

Use of Renewable Alcohols in Autocatalytic Production of Aspen Organosolv Lignins

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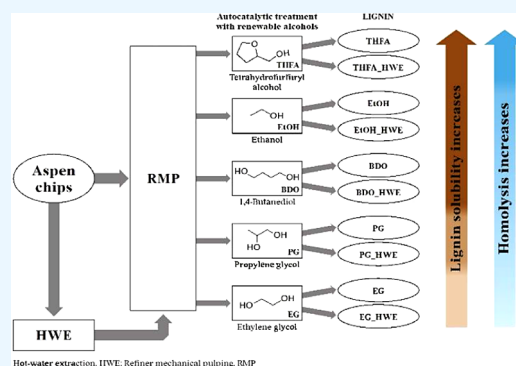
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ABSTRACT: This study aimed to investigate the intrinsic efficiency of renewable alcohols, applied under autocatalytic conditions, for removing lignin from aspen and hot-water-extracted aspen while substantially preserving the lignin structure so as to facilitate various valorization strategies. Ethylene glycol (EG), propylene glycol (PG), 1,4-butanediol (BDO), ethanol (EtOH), and tetrahydrofurfuryl alcohol (THFA) were evaluated based on their lignin solubilization ability, expressed as the relative energy difference (RED) following the principles of the Hansen solubility theory. The findings indicate that alcohols with a higher lignin solubilization potential lead to increased delignification, almost 90%, and produce a lignin with a higher content of β -O-4 bonds, up to 68% of those found in aspen milled wood lignin, thereby indicating their potential for valorization through depolymerization. However, these alcohols also produce lignin with a higher content of β - β and β -5 bonds, resulting in a higher molecular weight and polydispersity, due to readily occurring homolytic reactions. Hot-water extraction reduced the delignification efficiency and resulted in a lignin with a lower β -O-4 bond content. The lignins produced in these experiments exhibited a superior UV-A absorption capacity compared with synthetic benzophenone, as well as a greater radical quenching ability than synthetic butylated hydroxytoluene, indicating their potential for use in the protection of polymers against degradation.



1. INTRODUCTION

In conventional chemical pulping processes, the delignification process aims to efficiently remove lignin and preserve cellulose to produce high quality pulp for paper and/or dissolving pulp. Therefore, lignin is considered a byproduct. The significant changes that lignin undergoes during delignification are disregarded because lignin is burned in the process of chemical regeneration, such as in the kraft process, which is the dominant chemical pulping process today (a low-value use of lignin at \sim \$0.18/kg¹). Recently, the recovery of kraft lignin for higher-value applications has become more common through new isolation technologies, such as LignoBoost and Ligno-Force. Using these technologies, lignin may be recovered at modest amounts, 10–15% of the total lignin in the black liquor, without negative effects on the energy balance in the mill.² Kraft lignin, however, contains residual sulfur in various forms including thiol and thiirane groups in the amounts of 1.5–3%,^{1,3,4} The presence of sulfur limits a vast array of lignin applications ranging from conversion to lignin platform chemicals to the use in high-value therapeutic applications.^{1,5} Thus, various methods of desulfurization of kraft lignin have been proposed.⁴ Nonetheless, the valorization of kraft lignin is further hindered because of its complex, condensed structure. There is a broad scientific consensus that kraft delignification

results in lignin that is considerably different from the native lignin in wood, although its structure is still largely unknown,^{2,6,7} Lignin condensation resulting from the formation of C–C linkages is especially detrimental for conversion to lignin monomers using different depolymerization strategies (the value of lignin when converted to useful chemicals has been estimated at \$1.08/kg¹). The C–C bonds are characterized by a higher bond dissociation energy (BDE) than the aryl ether bonds; thus, the energy required to break bonds in condensed lignin increases (BDE, kcal/mol: α -O-4, 50–56; β -O-4, 54–72; β -1, 65–69; 4-O-5, 78–83; β - β , 81; β -5, 104–108; 5–5, 115–118^{8,9}). As a result, the yield of monomers produced during catalytic depolymerization of lignin has often been found to be directly correlated with the content of the β -ether bonds.^{9,10} Lignin condensation may also hinder functionalization strategies in lignin valorization by decreasing the number and/or accessibility of reactive sites.¹¹

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To facilitate valorization of kraft lignin, fractionation and purification steps have been proposed,¹² although they may involve the use of chlorinated solvents,¹³ thereby increasing the cost of lignin.

In emerging integrated biorefineries, all lignocellulosic constituents should be explored for valorization and as biogenic carbon candidates to substitute fossil carbon products, with the overarching goal of creating a “zero-waste” process.¹⁴ Therefore, less invasive alternatives to mature chemical pulping technologies should be developed to produce lignin with a better-preserved native structure. In this context, organosolv technologies have been considered promising for pretreatment^{15,16} and pulping,¹⁷ especially if conducted using renewable solvents produced by biomass conversion, potentially even at the same biorefinery site.¹⁸ Furthermore, compared with the kraft process, organosolv processes provide higher yields, release less malodorous volatiles, and result in inherently sulfur-free lignins.³ They have also been recognized as less capital intensive and more economical for small- and medium-sized enterprises;^{3,17} this aligns with forest restoration initiatives, while also promoting local development. Most importantly, organosolv lignin recovered from the spent liquor is relatively more pure and is characterized by lower polydispersity compared with kraft lignin.⁶ Despite these advantages, the well-known Alcell process, based on the use of aqueous ethanol (EtOH) as a low boiling point (LBP) solvent (boiling point temperature, T_{BP} 78 °C; temperature at flash point, T_{FP} 13 °C) without the addition of acid or alkali,¹⁹ still yields condensed lignin lacking the β -O-4 bonds that are dominant in native lignins [β -O-4 bonds/100 Ar, β -O-4, %: 5.3 in mixed hardwoods (HW) Alcell⁶ vs 60 in HW milled wood lignin (MWL) (average for MWLs isolated from 12 HW species)].²⁰ To produce lignin closer in structure to that of native lignin, with a higher content of the β -O-4 bonds, new strategies have been proposed, including cyclic and mild extractions. A cyclic accelerated solvent extraction produced spruce EtOH lignin with fully preserved β -O-4 bonds and a yield of 67% of original lignin in spruce.²¹ A mild extraction at reflux temperature and at 120 °C resulted in lignin yields of 28.7% and 73.9%, respectively, based on the original beech lignin. The β -O-4 content in the reflux EtOH lignin was an outstanding 82%, while the lignin produced at 120 °C contained 45% with frequent α -ethoxyl groups (\sim 78% of the total β -O-4 bonds were α -ethoxylated; 35 β' -O-4 bonds/100 Ar) but also contained 32% of condensed syringyl units (S_{cond}).²² Thus, seemingly conflicting goals related to maximizing delignification and producing lignin of high yield with a maximally preserved native structure have become imperative for developing novel sustainable organosolv delignification processes.^{21,23–25}

Compared to LBP solvents, high boiling point (HBP) solvents present some advantages that are mostly attributed to the low pressure generated during cooking, eliminating the need for costly high-pressure-resistant reactors, which are required for treatments with LBP solvents such as EtOH (\sim 18 bar at 180 °C in 50% EtOH).²⁶ The low volatility and lower flammability of HBP solvents offer additional advantages in terms of improved safety and reduced risk of explosion. Considering that HBP solvents consume more energy in the evaporation step during recovery,²⁷ other recovery operations should be explored, including membrane separation and adsorption.^{28,29} In the context of an emerging bioeconomy, renewable HBP solvents, potentially produced at the same

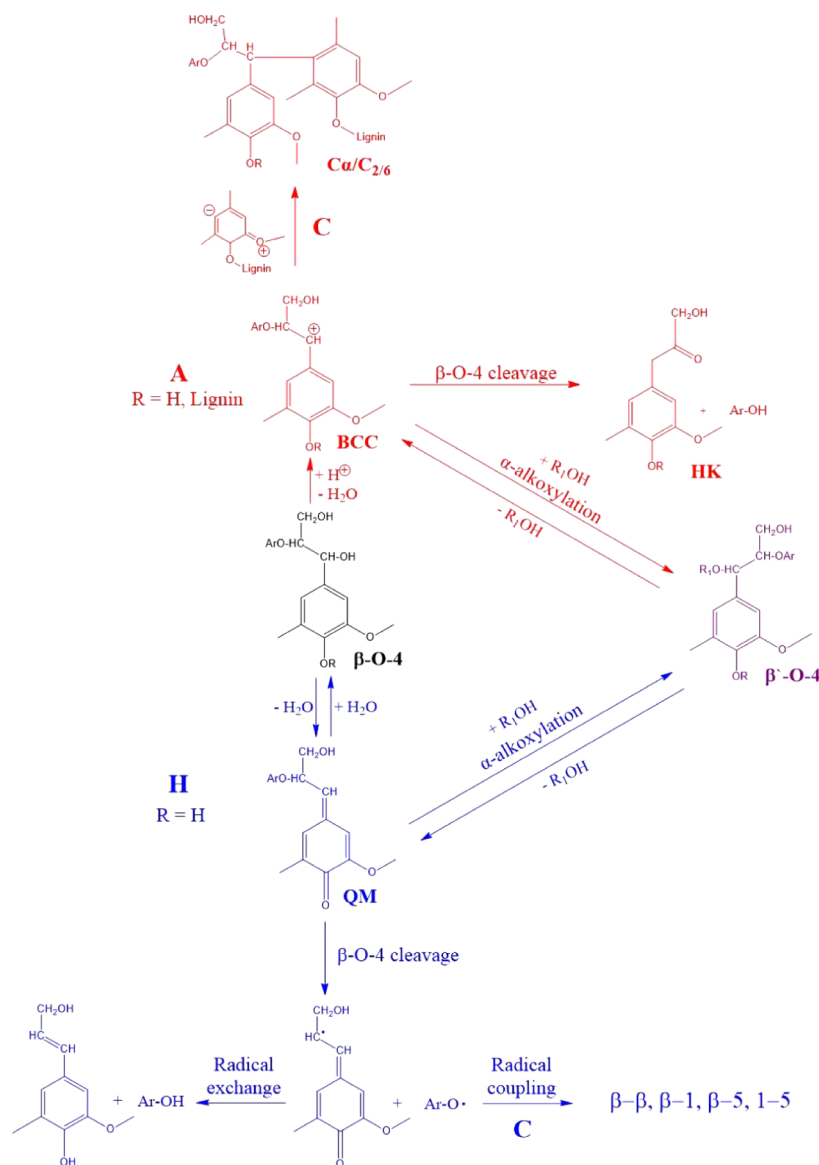
biorefinery site, would facilitate more sustainable and environmentally benign delignification processes.¹⁸ Furthermore, with delignification conducted under autocatalytic organosolv conditions, i.e., without the use of acid catalysts, corrosion would be less likely, thereby reducing reactor specifications and cost. As an additional benefit, eliminating acid catalysts would potentially require less complicated processes for the recovery and recycling of solvents.^{30,31} Although autocatalytic processes require higher temperatures,³² they result in higher pulp yields. In the presence of acid catalysts, pulp yields are inevitably decreased because glycosidic bonds, especially in hemicelluloses, are more readily cleaved and more polysaccharides are solubilized compared to autocatalytic reactions without catalysts.³³ Moreover, the native structure of lignin is better-preserved with autocatalytic processes because lignin condensation increases at higher severities in the presence of acid catalysts,^{9,11,16,34,35} It should be noted that different acids cause different effects, and condensation is more severe with H_2SO_4 than with HCl,²² although H_2SO_4 is the most widely used acid catalyst because of its high efficiency and tremendous synergy with organic solvents/ethanol.³⁶ Furthermore, lignin recovered from autocatalyzed organosolv delignification processes is more readily upgraded in valorization processes,^{16,26,30} Despite these benefits and positive experience by Repap Enterprises Inc., which produced high quality Alcell pulp and Alcell lignin (“golden lignin standard”³⁷) in autocatalytic aqueous EtOH pulping for several years in the 1990s,^{19,38} organosolv delignification and pretreatment studies have been conducted more frequently in acid-catalyzed conditions. For example, recent studies using eucalyptus have explored acid-catalyzed organosolv treatment with renewable alcohols, including EtOH, 1,4-butanediol (BDO), and ethylene glycol (EG) to produce lignin with a preserved structure. These studies demonstrated that an increase in acid charge and/or solvent concentration resulted in lignin condensation that advanced further in EtOH than in BDO treatment. In addition, 80% diols (BDO and EG) were found more effective than 80% EtOH in acid-catalyzed delignification at lower temperatures (120 °C), although all three solvents resulted in almost complete delignification at higher temperatures (170 °C, 1 h, 20 mM H_2SO_4). Lignin produced in these studies had slightly less than 24% β -O-4 bonds compared with \sim 43% β -O-4 bonds in the eucalyptus reference lignin (cellulolytic enzyme lignin, \sim 55% of the β -O-4 bonds preserved).^{23,39} However, the intrinsic effects of organic solvents without catalysts have been addressed only sporadically in studies using wood; e.g., autocatalyzed delignification of southwest birch (*B. alnoide*),³⁰ eucalyptus (*E. globulus*),^{31,40} and radiata pine (*P. radiata*)^{41,42} in EtOH, eucalyptus (*E. globulus*) in γ -valerolactone (GVL),³⁸ todo fir (*A. sachalinensis*) in BDO,^{43,44} and a softwood mixture in EG.³⁵ Additionally, a hybrid steam explosion/EtOH method has been explored in studies using silver birch (*B. pendula*). This method included autocatalytic and acid-catalyzed treatments and characterization of recovered lignins.^{45,46}

Solubility properties of solvents inherently regulate their ability to solubilize lignin in organosolv treatments. They may be interpreted through various empirical parameters, including the Hildebrand and Hansen solubility parameters.⁴⁷ The Hildebrand solubility parameter theory postulates that materials of similar solubility parameters, δ , interact more readily with each other resulting in their increased compatibility (δ , (cal cm⁻³)^{1/2}, (MPa)^{1/2} is equal to the square root of the cohesive energy). In the pioneering work on lignin

Table 1. Some Properties of the Select Renewable Alcohols, Organic Solvents (OS) and Lignin

lignin/OS	μ (mPa·s)	T_{BP} (°C)	T_{FP} (°C)	solubility parameter (MPa ^{1/2})				RED (R_u/R_o)		
				Hildebrand	Hansen			$R_u(R_o)$	OS	OS:water (6:4) ^a
					δ^*	δ_D	δ_P			
lignin ⁴⁹		---	---	31.1	21.9	14.1	16.9	(13.7)		
ethylene glycol (EG) ⁴⁷	16.0	197	111	33.0	17.0	11.0	26.0	13.7	1.00	1.40
propylene glycol (PG) ⁴⁷	42.0	188	99	30.2	16.8	9.4	23.3	12.9	0.94	1.32
1,4-butanediol (BDO) ³⁹	84.9	235	135	31.3	16.6	15.3	21.7	11.7	0.85	1.27
ethanol (EtOH) ⁴⁷	1.20	78	13	26.5	15.8	8.8	19.4	13.5	0.99	1.26
tetrahydrofurfuryl alcohol (THFA) ⁷⁶	6.24	178	74	23.5	17.8	8.2	12.9	10.9	0.80	0.95
water ⁷⁶	0.89	100	---	47.8	15.5	16.0	42.3	28.5	2.08	---

^aSolvent mixture⁷⁵: $\delta = \sum_i \phi_i \delta_i$; $\delta^* = (\delta_D^2 + \delta_P^2 + \delta_H^2)^{1/2}$.

