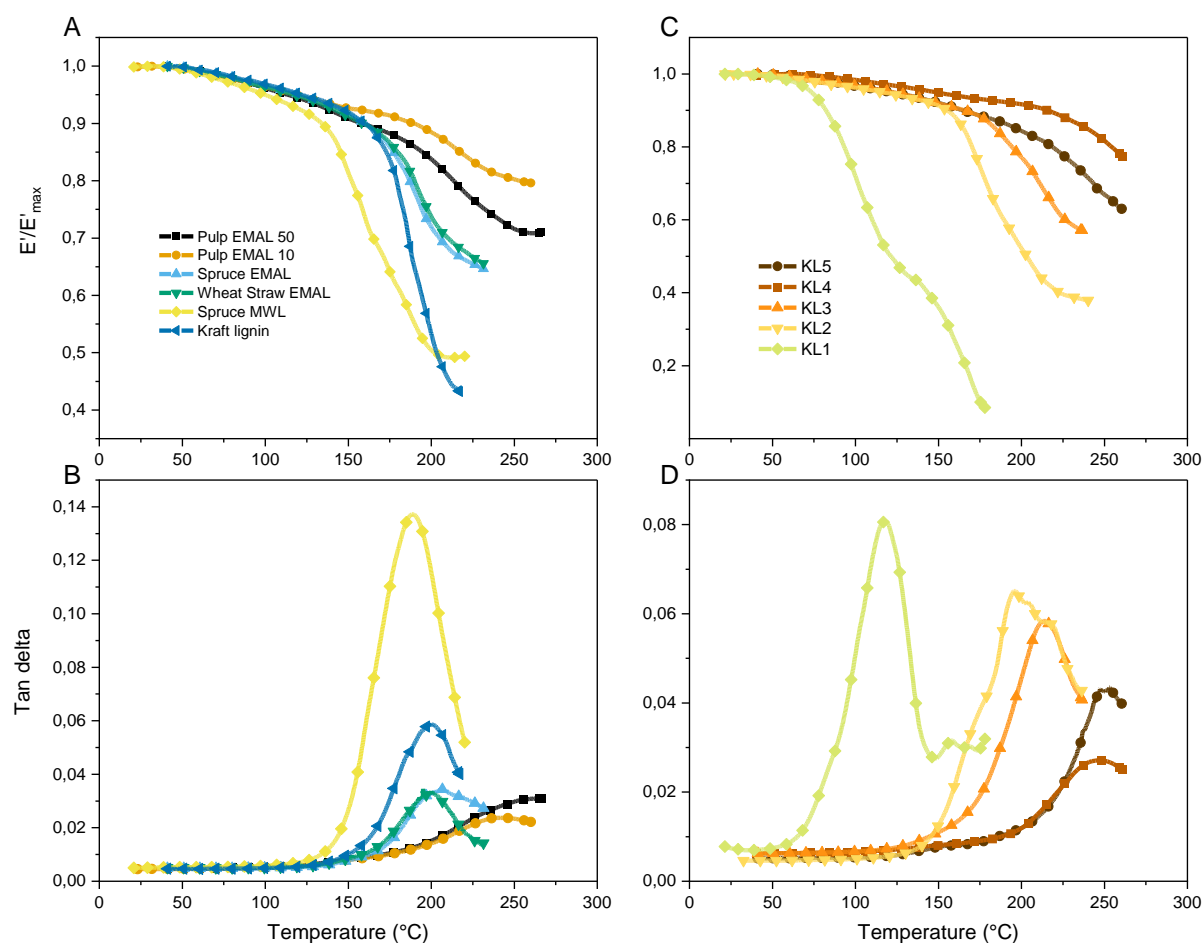


Figure 12. DMA thermograms of fractionated kraft lignin (A and B) and isolated lignin (C and D) with E' (top) and tan delta (bottom). E' has been normalized to the maximum value in the observation. The symbols are for guidance and do not represent data points.

Another novel observation is the broadness of the transitions. Many samples have glass transitions in tan delta that spans over 100 °C, which is broader than what is typically observed for synthetic polymers. In synthetic polymers, broad glass transitions are commonly associated with materials featuring nanoscale compositional variations, as can be seen in specific types of copolymers or polymer blends (Mok et al., 2008; Li et al., 2018). Given the heterogeneous composition and structure inherent to lignins, it appears reasonable to assume that their broad transitions result from similar variations in nanodomains. As thermoplastic processing is performed beyond the T_g , this is an obstacle to lignin utilization, but

polymers with broad glass transition are often very good acoustic and vibrational dampeners.

