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Research Article

Influence of Ethanol Organosolv Pulping Conditions on Physicochemical Lignin Properties of European Larch

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Over the years, the organosolv pulping process has proven to be a valuable pretreatment method for various lignocellulosic feedstocks. The objective of this study was to characterize and assess the potential applicability of the organosolv lignin fraction from European larch sawdust, as no research has been conducted in this field so far. Eight different samples were prepared from the European larch sawdust under varied reaction conditions and one milled wood lignin sample as reference. The reaction temperature and sulfuric acid loading were varied between 420 and 460 K and 0.00 and 1.10% (w/w on dry wood basis) H_2SO_4, respectively. The antiradical potential (via DPPH$^\bullet$ method), chemical structure (via ATR-FTIR, ^{1}H NMR, ^{31}P NMR, and thioacidolysis), as well as the molecular weight distribution of the isolated lignins were analyzed and compared. Results from thioacidolysis show a direct correlation between the amount of β-ether bonds broken and pulping process severity. Similarly, both antiradical potential and phenolic hydroxyl group content exhibit a direct relationship to reaction temperature and catalyst loading. On the contrary, the content of aliphatic hydroxyl groups and the average molecular weights both decreased with increasing process severity. The high content of phenolic hydroxyl groups and antioxidative potential of the larch organosolv fractions, especially for the sample isolated at 460 K and 1.10% H_2SO_4 loading, indicate good applicability as antioxidants as well as feedstocks for further downstream valorization and require additional research in this area.

1. Introduction

The impending depletion of fossil resources has incited a rise of interest in converting renewable resources, such as lignocellulosic feedstocks, to chemicals and fuels. Lignin is an excellent candidate to fulfil that role, being the largest naturally occurring source of aromatic compounds and a major component of lignocellulose. Lignin is built up from methoxylated hydroxy phenylpropanoid units via random radical polymerization, leading to a complex, amorphous, and water-insoluble polymer with a multitude of chemically distinct binding motifs [1].

The organosolv pulping process is one option of biorefinery concepts that can efficiently separate biomass into cellulose-, hemicellulose-, and lignin-rich fractions. It was developed over 80 years ago by Kleinert and Tayenthal [2] for the fractionation of multiple types of lignocellulosic feedstocks, including wood and agricultural wastes, using low-boiling point water-miscible solvents as pulping agents. The resulting organosolv lignin has a low content of residual carbohydrates as well as high potential for valorization, besides producing a cellulose-rich fraction of high quality comparable to that of the kraft process [3].

Several studies have been conducted recently on the organosolv pulping of softwoods. Lesar et al. studied the uncatalyzed pulping of mixed softwood from recycling companies. The maximum lignin removal of 51% was achieved at 493 K with 196 min of reaction time and 65% aq. EtOH [4]. Nitsos et al. focused on acid-catalyzed organosolv pulping of spruce. They reached a feedstock delignification

of 62% at 456 K, 60 min, and 60% aq. EtOH and a sulfuric acid loading of 1% [5]. Løhre et al. tested a flow-through organosolv fractionation of mixed softwood. The maximum lignin removal of 83.1% was achieved at 448 K, 63% aq. EtOH with 6.00 mol·m^{-3} H_2SO_4 [6]. Smit et al. studied a novel acid-catalyzed acetone organosolv fractionation process of spruce and pine, amongst other lignocellulosic feedstocks, as pretreatment for enzymatic sugar production. However, the applied reaction conditions of 413 K, 50% aq. acetone, 120 min, and 40 mM of H_2SO_4 proved to be too benign, as delignification was just 31.6% for pine and 29.7% for spruce, respectively [7].

Furthermore, multiple studies have been published recently on possible applications for organosolv lignins. It was proposed that it can be used as an adhesive in wood panel production [8], a monomer in a biopolymer [9], a possible antioxidant [10, 11], and a pesticide [10].