Scheme 1. Acidolytic (A) and Homolytic (H) Reactions of the β -O-4 Structure in Organosolv Treatment Quinone Methide (QM); Benzyl Carbocation (BCC); Hibbert Ketone (HK); Formation of C–C Bonds, Condensation Reactions (C)^{1–9}

solubility, Schuerch demonstrated that the ability of a solvent to dissolve lignin improves with the hydrogen-bonding capacity of the solvent if they are characterized by the δ value of ~ 11 (cal cm⁻³)^{1/2} (~ 22.5 (MPa)^{1/2}). The addition of water to organic solvents that have a lower hydrogen bonding

capacity and a δ value of ≥ 9 (cal cm⁻³)^{1/2} (≥ 18.5 (MPa)^{1/2}) was shown to facilitate lignin solubilization by favorable changes of solvent properties, especially solubility parameters and hydrogen bonding capacity.⁴⁸ The Hansen solubility parameters were postulated later by dividing the Hildebrand

solubility parameter into three separate parameters to represent different interactions that are considered critical for dissolution: dispersion interaction forces (δ_D), dipolar interaction forces (δ_P), and hydrogen bond interaction forces (δ_H). These parameters can be organized as three coordinates in the Hansen space to provide a distinct 3D map of individual solvents and polymers and assist in elucidating their interactions. These are evaluated by taking into consideration two parameters that are essential to the Hansen space: (1) the distance between the solvent and the polymer, R_a ; $(R_a)^2 = 4(\delta_{D2} - \delta_{D1})^2 + (\delta_{P2} - \delta_{P1})^2 + (\delta_{H2} - \delta_{H1})^2$ and (2) the empirical radius of a sphere encircling the polymer that defines its favorable interactions, R_o ; solubility sphere. In this respect, the distance between good solvents and a polymer, R_w , should be shorter than the radius of the solubility sphere, R_o , of the polymer, i.e., the relative energy difference (RED), which is the ratio between R_a and R_o ($RED = R_a/R_o$), should be less than 1 ($RED < 1$). In other words, all solvents that are located inside the solubility sphere in the Hansen space are likely to solubilize the polymer. Accordingly, lignin solubility increases in solvents characterized by a lower RED, i.e., with a higher affinity toward lignin, they are better lignin solvents.^{33,49}

Both Hildebrand and Hansen solubility theories have been applied in the screening of organic solvents for use in delignification and in the evaluation of the delignification efficiency.^{32,39,50–54} By contrast, the effects of the solvent affinity toward lignin on the chemical structure and properties of the recovered lignin have not been extensively explored.^{55,56}

In this study, we examined the effects of renewable alcohols characterized by a relatively good affinity toward lignin (Table 1) on the autocatalytic organosolv delignification of bigtooth aspen (*Populus grandidentata*) and the properties of the recovered lignin. The selected HBP alcohols, EG, propylene glycol (PG), BDO, and tetrahydrofurfuryl alcohol (THFA), and LBP EtOH were used as 60% aqueous solutions.^{19,30} Notably, in addition to facilitating effective lignin solubilization, a critical benefit of using alcohols for delignification is their ability to capture electrophilic benzyl carbon atoms, including benzyl carbocations (BCC) and C_α on quinone methides (QM), generated from acidolytic or homolytic dehydration of lignin units, respectively, by acting as potent nucleophiles.^{16,23,25,30,43,44,57–59} The structure formed by benzyl or α -alkoxylation is commonly designated as the β -O-4 structure (Scheme 1). α -alkoxylation is considered vital in mitigating lignin condensation by impeding the formation of lignin–lignin C–C bonds in the reaction between BCCs and electron-rich carbons of lignin aromatic rings, *ortho* or *para* to methoxy groups, $C_{2/6}$. Moreover, it has been suggested that α -alkoxylation stabilizes the β -O-4 aryl ether linkages against cleavage (benzyl ethers are less reactive than unprotected benzyl alcohols) and improves lignin solubility in alcohols.^{16,21,39} We hypothesized that the synergy between the excellent lignin solubilization properties of these alcohols and their proficiency in α -alkoxylation would result in efficient delignification and the recovery of lignin, preserving its native structure, and achieving high yields. In this study, we also examined the effects of organosolv pulping on aspen that had been subjected to hot water extraction (HWE); aspen_HWE. HWE (160 °C, 2 h, P-factor 540 h) is a mild hydrothermal pretreatment (HTP), which is performed to remove most of the hemicelluloses and reduce the recalcitrance of lignocelluloses.⁶⁰ Hydrothermally pretreated wood might undergo delignification more readily than untreated wood and produce

lignin of higher purity because HTP results in a relatively disrupted wood structure of higher porosity and a likely deconstructed lignin-carbohydrate complex.^{21,37,60–62} This has been demonstrated in studies of hydrothermally treated birch in which up to ~86% of the original lignin was extracted in acetone/water (9:1, v/v) at room temperature with yields dependent on the severity of HTP.³⁷ It has also been documented that delignification of sugar maple in acetone–water–oxygen (150 °C, 2 h) notably improves after HWE.⁶³ Moreover, the purity of MWL isolated from sugar maple improved after HWE.⁶⁴ In studies on softwoods (radiata pine), the delignification during autocatalytic EtOH treatment improved after a low-severity HTP, in contrast to being hindered by the increase in severity of the HTP.⁴¹ The delignification inhibition has been attributed to the increase in lignin condensation during HTP,⁴¹ underscoring a need to adjust the conditions of HTP to minimize negative effects. Nevertheless, it is important to note that carbohydrates and carbohydrate degradation products removed during HTP (mostly xylan-based) have been extensively investigated as the source of added value for wood-based biorefineries following separation and biological and/or chemical conversion.⁶⁵ In this context, a two-step sequential mild HTP and organosolv treatment would be in line with the goals of “zero-waste” processes,¹⁴ focused on the efficient use of all lignocellulosic/wood constituents, sometimes labeled as “Lignin-First”³⁷ or “Consolidated Lignin”²¹ biorefineries.

After organosolv treatment and lignin isolation, aspen organosolv lignins (OSL and OSL_HWE lignins from aspen and from hot-water-extracted aspen, respectively) were characterized with respect to their purity and structure. In addition, we investigated the UV-absorption, radical quenching, and glass transition temperatures of isolated lignins as key properties in novel lignin applications of recent interest (e.g., sunscreens, stabilizers, packaging applications).⁶⁶

2. EXPERIMENTAL SECTION

2.1. Pulp Preparation and Pretreatment. Bigtooth aspen (*Populus grandidentata*) chips were produced for earlier studies⁶⁷ and stored in frozen storage at the USDA Forest Service, Forest Products Laboratory (FPL), Madison, WI. Hot-water extraction of aspen chips was performed at 160 °C for 2 h at a low P-factor⁶⁰ (eq 1) of 555 h (including the heating ramp). Aspen chips, 2 kg, and 10 L water were placed in a 20 L stainless steel pressure digester equipped with steam jacket, circulating pump and heat exchanger. The chips and liquor were heated to 160 °C over 20 min and the temperature was maintained for 2 h. Hydrolysate (5.8 L) was removed at temperature through a pressurized cooling coil. The hydrolyzed chips were washed twice by circulating with 15 L water at 100 °C for 30 min.

$$P\text{-factor} = \int_0^{t, \text{hr}} e^{(40.48 - \frac{15,106}{T, K})} dt \quad (1)$$

These chips were refined at 60 °C with a 30.5 cm atmospheric disc refiner operating with a gap of 125 μm . The resulting pulp was press filtered, fluffed, and placed in cold storage (1.38 kg dry weight, 68.8% yield). The hydrolysate pH was reduced from 3.5 to 2 using H_2SO_4 to precipitate lignin that was isolated by centrifugation and freeze-dried (32.5 g, 1.6% yield). Aspen refiner mechanical pulp (mechanical pulp) was prepared for organosolv by two-pass refining of aspen

using hot-water at 60 °C with a 30.5 cm atmospheric disc refiner operating with a gap of 1.25 mm followed by 125 μm. The resulting pulp was press filtered, fluffed, and placed in cold storage. The chemical composition of aspen and aspen_HWE mechanical pulps was then analyzed.

2.2. Organosolv Delignification. Organosolv treatment of aspen and aspen_HWE mechanical pulps was conducted using alcohols EG, PG, BDO, EtOH and THFA. Typically, 36 g (oven-dry (OD) equivalent) was placed in a 2 L Parr reactor equipped with a tapered-screw mixer. Alcohol and water were added such that the treatment mixture had a ratio of 6:4 alcohol:water and a total volume of 1.2 L. This was heated to 195 °C over a period of 1 h, held at temperature for the treatment time of 2 h, and cooled rapidly in a water bath. The treatment H-factor⁶⁸ was 14,100 h (including heating ramp); calculated according to eq 2. The treated pulp was filtered to a wet mat that was initially washed via flow of an additional 180 mL of 6:4 alcohol:water mixture pulled through the pulp mat with vacuum. The treatment liquor pH was measured at room temperature and set aside for lignin isolation. The remaining pulp was then washed thoroughly with deionized water, pressed, and freeze-dried to determine pulp treatment yield.

$$H\text{-factor} = \int_0^{t,\text{hr}} e^{(43.2 - \frac{16,117}{T,K})} dt \quad (2)$$

2.3. Lignin Isolation. Organosolv lignin was precipitated from the treatment liquors by dilution into nine equivalents of water that had been previously acidified to pH 2 using H₂SO₄. The precipitated lignin was allowed to settle overnight, the supernatant decanted, and the remaining lignin suspension concentrated by centrifugation for 15 min at 5000 G. The lignin was further washed multiple times by suspending in water that was acidified to pH 2 with H₂SO₄ followed by centrifugation. The lignin was then freeze-dried to determine yield and store.

Aspen MWL was prepared from dry, ground, acetone/water (9/1, v/v) extracted wood. The wood was milled using a vibratory stainless steel custom-made ball-mill over 5 h for a total milling time of 150 min with intermittent cooling periods. The wood flour was then extracted once with 100% 1,4-dioxane then twice with 96% dioxane/water and the combined extracts were dried to yield MWL amounting to 15% based on the wood lignin content.⁶⁹

2.4. Chemical Composition of Pulps and Lignin. The chemical composition of aspen and aspen_HWE mechanical pulps, organosolv pulps and OSL and OSL_HWE lignins was determined by conducting the conventional two-step acid hydrolysis (H₂SO₄; 1. 72% (v/v) at 30 °C for 1 h; 2. 3.6% (v/v) at 120 °C for 1 h) followed by the determination of monosaccharides in the hydrolysate using the high-performance anion exchange chromatographic method with pulsed amperometric detection (HPAEC/PAD) in a Dionex ICS-3000 system, equipped with a CarboPac PA1 analytical and guard columns at 20 °C.⁷⁰ The content of polysaccharides was calculated from the amount of corresponding monosaccharides using anhydro corrections factors; 0.88 for pentoses (C₅ sugars; 132/150) and 0.90 for hexoses (C₆ sugars; 162/180). The total lignin content was calculated as a sum of the acid-insoluble lignin determined gravimetrically and the acid-soluble lignin determined by measuring the absorbance at the wavelength of 205 nm (spectrophotometer Genesys 180 UV-vis) and using 110 L(g cm)⁻¹ absorption coefficient.⁷¹

2.5. Lignin Acetylation. Acetylation of MWL and organosolv lignins (100 mg) was by treatment with a 1/1 mixture of acetic anhydride/pyridine (2 mL) at room temperature overnight. The material was dried to constant weight by coevaporation on a rotary evaporator with toluene followed by toluene/acetone mixes and finally acetone. The light brown foam was further dried in a vacuum oven at 40 °C.⁶⁹

2.6. NMR Spectral Analysis. A Bruker BioSpin AVANCE III 500 MHz NMR spectrometer fitted with a 5 mm, z-gradient Prodigy TCI cryoprobe was used to acquire spectra at 298 K. 2D ¹³C–¹H short-range correlation (heteronuclear single-quantum coherence, HSQC) experiments used the Bruker standard pulse program hsqcetetgpcisp2.3. Spectra were acquired using 30 scans and 1 s interscan delay for a total time of 145 min, with 12 ppm sweep width (SW) in F2 (1H) using 1024 data points for an acquisition time of 85 ms and 215 ppm of SW in F1 (¹³C) using 512 increments with 50% NUS sampling density. The central acetone solvent peak was referenced at δ ¹³C, 29.83 ppm; δ ¹H, 2.04 ppm. Samples for 2D NMR were prepared using 70.0 mg of acetylated lignin dissolved in a stock solution of deuterated acetone containing methyl ethyl ketone such that each sample contained 2.0 mg of internal standard (IS).