In the beginning of the chapter, the claim was made that lignins isolated with higher yields would be better representatives of *in situ* lignin. The softening temperature of dry Norway spruce is yet to be determined, but pine and some different hardwoods, have been found to have $T_{g,E''_{max}}$ between 205-235 °C (Startsev et al., 2017). These transitions are likely a combinatory effect of the cooperative or individual transitions of some of the amorphous wood components (lignin, hemi- and unordered cellulose), but it indicates that *in situ* lignin has a T_g over 200°C (Salmén, 1982). Thus, the $T_{g,E''_{max}}$ of 203 °C spruce EMAL, is much closer to a reasonable T_g for *in situ* lignin, than the $T_{g,E''_{max}}$ of spruce MWL of 179 °C.

4.2 Plasticization of lignin

In the previous section, we saw that chemical structure and molecular weight had a large impact on the viscoelastic properties of lignin. However, the question remains if the structural variations are so large, that they respond differently to different kinds of modification. In this section, four of the lignins, selected due to their discrepancy in structure, will be subjected to different kinds of plasticization. The four lignins are: wheat straw EMAL, spruce EMAL, kraft lignin, and pulp EMAL 50. Pulp EMAL 50 is from now on referred to as only Pulp EMAL. The first part of this section focuses on external plasticization and the second part on internal plasticization by acetylation.

4.2.1. External plasticization

The four lignins were plasticized to 10 and 20 wt% with glycerol (GLY), triacetin (TA) and diethyl phthalate (DEP). These plasticizers were selected as they have different functionalities: GLY can accept and donate hydrogen bonds, TA and DEP can only accept, and DEP has an aromatic ring. Polar molecules are needed to achieve lignin plasticization, and aprotic plasticizers are often found to be the most efficient (Bouajila et al., 2006; Ayoub et al., 2021; Laborie et al., 2004). By selecting

two plasticizers of similar structure, GLY and TA, we hope to elucidate this mechanism. DEP was selected as it could potentially have aromatic interactions with lignin. In this section, the compatibility, efficiency, and rigidity of the plasticized lignins are investigated.

Previous studies found DEP and GLY to be efficient plasticizers for kraft lignin (Ayoub et al., 2021; Sakata and Senju, 1975). In our study, they proved to lower the T_g effectively as well (**figure 13**). TA has not been used for lignin previously but has been used as a plasticizer for many other polymers, both biobased and synthetic (Gama et al., 2019; Phuong and Lazzeri, 2012). TA is equally efficient in our study but appears to mediate a more linear reduction in T_g .

The plasticizers are equally efficient at reducing the T_g based on the weight added, but when their molar content is taken into consideration, GLY performs much worse. As the molar mass of GLY is about half of that of TA and DEP, its molar efficiency is about half. The reason for this poor molar performance could be due to the hydrogen bonding potential of GLY. GLY can accept hydrogen bonding from lignin OH groups, but also donate hydrogen bonding to lignin OH, ether and carbonyl groups. TA and DEP likely reduce the hydrogen bonding density of the system, whereas GLY is more likely to maintain it. In Paper II, the activation energy of the glass transition was calculated for plasticized Spruce EMAL, based on frequency sweeps, and GLY was found to retain it, whereas the other two plasticizers significantly decreased it. This further strengthens the case that GLY has antiplasticizing interactions with lignin.