European larch (*Larix decidua* Mill) is one of the most important wood species of Central Europe that mainly occurs in mountainous regions (Alps, Carpathians, and Sudetes). It typically reaches 45 m with a diameter of up to 1.5–2.5 m and a lifespan of up to 800 years [12, 13]. Its natural habitat spans approximately 5000 km^2 with a further 5000 km^2 of plantations in the Central and Western Europe together with Japanese and hybrid larch species [14]. The European larch is one of the fastest growing conifer trees with more than 10 m^3·ha^{-1}·y^{-1} and additionally possesses a low susceptibility to pests, high wood durability, and good fiber characteristics [12, 13]. It is largely used in carpentry, façade building, as well as naval construction [15]. Several studies have been conducted on European larch wood, including lignin and extractive content [16] and distribution in the heartwood [17], as well as its correlation to decay resistance [18].

However, no study has been conducted yet on the influence of organosolv pulping conditions on the properties of the lignin fraction of European larch. Research into the valorization of side products of the wood industry, such as sawdust, seems promising due to the high relevance of larch timber on the European market.

Eight lignin preparations were extracted from the European larch under varied conditions using an ethanol organosolv process with conditions derived from the literature [19–21]. Additionally, one milled wood lignin sample was isolated as reference. Furthermore, the correlations between the pulping conditions and the physicochemical characteristics of the organosolv lignin samples were studied. The lignin structure was elucidated using ATR-FTIR, thioacidolysis, and HPSEC. The functional group content was assessed using ^{1}H NMR and ^{31}P NMR, and the antioxidative potential was determined via the DPPH method.

2. Materials and Methods

2.1. Feedstock. The European larch sawdust was provided by Sägewerk/Hobelwerk Alfred Seebacher GmbH & Co. KG located in Gnesau (Carinthia), Austria. The trees were harvested at approximately 100 years of age between November 2016 and March 2017 from the Nockberge region, Carinthia, at least 1000 m above sea level. The logs were sawn in October 2017 without previous debarking.

The sawdust was directly collected and transported to our laboratory in a 100 L plastic bag. The sawdust was screened, and the fraction smaller than 1.5 mm × 1.5 mm and larger than 100 μm × 100 μm was collected as feedstock for the organosolv fractionation experiments and stored in a refrigerator at 277 K in closed glass containers. Part of the sawdust was ground using an MF 10 basic microfine grinder (IKA, Germany) equipped with an MF 10.1 cutting-grinding head, from which the fraction smaller than 0.5 mm was retained for chemical analysis.

2.2. Organosolv Fractionation. Organosolv pretreatment of the pinaceous sawdust was performed in a 0.5 L stirred batch reactor, made of HastelloyC® (Paar Instrument Company, Series 4575A HP/HT). A split ring closure system with a flexible graphite gasket was used to maintain the reaction pressure and a thermowell to measure the internal temperature. The reactor was loaded with 36 g of sawdust on dry-wood basis, filled with 250 mL of a 75% v/v aqueous ethanol solution (liquid-to-solid ratio 7 : 1 v/w) with either sulfuric acid as a catalyst (0.75–1.65% w/w) or autocatalytic. The reactions were performed at 440, 450, 455, and 460 K, respectively, at a heating rate of 8–10 K/min, with 30 min of reaction time at the respective temperature [19–21]. After the fractionation process, the reaction mixture was filtered over a Whatman No. 5 filter paper in a Büchner funnel to separate the solid cellulose-rich substrate from the lignin-rich liquor. The solid phase was washed with a total of 165 mL (3 × 55 mL) of warm (333 K) aqueous ethanol solution and combined it with the initial filtrate, followed by warm (333 K) water washing (3 × 50 mL) which was discarded. The combined liquid phase was poured into three times the volume of distilled water (1.25 L) to precipitate the ethanol organosolv lignin fraction. In the case of the autocatalytic reaction, the water was acidified to pH 3 with conc. HCl to ease lignin precipitation. The crude larch organosolv lignin was separated from the liquid fraction once again via filtration and consequent warm water washing (333 K, 3 × 75 mL). Afterwards, both the lignin fraction and pulp fraction were freeze dried for 24 h and stored for further analysis (Figure 1).