2D ¹³C–¹H long-range correlation (heteronuclear multiple bond coherence, HMBC) spectra were recorded using the standard Bruker hmbcetgpl3nd pulse program. Spectra were acquired for 60 scans with 30% NUS sampling density for a total experiment time of 3.25 h. An 83 ms delay was used for the evolution of long-range couplings with a 1 s interscan delay. The same SWs used were as for the HSQC as well as the solvent reference.

Quantitative ¹³C spectra were obtained using the Bruker standard pulse program zgpg with D1 of 15 and 4200 scans for a run time of 18 h. The central CDCl₃ peak was referenced at δ ¹³C, 77.00 ppm; δ ¹H, 7.24 ppm. Baseline minima were used to integrate the phenolic, secondary, and primary carbonyl regions against the acetylated pentachlorophenol internal standard peak at 166.5 ppm. Samples containing 70.0 mg of the acetylated lignins were prepared with a stock solution of CDCl₃ containing acetylated pentachlorophenol such that each sample contained 4.0 mg of IS.

2.7. Molecular-Weight Distribution. Analytical GPC (Gel Permeation Chromatography) was performed on a Shimadzu Prominence LC20 with a photodiode array detector (SPD-M20A). Separation was performed using a PSS PolarSil Linear S column (7.8 mm ID × 30 cm L × 30 μm particle size) in a 50 °C oven, with 0.1 M lithium bromide (LiBr) in *N,N*-dimethylformamide (DMF) flowing at 1.0 mL min⁻¹ for 20 min. The samples were prepared as 0.33 mg mL⁻¹ solution of lignin in 0.1 M LiBr in DMF. The molecular weight distribution was calibrated at λ = 270 nm using PSS Polystyrene ReadyCal Standard Set *M*(p) 370–2 520 000 Da (P/N PSS-pskitr4; PSS-Polymer Standards Service, Amherst, MA, USA) and acetovanillone (166 Da). Aspen MWL, OSL, and OSL_HWE lignins and hardwood kraft lignin (HW kraft) from the FPL collection of technical lignins were analyzed.

2.8. UV-Absorption. Lignin and reference compounds, ferulic acid, quercetin, coniferaldehyde, sinapaldehyde, and benzophenone, were dissolved in 9:1 dioxane:water (500 mg/mL) then diluted to a concentration of 10 mg/mL. Absorption

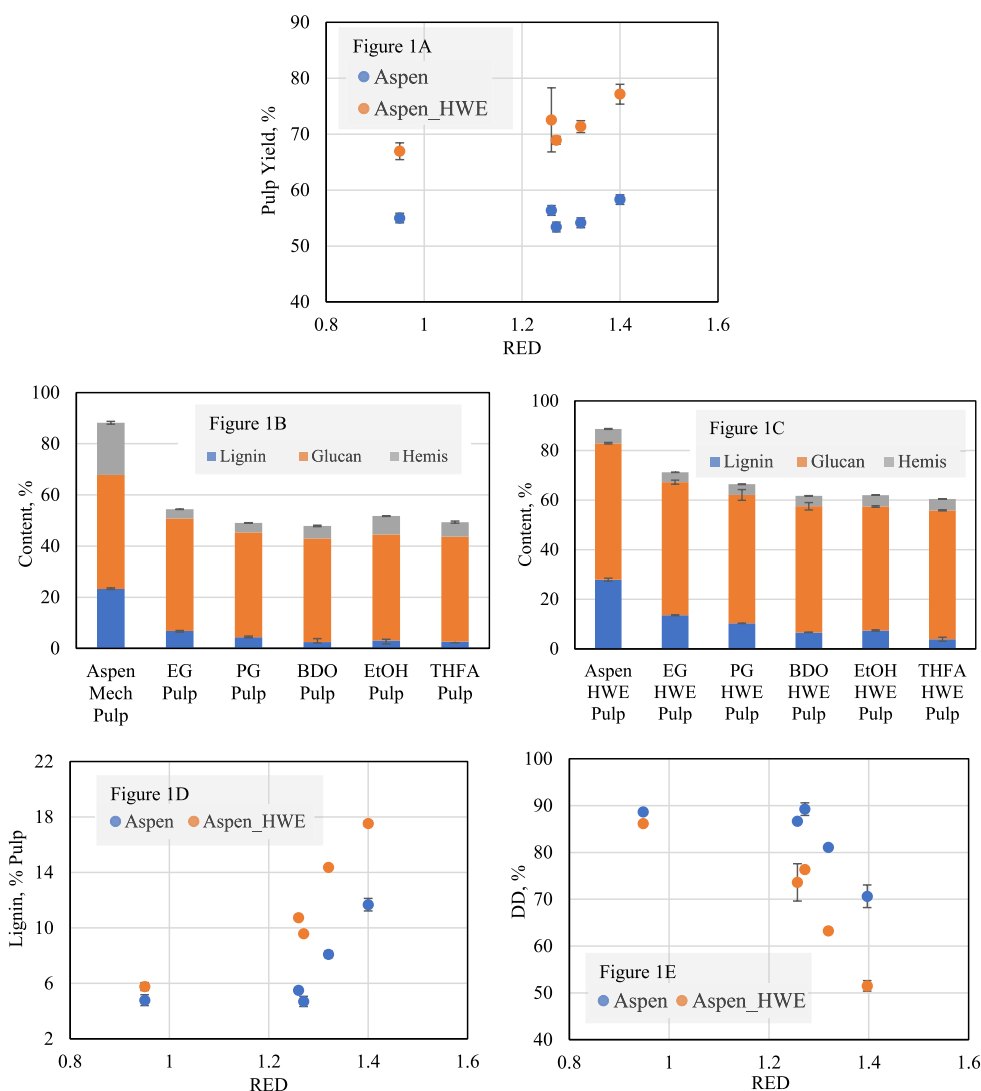


Figure 1. Organosolv delignification results. (A) Yield from Aspen pulp or Aspen_HWE pulp; (B) chemical composition of Aspen organosolv pulps; (C) chemical composition of Aspen_HWE organosolv pulps; (D) lignin content of oven-dry Aspen and Aspen_HWE organosolv pulps; (E) delignification degree⁶⁰ (eq 5) in Aspen and Aspen_HWE organosolv treatments.

spectra in the range from 220 to 400 nm were acquired using a UV-1900i UV-vis spectrometer (Shimadzu, Japan).

The cumulative absorption within the UV-A and UV-B regions was determined using eqs 3 and 4, respectively, where $A(\lambda)$ represents the absorbance at wavelength λ . The reported results represent the average of three measurements.

$$\text{UV-A} = \int_{315\text{nm}}^{400\text{nm}} A(\lambda)d\lambda \quad (3)$$

$$\text{UV-B} = \int_{280\text{nm}}^{315\text{nm}} A(\lambda)d\lambda \quad (4)$$

2.9. Radical Quenching Activity. Radical quenching ability of lignins was measured following a colorimetric assay using DPPH (2,2-diphenyl-1-picrylhydrazyl) as the free radical generator.⁷² IC_{50} , $\mu\text{g/mL}$, i.e., the concentration required to reduce or “inhibit” 50% of the DPPH was calculated. Natural antioxidizing compounds, ferulic acid and quercetin, and a typical synthetic antioxidizing agent, butylated hydroxytoluene (BHT) were used as reference compounds in these experiments.

2.10. Glass Transition Temperature (T_g). The glass transition temperatures (T_g 's) of the lignins were determined by temperature-modulated differential scanning calorimetry (TM-DSC) using a Q20 Differential Scanning Calorimeter (TA Instruments; New Castle, DE). In TM-DSC, the total heat flow curve can be separated into heat flows from reversible and nonreversible processes by overlaying a modulated temperature regiment onto the temperature ramp.⁷³ Consequently, irreversible processes such as enthalpic relaxation (commonly present in lignin powders) are removed from the total heat flow curve. This allows the T_g to be more accurately characterized from the remaining reversible heat flow curve.⁷⁴ A modulation amplitude and period of 1 °C and 60 s, respectively, and a ramp rate of 3 °C/min were used. HW kraft lignin was used as a reference lignin in TM-DSC studies.

3. RESULTS AND DISCUSSION

3.1. Delignification Results. Organic solvents were utilized in these investigations as 60% aqueous solutions. These included renewable alcohols, linear, LBP EtOH and ring-containing HBP THFA of lower viscosities, as well as

HBP diols with higher viscosities, linear EG and BDO, and bulky PG. Corresponding RED values these solvent/water mixtures ranged from 0.948 to 1.397⁷⁵ as detailed in Table 1,^{39,49,76} the pulp yields were found to depend on the solvent, with lower average yields observed for aspen pulps [% OD aspen/aspen_HWE: Aspen_Pulp_{av} = 55.4 (standard deviation, SD 1.938) vs Aspen_HWE_Pulp_{av} = 71.4 (SD 3.887)] (Figure 1a). The chemical composition relative to those of aspen and aspen_HWE mechanical pulps can be seen in Figure 1b,c, respectively. Treatments using alcohols with lower RED produced pulps with less residual lignin, demonstrating the superior performance of better lignin solvents; aspen pulps produced using BDO, EtOH, and THFA contained on average 5.0% lignin (SD 0.439). Less potent lignin solvents, PG and EG produced pulps with higher residual lignin contents of 8.1% and 11.7%, respectively (Figure 1d).

We compared the results of these experiments, conducted at 195 °C for 2 h (H-factor = 14100), with those from similar experiments reported in the literature. For example, an autocatalytic 50% EtOH treatment of *E. globulus* chips at a similar severity (200 °C, 75 min, H-factor 13,400 h) yielded pulp containing 6.8% Klason lignin.⁴⁰ This is approximately 30% higher than the average lignin content in BDO/EtOH/THFA aspen pulps, demonstrating a lower delignification efficiency of alcohols with lower affinity toward lignin (50% EtOH, RED 1.37 vs RED < 1.3 for BDO/EtOH/THFA). In contrast, the autocatalytic 60% EtOH (RED 1.26) treatment of ground birch (*B. pendula*) at a much lower severity (200 °C, 15 min, H-factor 2,360 h) generated solids containing 9.1% lignin. Following the explosive discharge, the lignin content decreased to 7.8%⁴⁵ or just below the content of lignin in aspen PG pulp (RED 1.32; lignin content 8.1%). It should be noted, however, that autocatalytic EtOH treatment has shown a variable delignification extent, even at higher severities. Delignification efficiency depends strongly on wood species and preparation of wood, including particle size and pretreatment/preextraction; e.g., autocatalytic 60% EtOH studies conducted at a high severity (200 °C, 2 h, H-factor 18,850 h) using toluene-ethanol (2:1, v/v) preextracted ground birch (*B. alnoide*) (0.70–0.35 mm) produced pulp containing a comparatively high content of residual lignin of 14.2%.³⁰

The aspen_HWE pulps consistently contained more lignin than the corresponding aspen pulps (Figure 1d). The observed differences in the residual lignin content are in line with differences in delignification degrees (Figure 1e). A relatively less efficient lignin removal from aspen_HWE may be related to the intrinsic negative effects consistent with the more condensed lignin containing fewer β-O-4 bonds in the wood after HWE.^{41,61,77,78} Despite being performed at a relatively low severity represented by P-factor <600 h⁶⁰, these negative effects exceeded potential positive effects of HWE related to the increase in the content of free phenolic hydroxyl (PhOH) groups, porosity, and the cleavage of lignin-carbohydrate bonds.^{21,37,60,62,63,78} Nevertheless, better lignin solvents were more efficient in the delignification of aspen_HWE; specifically, THFA (RED 0.95) produced only a slightly higher DD on aspen than on aspen_HWE (DD, 88.6% for aspen vs 86.2% for aspen_HWE), in contrast to EG (RED 1.40), which was substantially more efficient in delignification of aspen than of aspen_HWE (DD, 70.6% for aspen vs 51.5% for aspen_HWE).

In comparison, earlier studies have reported a less efficient delignification in less severe autocatalytic treatment of

eucalyptus (*E. grandis* × *E. urophylla*), 50% DD, using 50% EtOH (180 °C, 60 min, H-factor 2,100 h, RED 1.37),⁷⁹ and birch, 80.5% DD, using 60% EtOH (200 °C, 30 min, H-factor 4,700 h) in combination with the explosive discharge.⁴⁵ It is interesting that 65% BDO (RED 1.21) treatment of poplar sawdust (170 °C, 1 h, H-factor 940 h) resulted in a relatively low DD of 22.2%,²⁹ indicating that autocatalytic organosolv processes require higher temperatures/severities to advance lignin removal.³² In these studies, 90% DD has been achieved using an acid catalyst (50 mM HCl); surprisingly, 65% EtOH with a similar lignin solubility potential (RED 1.21) resulted in only 66.5% DD under the same conditions.²⁹ In contrast to these results, 60% GVL with a higher affinity toward lignin (RED 0.73) produced 95% DD in autocatalytic treatment of *E. globulus* at a relatively modest severity (180 °C, 3 h, H-factor 6,300 h) underscoring the benefits of a good lignin solvent³⁸ (GVL δ (MPa^{1/2}): δ_D = 19, δ_P = 16.6, δ_H = 7.4).⁵⁶

$$\text{DD, \%} = \frac{\text{lignin removed, g}}{\text{lignin in pulp before treatment, g}} * 100\% \quad (5)$$