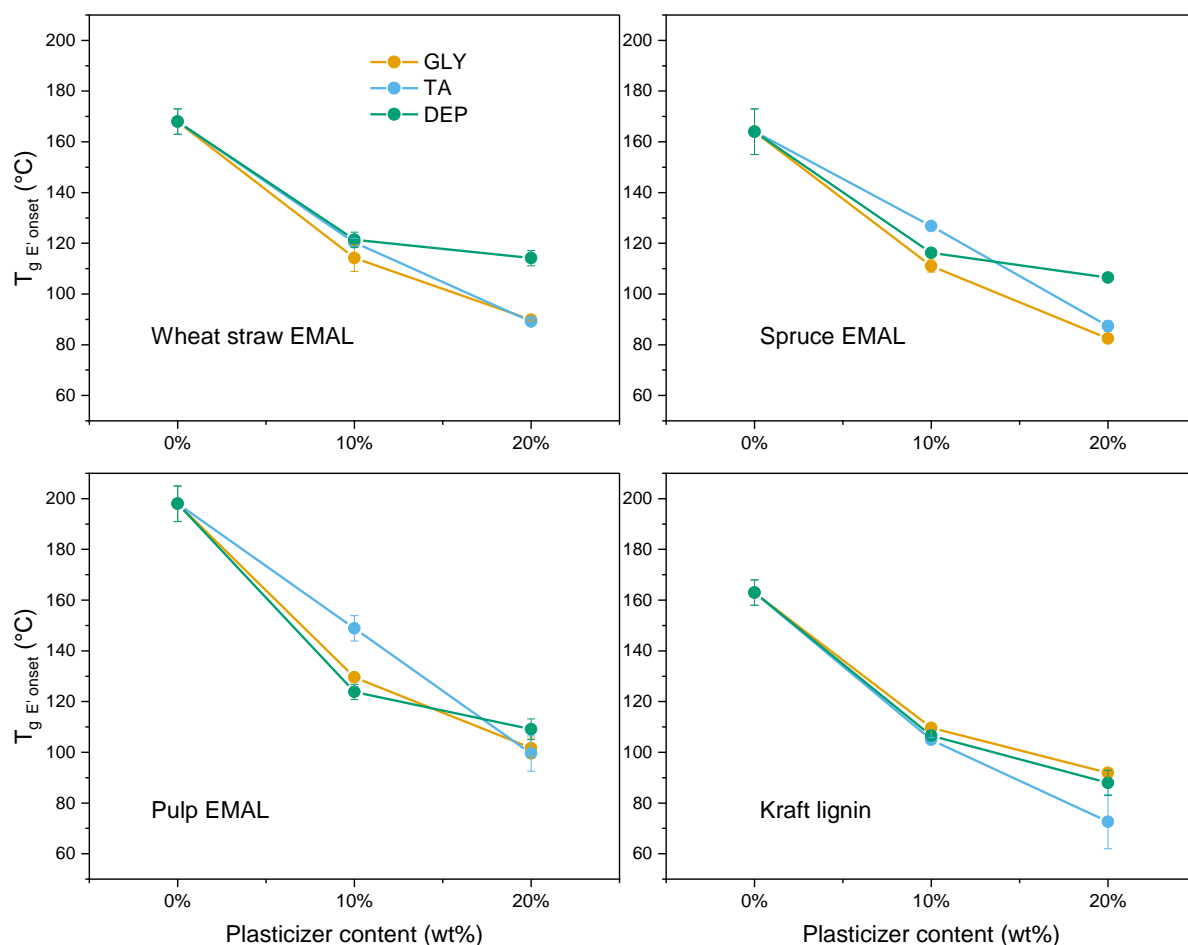


Figure 13. T_g determined by the E' onset plotted against the plasticizer content.

Tan delta profiles measured with DMA are useful to study the efficiency of plasticization (decrease in T_g), but also to investigate the compatibility of polymer and plasticizer. As explained in the previous section, compositional and configural heterogeneity give rise to broad transitions. In synthetic polymers, plasticization is often accompanied by a broadened tan delta peak, as the heterogeneity in the system increases. The plasticized lignins exhibited an opposite behavior. As the full glass transition for the lignins was not measured, since most lignins start to deteriorate over 240 °C and since some plasticizers are not stable over 200 °C, the transition widths were determined by calculating the difference between the maximum and the onset of the tan delta transition. The tan delta peak is sometimes slightly skewed for the plasticized samples, so the values are not direct measurements of transition width but give an indication. Pulp EMAL has its glass transition width reduced the most,

with approximately 50 % at 20 wt% plasticizer content. Spruce EMAL has its transition reduced the least, with approximately 25% at 20 wt%. If the reason for the broad glass transition is due to compositional heterogeneity at a nanoscale, the narrowing of the transition is likely due to an increased homogeneity.

Amorphous polymers pass from glassy to rubbery at the glass transition, but a transition from rubbery to liquid-like is also possible. Especially for low molecular weight polymers, as there are less entanglements that can hinder flow. The two lowest molecular weight lignins, wheat straw EMAL and kraft lignin, become liquid-like after plasticization: kraft lignin with all plasticizers at 10 wt%, and wheat straw EMAL at 20 wt% with TA and GLY. This transition is marked T_f in **figure 14**.