3.2. Lignin Yield. Based on the amount of wood, the average yields of organosolv lignin recovered from the spent liquors of aspen (OSL lignins) and aspen_HWE (OSL_HWE lignins) were comparable [% OD aspen/aspen_HWE, OSL_{av}, 12.9 (SD 0.675) vs OSL_HWE_{av}, 13.3 (SD 3.963)] (Figure 2a). Based on the amount of lignin, a higher average lignin yield was observed for aspen [% lignin in aspen/aspen_HWE, OSL_{av}, 56.0 (SD 2.90) vs OSL_HWE_{av}, 48.0 (SD 14.2)] (Figure 2b,c), probably because of the relative increase in lignin content in the wood after HWE, showing an increase of approximately 20% (lignin content, % OD wood, aspen_HWE, 27.9 (SD 0.583) vs aspen, 23.2 (SD 0.136)). The yield of lignins, based on the lignin removed from aspen and aspen_HWE wood, was approximately equal [67% lignin removed from aspen/aspen_HWE] (Figure 2b,c). The average yield of BDO, EtOH, and THFA lignins was ~57% (SD 1.221) based on lignin in wood and ~65% (SD 0.486) based on lignin removed (DD_{av} = 88.2%). Notably, a higher lignin yield of 63.4% has been achieved in autocatalytic treatment of *E. globulus* in 50% EtOH (RED 1.37) at higher severity (200 °C, 75 min, H-factor 13,400 h).³¹ In contrast, lower lignin yields have been obtained in autocatalytic birch EtOH and eucalyptus GVL studies; 50% to 60% of the extracted lignin was isolated from these spent liquors.^{30,38} Differences in lignin recovery yields among these studies and the literature stem from variations in lignin precipitation methods. For instance, unlike the precipitation method used in our study involving water dilution (10 times) and acidification (with H₂SO₄ to pH 2), GVL lignin was precipitated solely by water dilution (six times).³⁸

3.3. Lignin Chemical Composition. The chemical composition of lignins is presented in Figure 3a,b. Consistent with organosolv lignins renowned for their high purity,⁶ these lignins exhibited high lignin content, with the average lignin content ranging from 95.4% (SD 1.200) for OSL lignins to 95.8% (SD 1.264) for OSL_HWE lignins, and negligible ash content, averaging 0.10% (SD 0.072) and 0.14% (SD 0.123), respectively. These findings align with previous studies reporting <0.1% ash in EtOH lignins.⁶ Moreover, the lignins contained small amounts of carbohydrates, indicating cleavage of linkages between lignin and carbohydrates,^{30,80} probably benzyl ether bonds.⁴⁴ Notably, OSL_HWE lignins retained

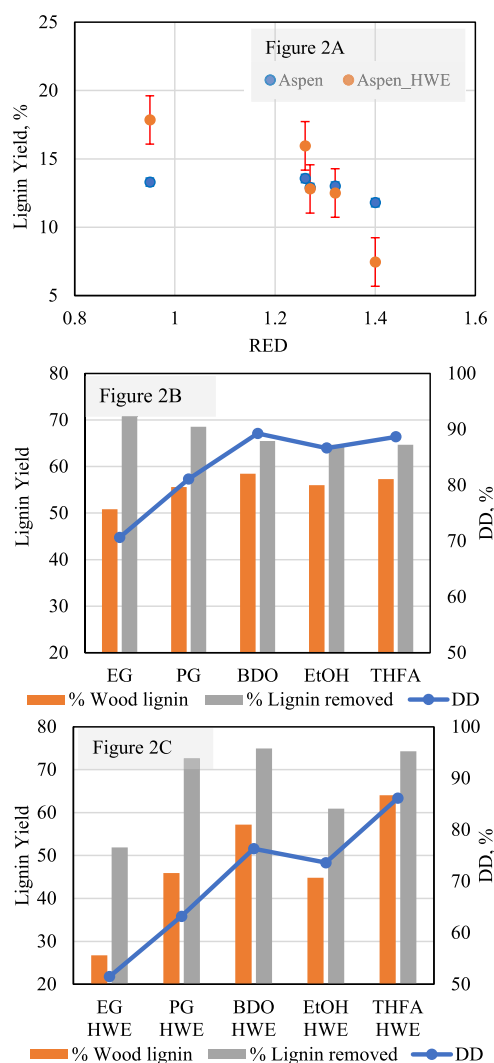


Figure 2. Lignin Yield. (A) OSL/OSL_HWE lignins: Lignin yield in % of OD aspen/aspen_HWE vs RED; (B) OSL lignins: Lignin yield in % of lignin in aspen and in % of aspen lignin removed vs DD, %; (C) OSL_HWE lignins: Lignin yield in % of lignin in aspen_HWE and in % of aspen_HWE lignin removed vs DD, %.

fewer carbohydrates than their OSL counterparts (polysaccharide content, 0.15% (SD 0.044) for OSL_HWE_{av}, 0.34% (SD 0.238) for OSL_{av}). This underscores the beneficial impact of HWE on lignin purity, as HWE led to the removal of approximately 80% of xylans and probably weakened the LCC.^{21,37,62} The remaining carbohydrate fraction in lignins primarily comprised xylans and glucans, with xylans being dominant. Additionally, the significant reduction in xylans during HWE is evidenced by the decline in the xylan-to-glucan ratio in lignins from 3.8 for OSL_{av} to 0.9 for OSL_HWE_{av} lignins (Figure 3c,d).

3.4. Characterization of Lignins: HSQC. The 2D ¹H-¹³C NMR HSQC analysis was conducted to elucidate the structural characteristics of OSL and their counterpart OSL_HWE lignins and to compare them with aspen MWL. The HSQC spectra illustrating the aliphatic/side chain and aromatic regions of MWL, BDO, and BDO_HWE lignins are presented in Figure 4A–C. The HSQC spectra of other lignins can be found in Figure S1:1–8. Assignments of cross-peaks, based on the chemical shifts of lignin model compounds,^{81,82} and the results of semiquantitative analysis of various lignin units and

structures are provided in Table S1, along with corresponding equations.

3.4.1. Lignin Bonds and Units: β -O-4 Bonds. It is hypothesized that the cleavage of the β -aryl ether linkages during organosolv treatment, as evidenced by the increase in the content of PhOH groups in the residual lignin,⁴³ occurs through two primary competing mechanisms: the acidolytic mechanism, prevalent in the presence of acid catalysts, and the homolytic mechanism, dominant under autocatalytic conditions^{58,83} (Scheme 1). The presence of various guaiacol- and syringyl-type homobenzaldehydes and arylpropanones, known as Hibbert's ketones (HKs) (HKs are typically represented by 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)- and 3-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)- 2-propanone) in the organosolv liquor and/or of lignin-bound HKs in organosolv lignin suggests the occurrence of the acidolytic mechanism initiated by the formation of BCCs. The BCCs also undergo reactions with electron-rich aromatic carbons, forming C _{α} -C_{2/6} condensed lignin units,^{16,58,59,84,85} Conversely, the homolytic, radical mechanism, limited to phenolic β -aryl ethers, progresses via QM intermediates. These intermediates homolytically split to form phenoxy radicals, which then undergo radical transfer and recombination, resulting in typical homolytic products, including coniferyl alcohol in organosolv liquor and newly formed C–C bonds, such as β -1, β -5, and β - β bonds in organosolv lignin (Scheme 1).^{44,58,83,86} A notable decrease in the content of the β -aryl ether linkages and/or an increase in the content of C–C bonds in organosolv lignin indicate significant alterations in the native lignin structure, which are essential for determining lignin valorization potential⁴⁶

The presence of β -aryl ether linkages was confirmed in all OSL and OSL_HWE lignins based on the typical C/H correlation signals observed in the side-chain region of the spectra (Figures 4, S1:1–8 and Table S1). The effect of RED on the content of β -aryl ether structures is illustrated in Figure 5a (Table S1).

The OSL_HWE lignins exhibit a lower content of β -O-4 linkages compared with their respective OSL counterparts, except for EG_HWE lignin, which displays a higher content of β -O-4 linkages than EG lignin (β -O-4 content: 19.2% for EG_HWE, 13.7% for EG, Δ 28.6%). However, the EG treatment of aspen_HWE results in less effective delignification (EG DD: 51.5% for aspen_HWE vs 70.6% for aspen, Δ 27.1%), suggesting that a readily accessible fraction of lignin in aspen_HWE is also enriched in the β -O-4 linkages. Nevertheless, the reduced retention of β -O-4 bonds in most OSL_HWE lignins aligns with the cleavage of these bonds during HWE.^{37,61,63,78} Better lignin solvents yielded lignin with more β -O-4 linkages (Figure 5a).

Our results suggest that among this series of renewable alcohols, those leading to higher lignin removal also produce lignin with better preserved β -aryl ether bonds (Figure 5b,c). THFA, exhibiting the highest affinity for lignin, results in a high DD (THFA DD: 88.6% for aspen, 86.2% for aspen_HWE (Figures 5b and 1e)), yielding more than half of the total lignin in wood (57.3% for THFA and 64.0% for THFA_HWE (Figures 2b,c and 5c)) with relatively well-preserved β -O-4 linkages (β -O-4:36.9% for THFA, 25.5% for THFA_HWE, i.e., 68.8% and 47.5% of the β -O-4 linkages present in MWL, respectively).

In contrast to our findings, the autocatalytic GVL treatment of *E. globulus* wood chips using 50% to 60% GVL (RED 0.91/

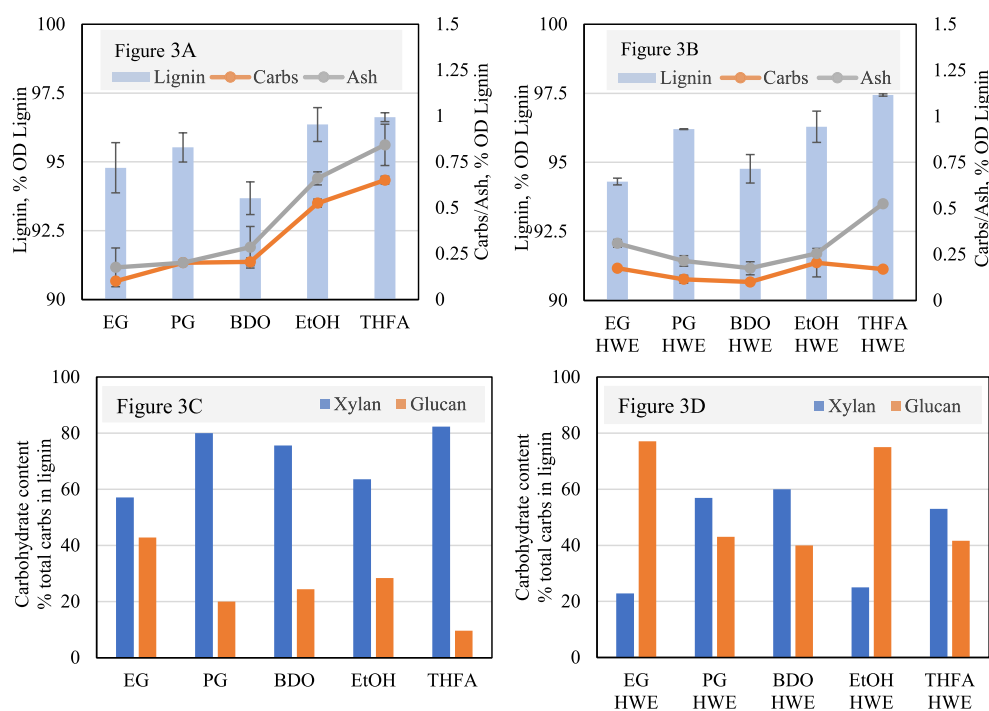


Figure 3. Chemical Composition of Lignins. (A) OSL lignins: Lignin, carbohydrates, and ash content; (B) OSL_HWE Lignins: Lignin, carbohydrates, and ash content; (C) OSL lignins: Xylan and glucan content in the remaining carbohydrates; (D) OSL_HWE lignins: Xylan and glucan content in the remaining carbohydrates.

0.73) (180 °C, 2 h, H-factor 4,200) resulted in lignin containing 12% β -O-4 linkages; 60% of the dissolved lignin was recovered.³⁸ In contrast, acid-catalyzed GVL investigations using hybrid poplar wood chips (90% GVL (RED 0.67), 85 mM H₂SO₄, 120 °C, 1 h) reported a recovery of ~47% of the original lignin containing 32.6% of β -O-4 linkages, representing ~47.3% of the original β -O-4 linkages.²⁴ Further, a 95% EtOH (RED 1.00) treatment (0.2 M HCl, 110 °C, 6 h) of beech produced a lignin containing 43% β -O-4 linkages, with the yield of 42.4% of the original lignin.¹⁶ Additionally, experiments on eucalyptus using EtOH, BDO, and EG under the same conditions (80% alcohol, 100 mM H₂SO₄, 120 °C, 4 h) resulted in the recovery of ~80% of the removed lignin.²³ Notably, 80% diols, BDO (RED 1.08) and EG (RED 1.19), achieved near-complete lignin removal and exhibited an equal β -O-4 content of ~23.5%, retaining ~55% of the original β -O-4 linkages.²³ Unexpectedly, 80% EtOH (RED 1.08) removed only 37% of the total lignin yet demonstrated a comparable β -O-4 content of 23.4%.²³ Discrepancies between our findings on aspen with autocatalyzed 60% alcohol treatments and previous studies on eucalyptus with acid-catalyzed 80% alcohol treatments²³ may stem from differences in wood species and preparation protocols, solvent properties, treatment severities, and lignin degradation pathways, i.e., the extent of homolytic and acidolytic pathways in these treatments.^{16,44,58,59,80,83}

It is evident that aspen lignins produced in our autocatalytic experiments using 60% renewable alcohols exhibited favorable recovery yields and well-preserved β -O-4 bonds. Superior lignin solvents, i.e., BDO, EtOH, and THFA, enhance the lignin yield and quality in terms of β -O-4 protection.