Overall, the plasticizers appear close to equally efficient, and apart from the differences already mentioned, no reproducible differences in the tan delta curves were found. This would indicate that the structural semblance of the lignins is such that their response to different plasticizers is the same. However, there is one systematic difference between the efficiency of plasticizing condensed and native lignin. The condensed lignins had a much greater reduction in T_g , 75-100 °C at 20 wt%, whereas the isolated native lignins were only reduced with 60-80 °C at the same concentration (**figure 13**). Condensed lignin has less free volume than native lignin, as indicated by the Flor-Fox modeling, and it appears as if they were better aided by the introduction of free volume by plasticization.

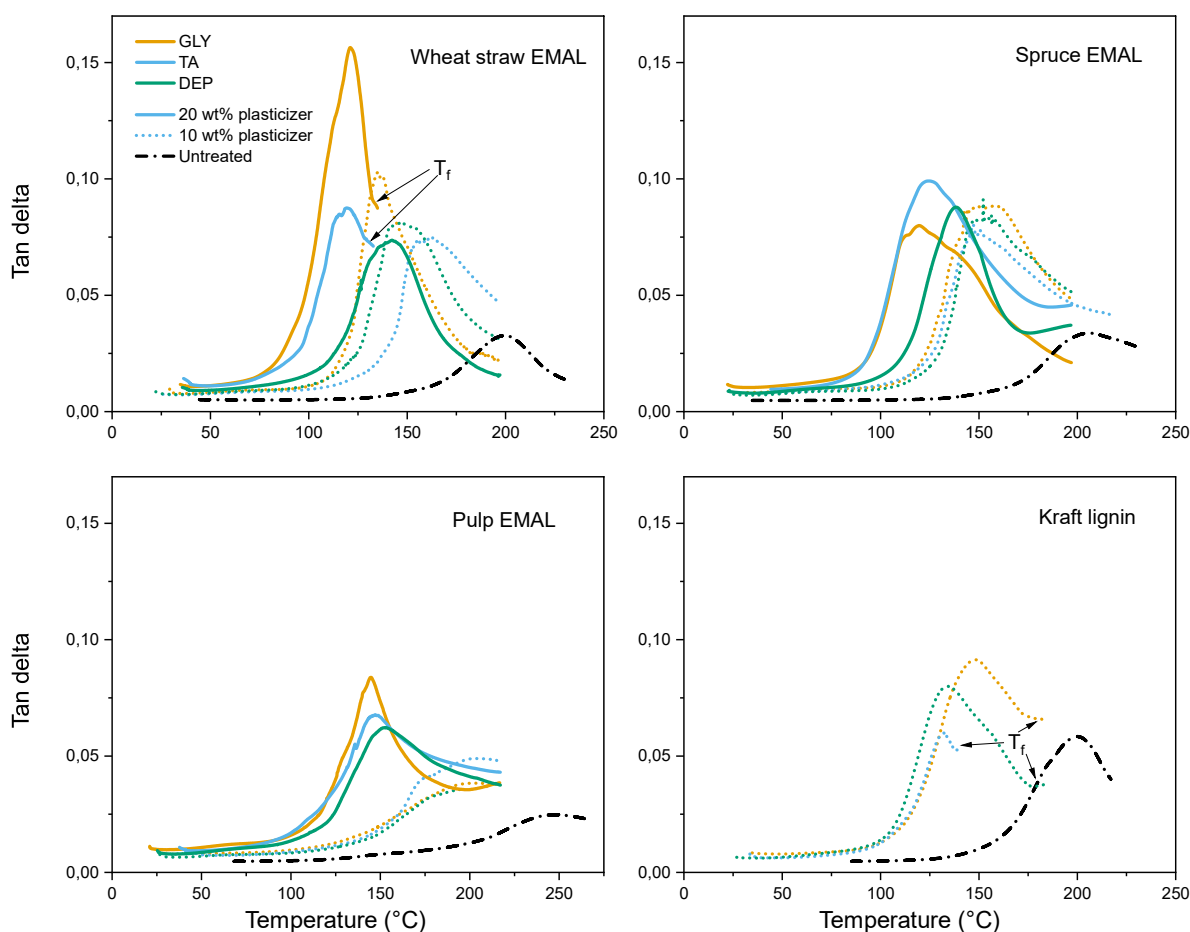


Figure 14. The mechanical damping (tan delta) behavior of plasticized lignins as a function of temperature. Kraft lignin plasticized with 20% is not shown, as the material started to flow out of the sample holder at low temperatures. T_f refers to the temperature where the blend starts to flow.