3.4.2. Lignin Bonds and Units: β' -O-4. The occurrence of α -alkoxylation in the β -aryl ether structures (Scheme 1) has been noted in alcohol organosolv treatments, and the abundance of β' -O-4 structures is dependent on the species and various treatment parameters. For example, the β' -O-4

content in birch lignin produced using 60% EtOH (200 °C, 15 min) in autocatalytic treatment and in the presence of 0.2% and 1.0% H₂SO₄ (w/w biomass) was 15.0%, 24.6%, and 52.8% of the total β -O-4 bonds, respectively, demonstrating that acid conditions promote α -alkoxylation.⁴⁶ In experiments using walnut, pine, beech, and cedar, lignins generated with 80% EtOH at lower temperatures (up to 120 °C) in the presence of HCl (0.24 M) or H₂SO₄ (0.12 M) contained on average 36.3% (SD 7.86) β' -O-4 structures, corresponding to 67.1% (SD 16.25) of the total β -O-4 bonds.²² Eucalyptus lignin produced using EtOH, EG, and BDO (80% solvent, 120 °C) in the presence of H₂SO₄ (100 mM) contained 21.8% β' -O-4 structures (SD 2.80), equivalent to 93.2% (SD 11.84) of the total number of β -O-4 bonds, with essentially all β -O-4 bonds present as β' -O-4 structures in diol-based, EG and BDO lignins, and 80% of the total β -O-4 bonds present as β' -O-4 bonds in the EtOH lignin.²³ In addition, eucalyptus EtOH lignin produced in the presence of H₂SO₄ at higher temperatures contained 3% β' -O-4 bonds (18.75% of the total β -O-4 bonds).¹⁰ The incorporation of alcohols via α -alkoxylation has been proposed to enhance delignification by suppressing lignin condensation, preserving the β -O-4 structures, and promoting lignin solubility.^{16,21–23,25,44} Through HSQC and HMBC spectra analysis, the presence of β' -O-4 structures indicative of α -alkoxylation, was confirmed in most of our lignins, as illustrated by the HSQC-HMBC overlaid spectra of OSL lignins in Figures S2:1–5. Specifically, β' -O-4 _{α} and the methylene CH₂ of the alcohol ether-bonded to β' -O-4 _{α} were identified. The contribution of β' -O-4 structures to the total content of β -aryl ether structures in OSL and OSL_HWE is depicted in Figure 6.

The yields of the recovered lignin (Figures 2b,c and 5c) increased with the incorporation of alcohol via α -alkoxylation. However, even in the case of BDO lignin, which represents the incorporation of a four-carbon alcohol (MW_{BDO-1} = 73 g/mol)

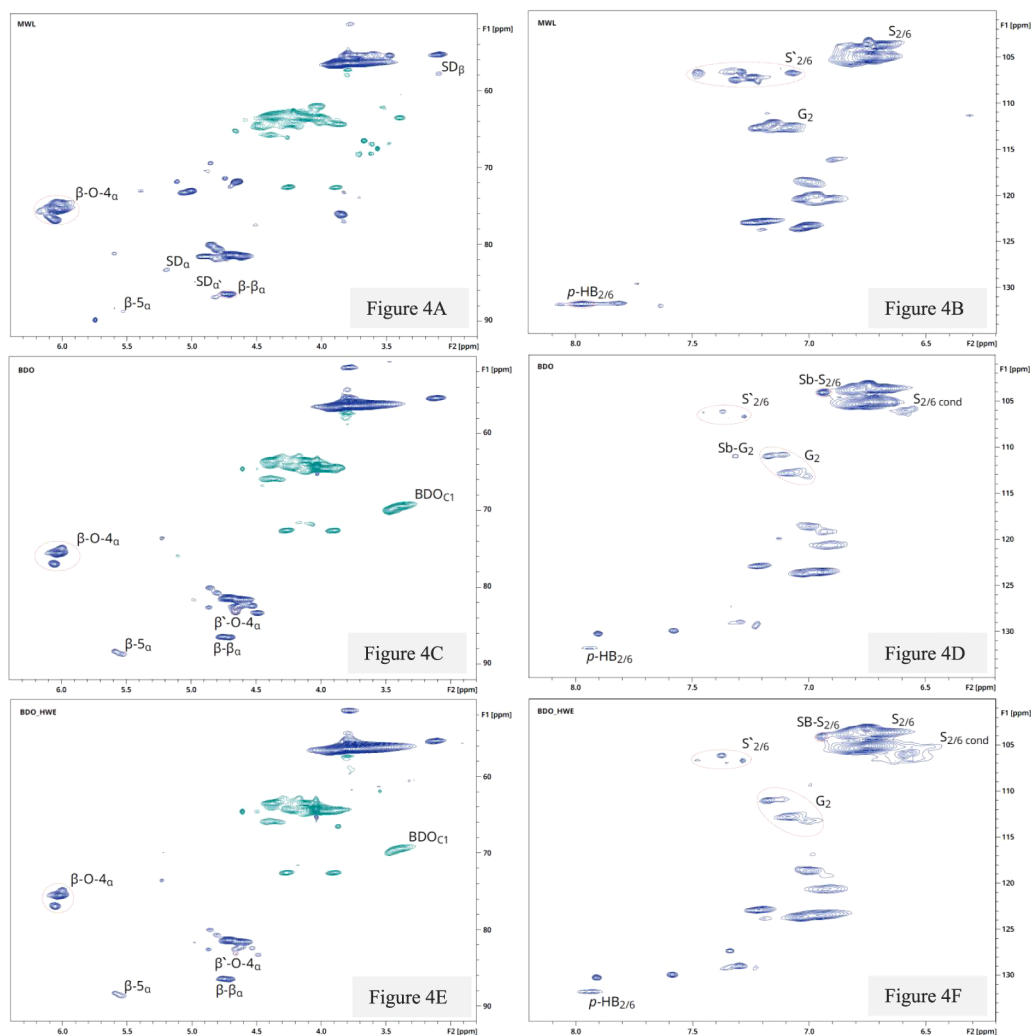


Figure 4. HSQC spectra of acetylated aspen lignins in acetone- d_6 . (A) MWL side chain; (B) MWL aromatic ring; (C) BDO lignin side chain; (D) BDO lignin aromatic ring; (E) BDO_HWE lignin side chain; (F) BDO_HWE lignin aromatic ring.

and exhibits the highest level of alkoxylation (β' -O-4, 8.1%), the increase in yield is estimated to be less than 3.8% (based on the lignin recovered). In this context, the lower purity of BDO (lignin content 93.7%) and EG_HWE (lignin content 94.3%; β' -O-4 4.3%) lignins compared with that of the other OSL and OSL_HWE lignins, respectively (lignin content: 95.8% (SD 0.835) for OSL_{av}, 96.2% (SD 1.096) for OSL_HWE_{av}), may be attributed to the loss of BDO and EG during the Klason lignin procedure, a two-step acid hydrolysis, where benzyl ether bonds are cleaved; a relatively lower lignin content is measured for lignins with higher degrees of alkoxylation (Figures 3a,b and 6).

The average degree of alkoxylation was lower for OSL_HWE lignins (β' -O-4_{av} 1.7% (SD 1.760)) than for the corresponding OSL lignins (β' -O-4_{av} 3.9% (SD 2.824)) (Figure 6), except for EG and EG-HWE lignins, which exhibited a modest degree of alkoxylation with a higher content of β' -O-4 structures in EG_HWE lignin (4.35% in EG_HWE vs 3.26% in EG lignin; Δ 33%). This result confirms the positive effect of α -alkoxylation on the preservation of β -O-4 bonds during the EG treatment of aspen_HWE²⁵ (β -O-4 content in EG_HWE was 19.2% vs 13.7% in EG). Conversely, the overall lower α -alkoxylation degree observed in the OSL_HWE lignins may provide further evidence for

condensation reactions (dehydration of C_α and formation of C_α - $C_{2/6}$ bonds) during HWE,⁶¹ which hinder further reactions at C_α . α -alkoxylation was not observed for PG_HWE lignin as demonstrated by HSQC-HMBC spectra showing no evidence of the methylene, PG_{Cl}, and β' -O-4_{av} correlations. Furthermore, PG lignin showed minimal α -alkoxylation with β' -O-4 content of only 0.23% (Figure 6). The limited nucleophilic activity of PG may be attributed to steric hindrance caused by its bulky structure. Similarly, THFA containing a tetrahydrofuran ring, showed a modest degree of α -alkoxylation in lignin, with β' -O-4 content of 3.43% and 1.17% in THFA and THFA_HWE lignin, respectively (Figure 6). Overall, BDO and EG, as linear diols, generated lignins with the highest β' -O-4 content among the OSL and OSL_HWE lignins, respectively: 8.10% in BDO (i.e., 29.5% of the total β -O-4 bonds) and 4.35% in EG_HWE (i.e., 22.6% of the total β -O-4 bonds), consistent with previous findings demonstrating the superior performance of diols in alkoxylation.²³ However, the α -alkoxylation degrees achieved in our autocatalytic experiments were significantly lower than those produced in acid-catalyzed organosolv alcohol studies, underscoring the critical role of acid catalysts in alcohol incorporation.^{25,46} Additionally, the relatively low α -alkoxylation degree of the lignins isolated in our studies may be

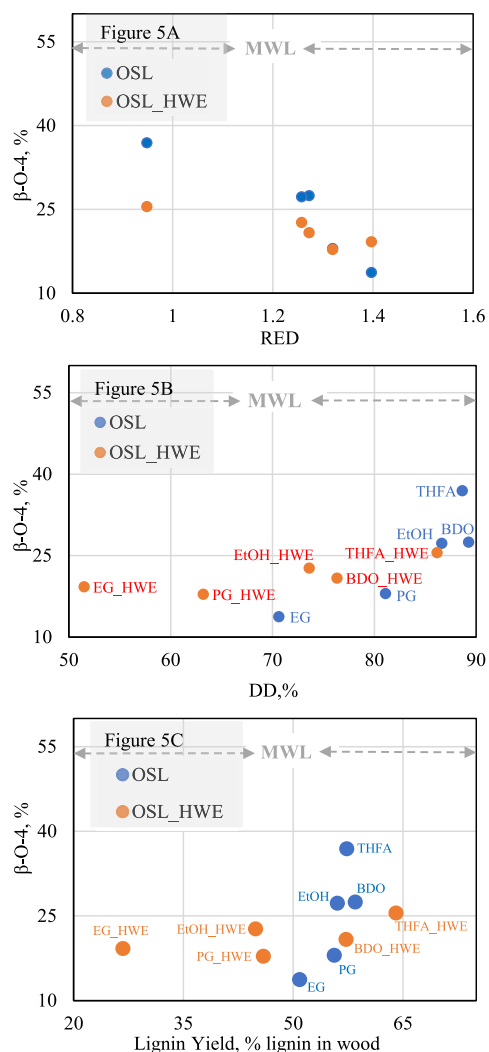


Figure 5. β -O-4 bonds (A) β -O-4, % vs RED; (B) β -O-4, % vs DD, %; (C) β -O-4, % vs lignin yield, % lignin in wood.

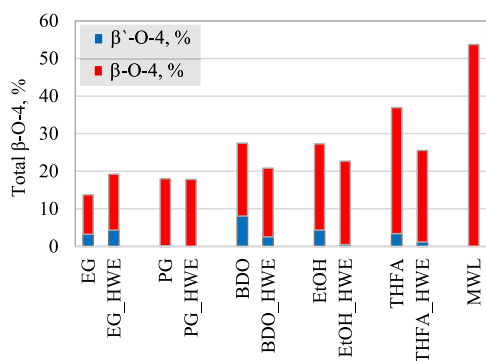


Figure 6. β' -O-4 structures.

attributed to the high treatment temperature of 195 °C, potentially leading to the cleavage of newly formed benzyl ether bonds owing to the reversible nature of the α -alkoxylation reaction.⁸⁷ Nevertheless, a lower α -alkoxylation degree combined with a relatively high content of β -aryl ether bonds in lignins recovered under high-temperature autocatalytic conditions may favor their use in depolymerization to aromatics by eliminating or reducing the need for de-etherification.¹⁶

3.4.3. Lignin Bonds and Units: β -5 and β - β Bonds. The OSL and OSL_HWE lignins contained β -5 and β - β bonds as typical lignin bonds, which are maintained as stable C–C bonds during organosolv treatments⁴⁴ (Figure 4, Figure S1:1–8 and Table S1). These bonds may be also formed through the homolytic cleavage of β -O-4 bonds (Scheme 1).^{80,86} The effect of RED on the content of the β -5 and β - β bonds is shown in Figure 7a,b, respectively. The average contents of β -5

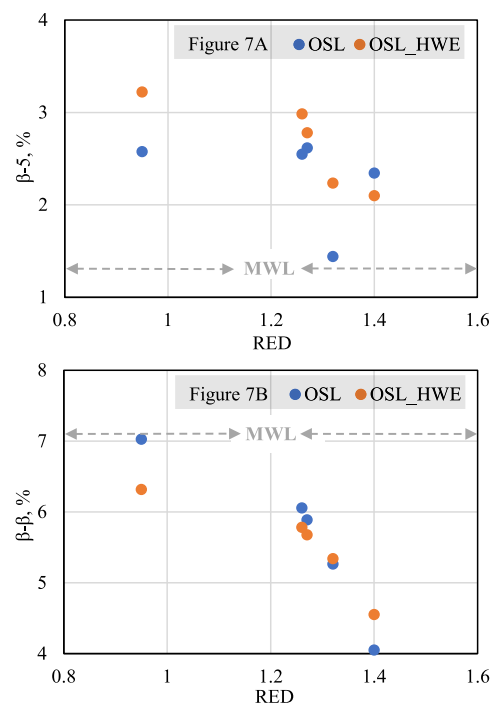


Figure 7. (A) β -5 and (B) β - β bonds.

bonds in OSL and OSL_HWE lignins were found to be similar and were double the β -5 content of 1.3% observed in MWL (2.3% (SD 0.494) for OSL_{av}, 2.7% (SD 0.482) for OSL_HWE_{av}). These results are consistent with the occurrence of homolytic reactions leading to the formation of new β -5 bonds⁸⁶ (Scheme 1), which become increasingly pronounced in solvents with lower RED (Figure 7a). The average content of β - β bonds was nearly identical in both lignin series, with OSL_{av} at 2.8% (SD 0.549) and OSL_HWE_{av} at 2.8% (SD 0.326), which was slightly lower than that observed in MWL (3.5%). Notably, the content of β - β bonds exhibited a steady increase with decreasing RED (Figure 7b). Although these results may indicate the progression of homolytic reactions in solvents with a greater affinity for lignin, they could also point to the enhanced capacity of these solvents to remove core lignin layers containing more β - β bonds, which result from dehydrodimerization reactions of cinnamyl alcohols during the initial phase of lignin deposition.⁸⁸ However, treatments involving THFA, EtOH and BDO yielded similar delignification degrees (Figures 1e and 2a,b), although THFA lignins presented the highest content of β - β bonds, suggesting the occurrence of more advanced homolytic condensation reactions (Scheme 1) during THFA treatment of both aspen and aspen_HWE. Specifically, OSL and OSL_HWE lignins derived from EG, PG, BDO, and EtOH treatments showed an average β - β

content of 2.8% (SD of 0.5492 and 0.3255, respectively), whereas the content of β - β bonds in THFA and THFA_HWE lignins was 3.5% (Δ 24%) and 3.2% (Δ 14%), respectively.