4.2.2. Internal plasticization through acetylation

The acetylation of lignin introduces free volume due to the hindrance of close packing by side chains, but it also weakens its secondary interactions by substituting OH groups with esters. Experimental evidence suggests that aliphatic OH groups in lignin exhibit stronger hydrogen bonding in lignin compared to phenolic OH groups, as the latter interacts with methoxy groups on the same ring (Kubo and Kadla, 2005). Quantitatively acetylated lignins were analyzed with DMA to explore the impact of acetylating lignins with varying OH group compositions and to assess how acetylation compares to external plasticization in terms of efficiency for different lignin structures.

The reduction in T_g was very similar for all lignins, with approximately 50 °C (**figure 15**). This would indicate that it is not more efficient to substitute aliphatic over phenolic OH groups. This drop in T_g is similar to what a previous study has found for quantitative acetylation of softwood kraft lignin (Bouajila et al., 2006).

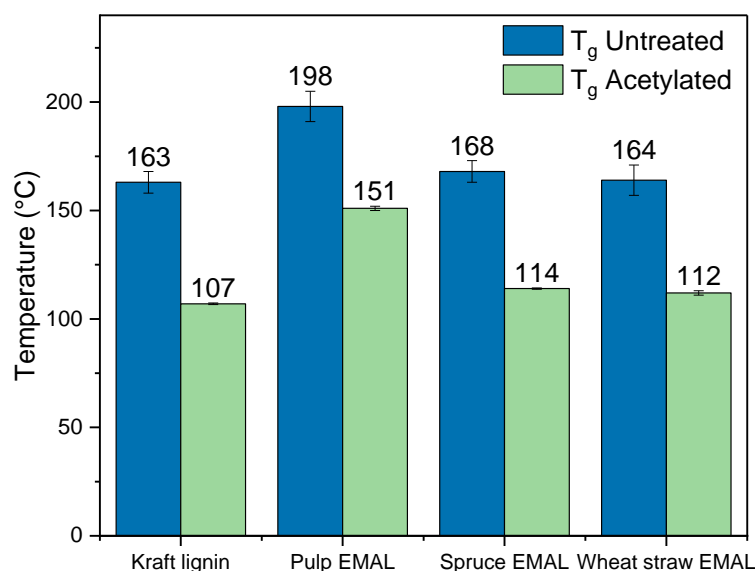


Figure 15. $T_{g,E'_{onset}}$ of untreated and acetylated lignins.

The mass increase for lignins with 8-10 mmol OH/g, that are fully acetylated, amounts to approximately 50%, which roughly translates to a 1 °C decrease in T_g for every wt%. In paper II, kraft lignin is plasticized to 50 wt% with TA, and the rate of the drop in T_g is 3.5 °C/wt%. The rate of decline in T_g for the other lignins at 20 wt% plasticizer, based on the values in **figure 13**, is 3-4 °C/wt%. Consequently, when comparing acetylation to external plasticization, the atomic efficiency of acetylation is notably low for all the lignins investigated in the study.

5. Conclusion and future remarks

This thesis set out to establish structure-property relationships for lignin that would increase the understanding of how different aspects of lignin structure and size interplay. The relationship between chemical structure, molecular weight and T_g could be spanned by isolating lignins of high yield from different biomasses and fractionating kraft lignin into molecular weight-groups. Both residual kraft pulp lignin and kraft lignins were found to have a lower apparent free volume than native lignins; thus, lignin structure is a strong determinant for T_g . However, the dynamic mechanical properties in the rubbery state were more influenced by molecular weight than chemical structure.

The second aim is related to how efficient modification of the viscoelastic properties of lignin can be achieved. The hypothesis that different lignin structures require different modifications to be efficiently plasticized was rejected. The compatibility, as examined with tan delta-curves and T_g reduction, was not different for different lignins with protic, aprotic, and aromatic additives. Similarly, quantitative acetylation reduced the T_g of the different lignins to the same extent, independent of the type of OH group that was substituted. This would suggest that the molecular structural similarities are of more importance than their differences in lignin plasticization.

The overreaching aim of this thesis is to increase the utilization potential of lignin. Two implications for the thermoformability of lignin and lignin-containing materials following from this work are that 1) depolymerization is an efficient method to improve the flow properties of lignin, but if followed by substantial condensation, the reduction in T_g will likely be limited, and 2) acetylating OH groups is not an efficient plasticizing treatment compared to adding free volume in the form of external plasticization.