3.4.4. Lignin Bonds and Units: *p*-HB. Aspen lignin (belonging to the *Salicaceae* family) is characterized by the presence of *p*-hydroxybenzoates (*p*-HB) as pendant groups on C₇ of S-units, particularly within fibers that contain lignin enriched in syringyl, S-units, in contrast to vessels with lignin abundant in guaiacyl, G-units. Previous studies have estimated the content of *p*-HB in poplar and willow lignin to range from 0.5 to 2.9%,⁸⁹ although higher amounts of up to 10% have been reported, specifically in aspen lignin.⁷⁷

Our analysis of aspen MWL revealed a *p*-HB content of 2.4%, which is consistent with recent findings indicating *p*-HB content in aspen MWL at 2.2%.⁶¹ The variation in *p*-HB content within lignins was observed to correlate with RED (Figure 8), demonstrating an average retention of ~60% of the

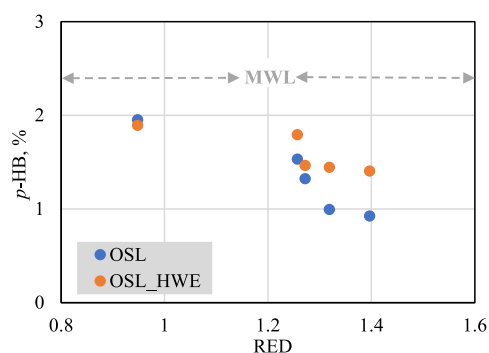


Figure 8. *p*-HB vs RED.

p-HB present in MWL. This result suggests the release of *p*-hydroxy benzoic acid (*p*-HBA) (pK_a 4.3⁶⁰) during treatments. The *in situ* generation of *p*-HBA may confer delignification benefits, with *p*-HBA acting as a protective agent and potentially inhibiting lignin condensation, as proposed in earlier studies of lignin dissolution during autohydrolysis of aspen (HTT).⁷⁷ Notably, treatments conducted with more effective lignin solvents exhibited higher retention of *p*-HB. In the context of integrated biorefineries, the *p*-HB pendant groups can be readily removed from lignin via mild alkaline hydrolysis and subsequently used in various applications, including as an alternative platform chemical to convert into a portfolio of commodity chemicals,⁸⁹ including *p*-aminophenol and paracetamol.⁹⁰

3.4.5. Lignin Bonds and Units: Stilbenes. Previous studies,^{30,44,79,80,86} have identified the presence of β -1 stilbenes in organosolv lignins, attributed to the acid-catalyzed elimination of formaldehyde from the β -1 structures.^{44,58} These structures are formed from the spirodienones via mild acid hydrolysis⁸² and from the β -aryl ethers through homolytic cleavage followed by β -1 radical coupling (Scheme 1).⁵⁸ Thus, spirodienones and β -aryl ether bonds are considered potential precursors of β -1 stilbenes. Diagnostic cross-peaks of S- and G- β -1 stilbenes (Sb_S and Sb_G, respectively)⁸¹ were observed in the HSQC spectra of both OSL and OSL_HWE lignins (superimposed HSQC spectra of EG lignin and LMCs representing β -1 stilbenes are provided in Figure S3 with the details of semiquantification shown in Table S1). The content of stilbenes in OSL lignins exceeded that in OSL_HWE lignins, with averages of 3.8% (SD 1.053) and 1.7% (SD

0.2853), respectively (Figure 9a–c). These findings suggest that stilbenes were probably produced from both spirodie-

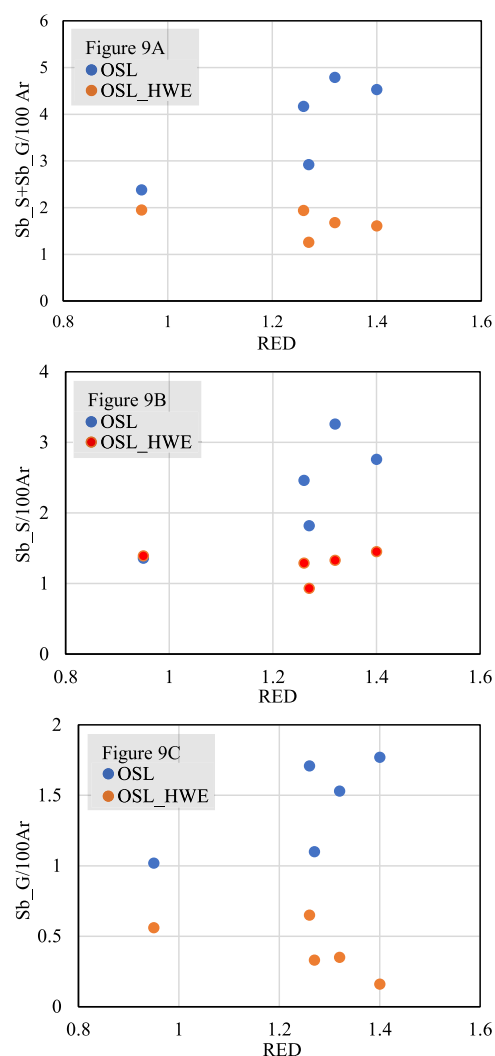


Figure 9. β -1 stilbenes. (A) β -1 stilbenes vs RED; (B) Sb_S vs RED; (C) Sb_G vs RED.

nones and β -aryl ethers, as evidenced by the presence of only ~1.8% spirodienones in MWL (Table S1), confirming the homolytic degradation of the β -aryl ether bonds during autocatalytic organosolv treatment.⁴⁴ Although spirodienones were not detected in the HSQC spectra of OSL and OSL_HWE lignins, diagnostic cross-peaks of β -1 structures were identified in all lignins, albeit not quantified because of overlapping correlations (Figures 4 and S1:1–8). The lower content of stilbenes observed in OSL_HWE lignins may be attributed to the substantial formation of stilbenes during HWE, indicating a decrease in both precursors, spirodienones and β -O-4 structures, in lignin remaining in wood as reported in earlier studies.⁷⁸ A decrease in the content of stilbenes in OSL lignins was noticed with decreasing RED (4.8% in PG (RED 1.32) compared with 2.4% in THFA (RED 0.95)), indicating better-preserved β -aryl ethers in better lignin solvents (Figure 5a). Conversely, the content of stilbenes in OSL_HWE lignins appeared unaffected by RED. Sb_S was the dominant stilbene type in all lignins, with Sb_S-to-Sb_G ratio

averaging 1.6 (SD 0.308) and 4.0 (SD 2.890) in OSL and OSL_HWE lignins, respectively.

3.4.6. Lignin Bonds and Units: Hibbert Ketones (HKs). The HSQC spectra of lignins did not display cross-peaks attributed to the lignin-bound G- and S-HKs ($G/S-C_{\alpha}H_2-C_{\beta}(=O)-C_{\gamma}H_2OH$) as representative products of acidolytic degradation of the β -O-4 lignin structures (Scheme 1)^{58,81,84} (Figure S1:1–8 and Table S1). Furthermore, no signals were detected in the region of nonconjugated C_{β} , δ , 201.59 ppm in the quantitative ^{13}C NMR. This result aligns with previous research establishing the dominant mechanism of homolytic cleavage of β -aryl ether bonds in autocatalytic organosolv delignification.⁴³ In contrast, the formation of HKs has been revealed in organosolv delignification under acid-catalyzed conditions,^{39,46} including mild extraction conditions using EtOH²² and dioxane/water.¹⁰

3.4.7. Lignin Bonds and Units: Condensed Syringyl Units (S_{cond}). Under acid conditions, lignin condensation reactions occur via electrophilic aromatic substitution reactions, creating C–C bonds between the electron-rich nucleophilic carbon located at the *o*- or *p*-position from the methoxy group ($C_{2/6}$) on the aromatic ring and the electron-deficient electrophilic benzyl carbocation, C_{α} (Scheme 1).^{10,16,24,25,39,59,79,80,85} Studies on the reactivity of lignin model compounds representing G and S units in treatments with various organic acids have demonstrated that aromatic carbons on S units are more reactive than those on G units because of the presence of two methoxy electron-donating groups,⁸⁵ resulting in more readily formed S_{cond} units. Even in alcohol organosolv treatments in which nucleophilic alcohols compete with aromatic carbons to trap benzyl carbocations and form benzyl ether bonds, suppressing lignin condensation, S_{cond} units are still formed, albeit to a lesser extent. For example, approximately 36% S_{cond} units were detected in lignin from acid-catalyzed EtOH treatment of eucalyptus, accounting for 43.9% of the total S units, and approximately 28% S_{cond} were observed in lignin from acid-catalyzed dioxane treatment of oak (dioxane/water (9:1), 0.2 M HCl, 1 h at reflux), representing 34.5% of the total S units.¹⁰ Condensation reactions resulting in S_{cond} units have also been identified during HTP of aspen,⁶¹ shedding additional light on our results regarding the less efficient delignification of aspen_HWE (Figure 1e) and the lower α -alkoxylation degree observed in OSL_HWE lignins (Figure 6). S_{cond} units are detected in the HSQC spectra by cross-peaks that may overlap with the $S_{2/6}$ of lignin-bound HKs (δ_C/δ_H 107.4/6.52, in DMSO- d_6).⁸⁴ The HSQC spectra of aspen OSL and OSL_HWE lignins displayed cross-peaks that were assigned to S_{cond} units (δ_C/δ_H 106.0–107.0/6.4–6.6) (Figures 4, S1:1–8 and Table S1), given the absence of HKs; no signals corresponding to the $C_{\alpha}H_{\alpha}$ (δ_C/δ_H 45.73/3.70) and $C_{\gamma}H_{\gamma}$ (δ_C/δ_H 68.26/4.77) of HKs were detected in the HSQC spectra (Figure S1:1–8). The effect of RED on the content of S_{cond} units is depicted in Figure 10a. OSL_HWE lignins contained more S_{cond} units than did OSL lignins, which is consistent with condensation reactions occurring during HWE and results in more condensed lignin in aspen_HWE.^{61,78} Lignins richer in β -O-4 linkages contained fewer S_{cond} units (Figure 10b). A similar inverse trend between the contents of β -O-4 linkages and S_{cond} units has been reported in recent studies on acid-catalyzed GVL treatment of poplar.²⁴

3.4.8. Lignin Bonds and Units: S/G Ratio. The S/G ratio of OSL and OSL_HWE lignins (4.0 (SD 0.958) for OSL_{av} and 5.0 (SD 0.615) for OSL_HWE_{av}) (Table S1 and Figure 11)

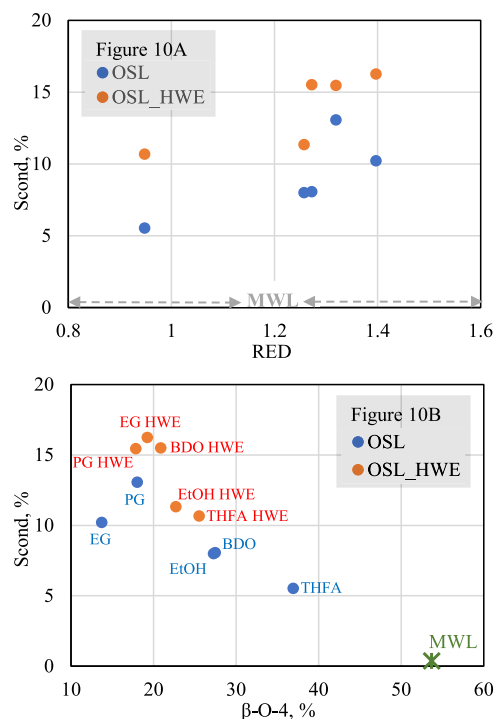


Figure 10. S_{cond} units. (A) S_{cond} % vs RED; (B) S_{cond} % vs β -O-4 %.

consistently exceeded that observed for MWL, which was 2.2. This result suggests that lignin enriched in S-units exhibited greater susceptibility to dissolution compared with lignin containing more G-units, with S-rich lignin typically found in fibers and G-rich lignin in vessels.⁸⁸ Inherently, S-enriched lignin is characterized by a more open, less condensed, more soluble structure. Better lignin solvents, i.e., alcohols with lower RED, yielded lignin with lower S/G ratios, i.e., lignin containing more G units. This finding aligns with their enhanced efficiency in removing lignin and higher DD (Figure 1e), affecting more G-enriched lignin fractions. The S/G ratio of EtOH lignin, measured at 3.5, was comparable with that reported for birch EtOH lignins generated in autocatalytic studies (50–70% EtOH, 200 °C, 30–60 min), averaging at 3.8 (SD 0.232).⁴⁶ Notably, earlier acid-catalyzed BDO experiments using eucalyptus produced BDO lignin with a similar S/G ratio to that observed in our experiments.²³

3.4.9. Lignin Bonds and Units: Hydroxyl Groups. The quantitative ^{13}C NMR analysis of acetylated lignins (Section

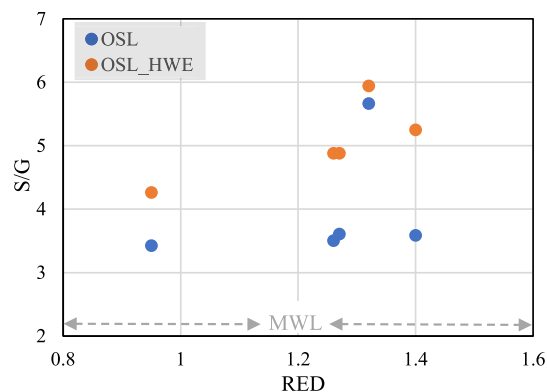


Figure 11. S/G ratio vs RED.

2.6) was conducted to quantify three distinct hydroxyl group types within lignin. These groups were discerned based on unique signals of carbons in acetoxy groups' carbonyl regions ($\text{CH}_3\text{-CO-O-}$), corresponding to primary groups (CH_2OH , 1°), secondary groups (CHOH , 2°), and phenolic groups (PhOH , Ph). Additionally, both BDO and BDO_HWE lignins exhibited a notable acetoxy signal at 171 ppm, indicative of the presence of CH_2OH groups from bound BDO (Figure 12). In

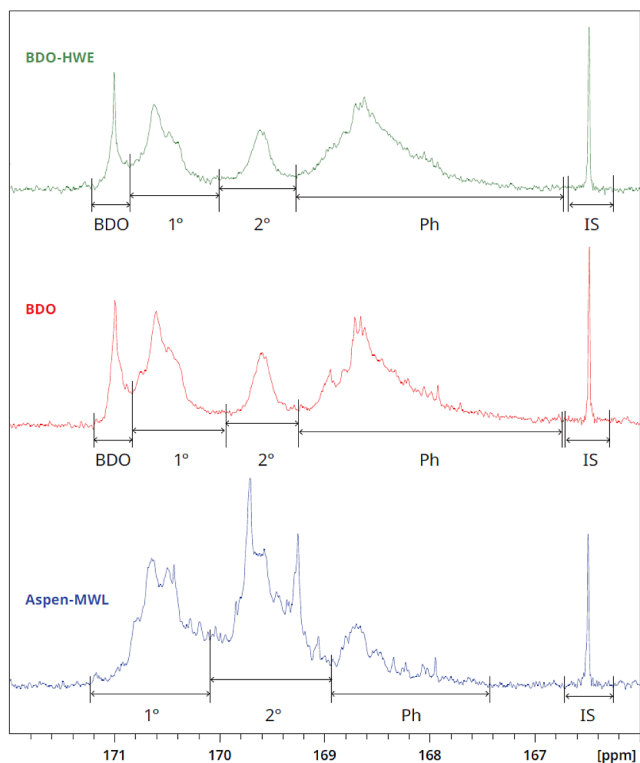


Figure 12. Acetoxy signals in ^{13}C NMR spectra of aspen MWL, BDO and BDO_HWE acetylated lignins; the quantification of hydroxyl groups was made using the selected integration ranges based on the arbitrary minima.

earlier studies, the higher content of aliphatic hydroxyl groups revealed by ^{31}P NMR analysis in BDO and EG lignins, generated under acid-catalyzed conditions from eucalyptus (120°C , $100\text{ mM H}_2\text{SO}_4$, 4 h), has been linked to the presence of free CH_2OH groups on the diol “tail”, incorporated into lignin through α -alkoxylation;²³ however, independent ^{31}P NMR signals corresponding to CH_2OH groups of diols, BDO and EG, could not be observed. Figure S4:1–4 presents the ^{13}C NMR spectra of all other acetylated aspen lignins investigated in this study. It is evident that, unlike in the ^{13}C NMR spectra of BDO and BDO_HWE lignins, these spectra display only signals associated with lignin in the acetoxy groups' carbonyl region.⁹¹ This hindered the estimation of the primary hydroxyl group content, CH_2OH groups originating from EG incorporated in EG and EG_HWE lignins. Figure 13a summarizes the contents of 1° , 2° , and Ph groups in lignins, whereas Figure 13b presents the content of PhOH groups in relation to the content of β -O-4 linkages in lignin. In organosolv treatments, the content of aliphatic hydroxyl groups in lignin decreased to less than 50% of the original content [ROH , mmol/g lignin: MWL, $8.8 > \text{OSL}_{\text{av}}$, 4.0 (SD 1.057) $> \text{OSL_HWE}_{\text{av}}$, 3.2 (SD 0.467)], as has been well

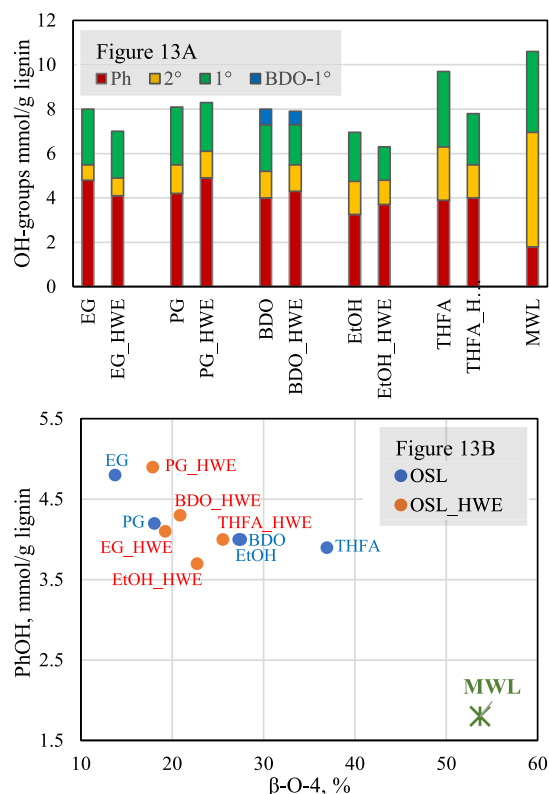


Figure 13. Hydroxyl groups. (A) Content of primary (1°), secondary (2°), and free phenolic (Ph) hydroxyl groups, mmol/g lignin; (B) Content of free phenolic (PhOH) hydroxyl groups, mmol/g lignin vs β -O-4, %.

documented in earlier studies of various organosolv treatments,^{23,24,91} This result points to various reactions, including cleavage of $\text{C}_\beta\text{-C}_\gamma$ bonds with the release of formaldehyde, leading to a decrease in the content of primary hydroxyl groups [$1^\circ/\text{CH}_2\text{OH}$, mmol/g lignin: MWL, $3.7 > \text{OSL}_{\text{av}}$, 2.6 (SD 0.513) $> \text{OSL_HWE}_{\text{av}}$, 2.0 (SD 0.327)], dehydration, α -alkoxylation, and $\text{C}_\alpha\text{-C}_{2/6}$ bond formation, leading to a decrease in secondary hydroxyl groups (Scheme 1) [$2^\circ/\text{CHOH}$, mmol/g lignin: MWL, $5.2 > \text{OSL}_{\text{av}}$, 1.4 (SD 0.622) $> \text{OSL_HWE}_{\text{av}}$, 1.2 (SD 0.251)]. The decrease in both primary and secondary hydroxyl groups was higher in OSL_HWE lignins than in OSL lignins, providing additional evidence for more intense alterations of aspen lignin throughout the two-step HWE-alcohol treatment than at the end of alcohol treatment performed independently. Also, alcohol treatments resulted in a notable increase in the content of PhOH groups consistent with earlier studies,^{23,24,91} corroborating the cleavage of β -O-4 bonds confirmed by HSQC studies [Ph/PhOH , mmol/g lignin: MWL, $1.8 < \text{OSL}_{\text{av}}$, 4.0 (SD 0.559) $< \text{OSL_HWE}_{\text{av}}$, 4.2 (SD 0.447)]. In accordance with the lower average content of β -O-4 linkage in OSL_HWE lignins, they had a higher content of PhOH groups than did OSL lignins. Our results further substantiate the notion that better lignin solvents lead to less modifications in lignin because they produced lignin of lower PhOH content, which aligns with their improved ability to preserve β -O-4 bonds (Figure 13b).

3.5. Molecular Weight Distribution (MWD). The molecular weight of lignins was evaluated using underivatized lignin samples (Section 2.7); chromatograms are presented in Figure S5. An increase in solvent affinity toward lignin corresponded to a gradual increase in both molecular weight

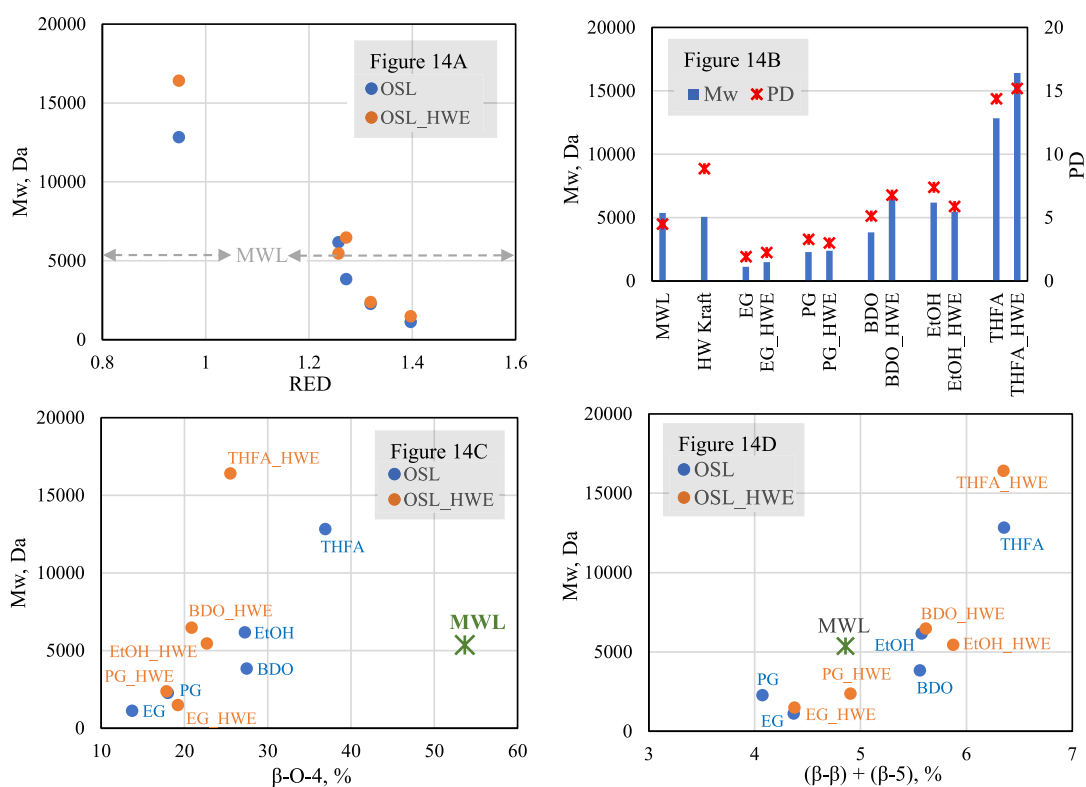


Figure 14. Molecular weight distribution. (A) Mw, Da vs RED; (B) Mw, Da and PD; (C) Mw, Da vs β -O-4, %; (D) Mw, Da vs $[(\beta-\beta) + (\beta-5)]$, %.

(MW) and polydispersity (PD), as depicted in Figure 14a,b. Less effective lignin solvents, EG (RED 1.40) and PG (RED 1.32), yielded lignins with lower MW and PD compared with MWL and HW kraft lignin [Mw_{av}, Da: (EG-PG) 1,703; (EG_HWE-PG_HWE) 1,940 vs MWL 5,361, HW kraft 5,051; PD_{av}: (EG-PG) 2.60, (EG_HWE-PG_HWE) 2.62 vs MWL 4.48, HW kraft 8.86]. This observation aligns with the significant cleavage of β -O-4 linkages observed during EG and PG treatments of aspen and aspen_HWE, resulting in fewer β -O-4 linkages in the respective lignins (<20%, Figure 14a). Conversely, more efficient lignin solvents produced lignins with better-preserved β -O-4 linkages and higher MW and PD. THFA (RED 0.95) yielded lignins with the highest content of β -O-4 linkages, alongside the highest MW and PD [Mw, Da, (PD): THFA 12,837 (14.38); THFA_HWE 16,411 (15.18)] (Figure 14c). The increased MW of THFA lignins corresponds with the increase in the content of β -5 and β - β bonds in these lignins (Figure 14d) $[(\beta-5 + \beta-\beta)$: 6.4% for both THFA and THFA_HWE lignins compared with 4.9% for MWL; Δ 31%], indicating advanced lignin condensation during THFA treatment of both aspen and aspen_HWE via homolytic pathways (Scheme 1).^{44,58,83}

In comparison, the MW of EtOH lignin [Mw, 6,176 Da, PD 7.40] is consistent with previous findings on EtOH lignin produced autocatalytically from birch [(60% EtOH, 200 °C, 30 min, H-factor 4,654 h), (Mw 8,000 Da and PD 5.6)],⁴⁶ although it surpasses the MW observed under even more severe autocatalytic conditions [(60% EtOH, 200 °C, 2 h), H-factor 19,011; Mw 3,140 Da, PD 2.52].³⁰ Discrepancies in MW across studies may arise from varying analysis methods, such as acetylation³⁰ or acetobromination,⁴⁶ ensuring lignin solubilization prior to GPC analysis. Nonetheless, lignins produced in our autocatalytic conditions exhibited higher MW and PD

compared with those produced with acid catalysts in similar conditions for various species, such as poplar, sweetgum (*Liquidambar styraciflua*), birch, and eucalyptus.^{23,46,72,87}

3.6. UV-Absorption. Photochemical degradation refers to the degradation of natural and synthetic materials caused by the combined effects of air and UV light, specifically UV-A light (315–400 nm), which is not absorbed by the atmosphere, and UV-B light (280–315 nm), which is capable of penetrating through the partially depleted ozone layer. Lignin, owing to its polyphenolic structure and the presence of various UV chromophoric moieties such as conjugated double bonds, carbonyl groups, and cinnamaldehyde units,⁹² possesses an inherent capacity to absorb UV light, thus safeguarding plants against photochemical degradation.⁹³ In the realm of emerging green technologies, lignin can also serve as a natural UV absorber in materials, mitigating the need for harmful synthetic UV-absorbing additives.^{92,93} Furthermore, considering that lignin exhibits cytotoxic effects on human cells only at high concentrations (>5 mg/mL) dependent on lignin type,⁵ researchers have explored its potential as a natural UV absorber in sunscreens.^{92,93} Hence, OSL and OSL_HWE lignins were analyzed for their efficacy in UV light absorption. The results revealed a significant loss of UV absorption ability exhibited by MWL during treatments (Figure 15a,b). This decrease in absorption in the 250–300 nm range may be attributed to the substantial loss of UV-absorbing chromophore moieties containing keto groups,⁹² such as C_α oxidized S units, S' units. Our HSQC studies showed a 5-fold decrease in the content of S' units in lignin after organosolv treatment of aspen and aspen_HWE; the content of S' units in MWL was 12.6%, whereas OSL and OSL_HWE lignins contained an average of less than 2.5% S' units (2.4% (SD 1.332) for OSL_{av}, 1.7% (SD 0.351) for OSL_HWE_{av}) (Table S1). Similarly, the

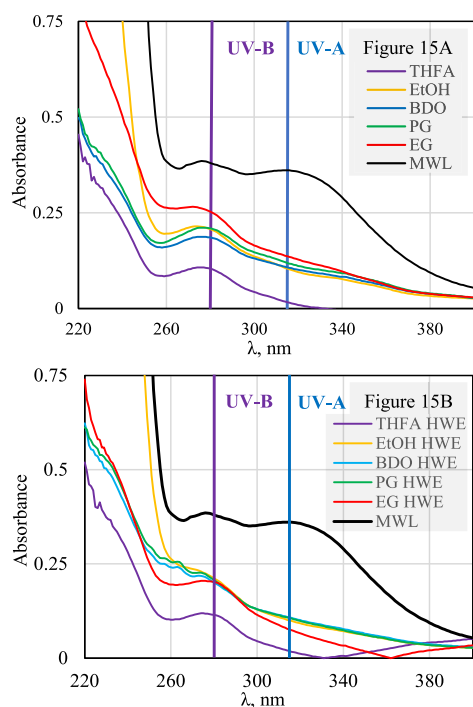


Figure 15. UV spectra of lignins in dioxane/water (9/1), 10 $\mu\text{g/mL}$. (A) OSL lignins vs MWL; (B) OSL_HWE lignins vs MWL.

removal of cinnamaldehyde end units (CA) that absorb light in the 300–340 nm region⁹² (Figure S6:1) contributed to the reduced absorption of lignin in this area; based on HSQC results, the content of CA decreased through treatments from ~3% in MWL to <1% in OSL and OSL_HWE lignins (CA: 0.86% (SD 0.233) for OSL_{av}, 0.62% (SD 0.238) for OSL_HWE_{av}) (Table S1).