An interesting aspect for future investigation would be how plasticization changes the ordering of lignin on the nano- and Ångström-scale. Dry lignin is often found

to form globular agglomerations, both in parts of the cell wall (Terashima et al., 2012), and when isolated (Vainio et al., 2004). The molecular ordering of lignin has also been suggested to depend on the interactions between aromatic rings (Li and Sarkanen, 2005; Ribca et al., 2023). X-ray scattering experiments could potentially probe the properties at these length scales, and studying plasticized lignins could aid both in the understanding of how plasticization is achieved, but also how lignin itself is ordered.

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Appendix 1. Method validation: powder sample holder for DMA

At 30 mg lignin sample loading, the powder pocket could reach 15 μm strain, before the force-maximum of the instrument of 18 N was exceeded. To test if these amplitudes were within the linear viscoelastic region, kraft lignin (KL) was subjected to an amplitude sweep at room temperature with a frequency of deformation of 1 Hz. The sweep was performed in two repetitions, and the curves can be seen in **figure A1**. The response is unchanged in the two sweeps, which implies viscoelastic linearity.

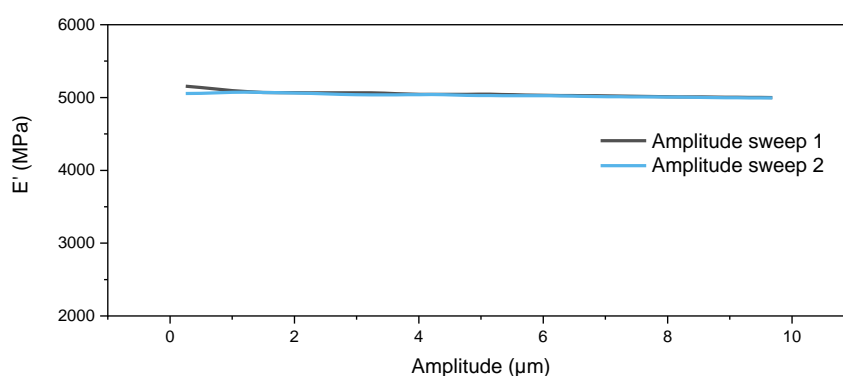


Figure A1. Amplitude sweeps of kraft lignin (30 mg) at room temperature.

To evaluate if the transition broadens in the powder sample holder due to friction between particles, a commercial poly(methyl methacrylate) (PMMA, Polyscience Inc.) was run both as a powder in the pocket and as a film (hot melt pressed) in tension mode (**figure A2**). The transition in the powder samples is about 7 $^{\circ}\text{C}$ broader in both $E'_{\text{onset}}-E'_{\text{endset}}$ and $\tan \delta_{\text{onset}}-\tan \delta_{\text{max}}$. It does not appear that friction between particles around the glass transition dissipates energy that broadens the transitions in a substantial way.

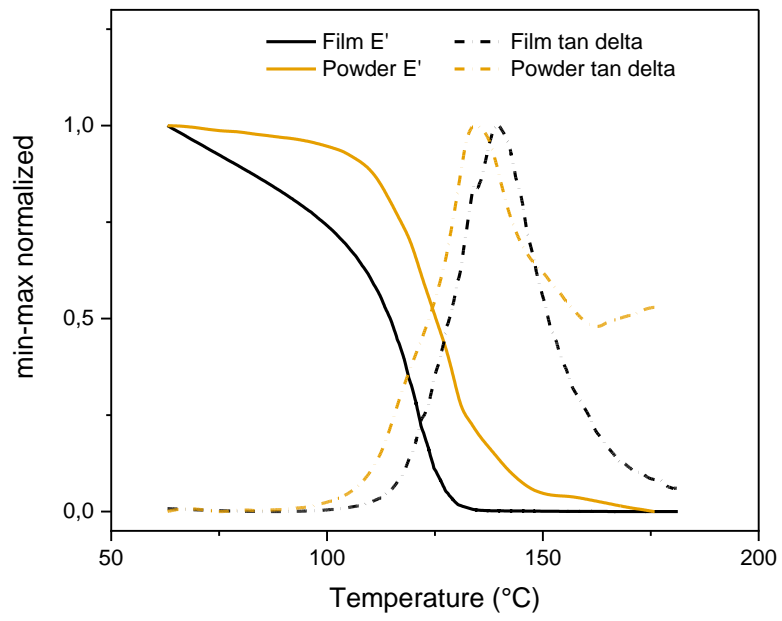


Figure A2. DMA thermogram of PMMA run as a film (tension) and as a powder (powder sample holder).

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