In addition, the cumulative absorption in the UV-A and UV-B ranges calculated for aspen lignins was compared with that exhibited by three natural UV absorbers, (poly)phenolics, ferulic acid, and quercetin,⁹⁴ and a synthetic UV absorber, benzophenone, diphenylmethane, *Bp-1*, which represents a typical class of UV-absorbers used to mitigate photo-degradation of polymeric materials, primarily in cotton and sunscreens.⁹⁵ The UV spectra of ferulic acid, quercetin, and *Bp-1* are presented in Figure S6:2–4. The results shown in Figure 16 indicate that the UV-A absorption of most OSL and OSL_HWE lignins is comparable with that of *Bp-1*, whereas

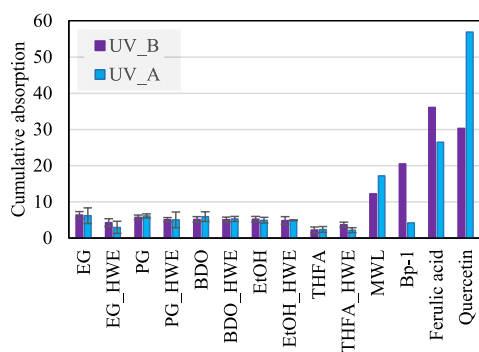


Figure 16. Cumulative UV-A and UV-B absorption of aspen lignins and natural and synthetic UV-absorbers in dioxane/water (9/1), 10 $\mu\text{g/mL}$.

Bp-1 provides, on average, a 5-fold higher UV-B absorption. Therefore, OSL and OSL_HWE lignins could be considered for use either independently or in conjunction with *Bp-1* in materials and sunscreens to reduce reliance on synthetic UV-absorbing agents. In contrast, the natural UV-absorbers, ferulic acid and quercetin, provide superior protection against UV-A and UV-B radiation compared with aspen lignins and *Bp-1*.

3.7. Radical Quenching Activity—DPPH. Lignin's capacity to stabilize polymers against degradation, based on UV shielding properties, is further reinforced by its radical quenching activity, antioxidizing activity (AOA), originating from its polyphenolic character.^{5,72,96} The radical quenching ability of lignin was evaluated in these studies using the standard DPPH method (Section 2.9).⁷² Results from this method are expressed as the concentration of the antioxidizing agent required to quench 50% of radicals in the solution, known as the IC₅₀ concentration; accordingly, lower IC₅₀ values correspond to more potent antioxidizing agents. Our results demonstrate that the AOA of our lignins ranges between that of potent antioxidizing agents, such as quercetin and ferulic acid, and the slightly less effective MWL, with IC₅₀ ($\mu\text{g/mL}$) values decreasing in the following order: quercetin < ferulic acid < OSL_HWE_{av} (74.9, SD 15.03) < OSL_{av} (88.2, SD 35.42) < MWL (Figure 17a). Moreover, our results indicate that these lignins have the potential to offer more efficient antioxidizing protection to materials compared with a synthetic antioxidizing agent, BHT, which is approximately two times weaker than MWL (Figure 17a).

The AOA of lignins increased with increase in PhOH content (Figure 17a) and S/G ratio (Figure 17b) and decreased with increase in the Mw of lignin (Figure 17c). These trends are consistent with documented effects of lignin structure on its AOA.^{5,72,96} The relatively lower AOA of MWL may be attributed to its low PhOH content, according to the pivotal role of PhOH groups in radical quenching ($\text{Ar-OH} + \text{X}^\bullet \rightarrow \text{Ar-O}^\bullet + \text{XH}$), and a relatively low S/G ratio, considering the higher AOA of dimethoxylated S units compared with monomethoxylated G units.⁹⁶ It may also be ascribed to the high content of the carbonyl groups in MWL (S' and CA units (Table S1)), because conjugated carbonyl groups have been shown to decrease AOA.⁹⁶ PG and PG_HWE lignins exhibited the highest AOA, consistent with their high PhOH content and S/G ratio, and low MW and PD (Figure 14). In contrast, THFA and THFA_HWE lignins displayed relatively lower AOA compared with most of the other lignins generated in this study, in line with their relatively low PhOH and S/G ratio and high Mw and PD (Figure 14). Nonetheless, the AOA of THFA and THFA_HWE lignins (IC_{50av} 108 $\mu\text{g/mL}$ (SD 32.88)) was still almost three times higher than that of BHT (IC_{50av} 300 $\mu\text{g/mL}$).

3.8. Glass Transition Temperature, T_g . Lignin, an amorphous polymer, exhibits a relatively high glass transition temperature (T_g) because of its strong intra- and intermolecular interactions. The T_g of lignin is primarily dictated by its chemical structure and plays a pivotal role in determining mechanical performance (e.g., stiffness) and physical properties (e.g., thermal expansion). Additionally, T_g impacts practical considerations such as thermal processing temperatures or the maximum temperature at which a material can be used.^{97,98}

The T_g values of our lignins and two reference lignins, aspen MWL and HW kraft lignin, were determined using TM-DSC (Section 2.10), with thermograms shown in Figure S7. In general, compared with HW kraft lignin, OSL lignins displayed

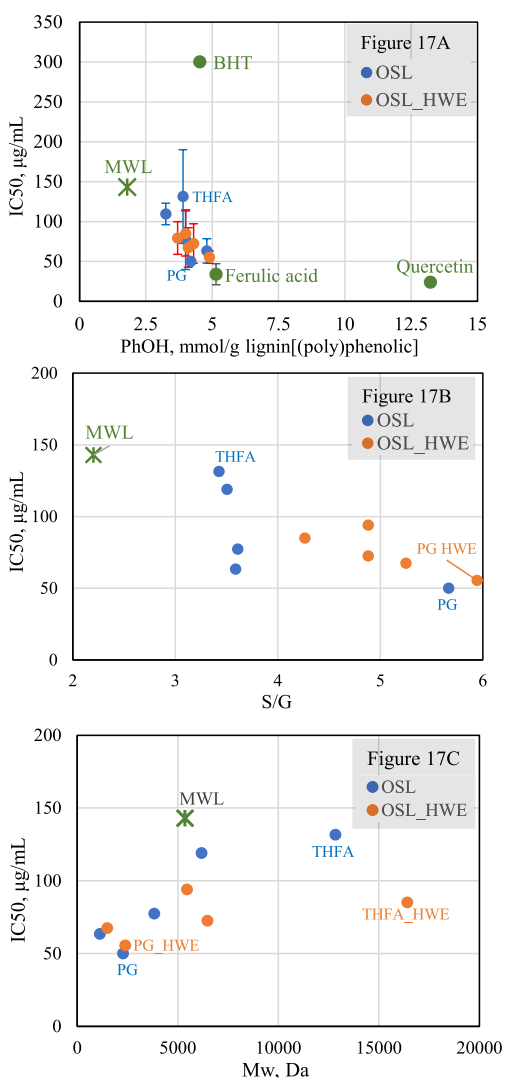


Figure 17. DPPH radical quenching ability—IC₅₀. (A) IC₅₀, µg/mL vs PhOH, mmol/g lignin [(poly)phenolic]; (B) IC₅₀, µg/mL vs S/G ratio; (C) IC₅₀, µg/mL vs Mw, Da.

lower T_g values, whereas those of OSL_HWE lignins were either similar or higher (Figure 18a). Additionally, lignins with fewer S_{cond} units demonstrated lower T_g values (Figure 18b). The exceptions to these trends were observed in THFA and THFA_HWE lignins, which displayed the highest T_g values within their respective groups, probably because of their elevated Mw and PD (Figures 14a,b and 18a) and a relatively high content of β - β and β -5 bonds (Figures 7a,b and 18c). In contrast, EtOH lignin exhibited a low T_g , comparable with that of MWL, resulting from various factors, including lower Mw and fewer S_{cond} , β - β , and β -5 bonds. Additionally, EtOH lignin contained benzyl ethoxylated lignin units as *in situ* masked hydroxyl groups (Figure 6), contributing to reduced intra- and intermolecular lignin interactions and consequently, a lower T_g .⁹⁷ However, α -alkoxylation (i.e., β' -O-4 structures) observed in BDO and BDO_HWE lignins (Figure 6) did not decrease T_g , indicating that the newly introduced BDO_{C4} primary hydroxyl groups participate in intra- and intermolecular lignin interactions, thus increasing T_g .

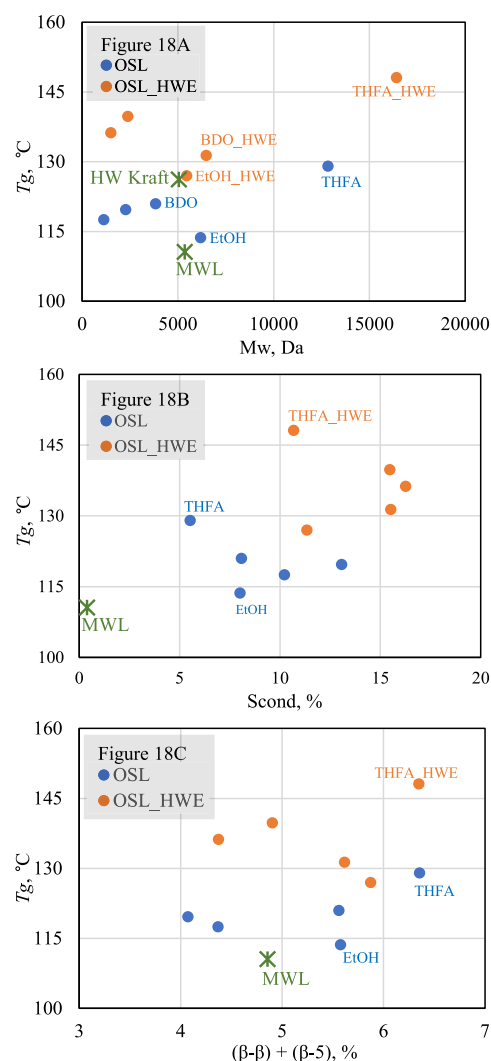


Figure 18. T_g . (A) T_g , °C vs Mw, Da; (B) T_g , °C vs S_{cond} , %; (C) T_g , °C vs $[(\beta\text{-}\beta) + (\beta\text{-}5)]$, %.

4. CONCLUSIONS

Renewable alcohols, with an increasing affinity toward lignin, EG < PG < BDO < EtOH < THFA, i.e., with decreasing RED, EG > PG > BDO > EtOH > THFA, were applied to aspen and hot-water-extracted aspen under autocatalytic conditions at a high temperature of 195 °C for 2 h. This resulted in increased delignification and yielded lignins with an increased β -O-4 content, following the same order of alcohol affinity toward lignin. Notably, THFA led to ~90% delignification, yielding more than 55% of the total lignin containing ~37% β -O-4 bonds, equivalent to a notable ~69% of the β -O-4 bonds present in aspen MWL. However, this lignin also exhibited a higher content of β - β and β -5 bonds than MWL, suggesting that homolytic reactions readily occur during THFA treatment and result in ~10% higher content of β - β and β -5 bonds than in MWL. This must be considered in valorization attempts through depolymerization strategies. The delignification degree of hot-water-extracted-aspen was consistently lower, and the corresponding lignins comprised fewer β -O-4 linkages and more S_{cond} units, showing a higher Mw, suggesting the occurrence of condensation reactions during HWE of aspen. Under autocatalytic conditions, alcohols resulted in a relatively weak α -alkoxylation, although diols, BDO and EG, were

relatively more efficient than other alcohols. Further, the α -alkoxylation degree improved in treatments of aspen compared with hot-water-extracted aspen. All lignins were characterized by higher UV-A absorption than benzophenone, a typical UV absorbing agent. They also exhibited a relatively high radical quenching ability, which depending on the lignin, was two to six times higher than that of BHT. The T_g of lignins, as an indicator of lignin thermal processability, increased with increasing MW of lignin. Lignins produced from aspen exhibited lower T_g than lignins produced from hot-water-extracted aspen and they all presented a lower T_g than HW kraft lignin, except THFA lignin.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.4c05981>.

The NMR and UV spectral data, GPC chromatograms, and DSC thermograms of studied lignins (PDF)

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Notes

The authors declare no competing financial interest.

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