Furthermore, catalyst loading, treatment temperature, and duration were merged into a combined severity factor (CSF), developed by Abatzoglou et al. [22], to be able to more easily compare different fractionation experiments, as shown in the following equation:

$$\mathrm{CSF} = \log_{10}\left[t \times \exp\left(\frac{T(t) - 373}{14.75}\right)\right] - \mathrm{pH}, \quad (1)$$

where t denotes the treatment time, $T(t)$ is the treatment temperature in K; 373 is the reference temperature in K, and 14.75 is a constant describing the role of temperature in a catalyzed reaction system [22].

2.3. Milled Wood Lignin. Milled wood lignin (MWL) was prepared based on a method published by Holtman et al.

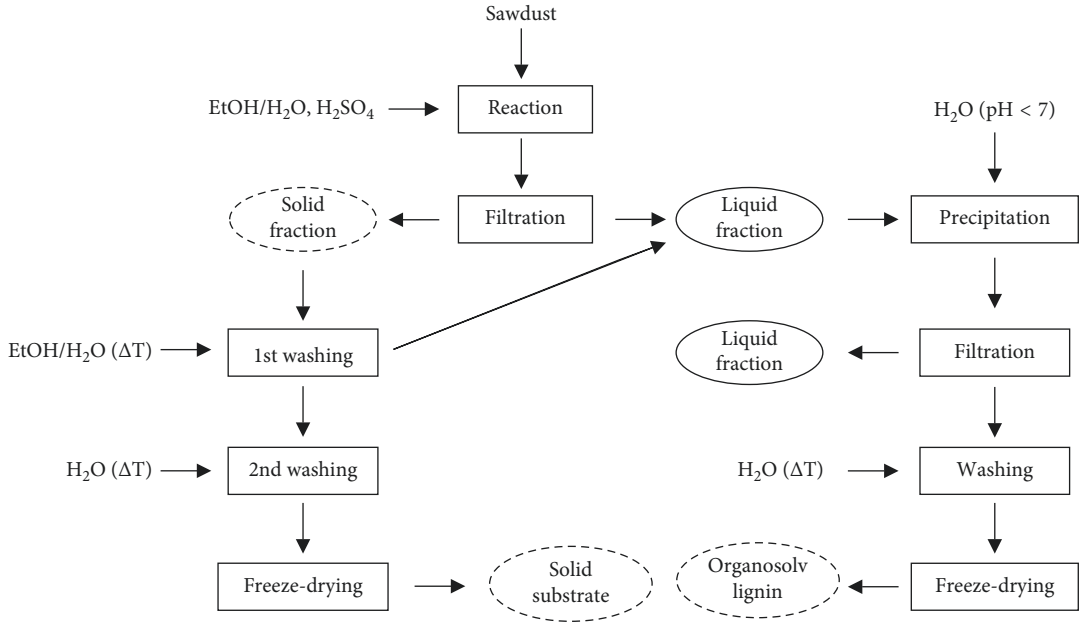

Figure 1: Schematic drawing of the organosolv fractionation and workup procedure. Product fractions are written in bold, dashed lines indicate solid fractions, whereas solid lines indicate liquid fractions.

[23] with minor modifications. For a detailed description of the method, please see Supplementary Materials. The yield of purified milled wood lignin was 4.0% based on dry extractive-free wood.

2.4. Analytical Protocols

2.4.1. Chemical Characterization. The humidity of the softwood sawdust was determined using an MA23 infrared moisture analyzer (Satorius AG, Germany). Klason lignin and acid-soluble lignin [24] of the feedstock and cellulose-rich organosolv fraction, hot-water- and acetone-soluble extractives [25], and ash content [26] of the feedstock were analyzed in accordance to standards published by the Technical Association of the Pulp and Paper Industry (TAPPI). Carbohydrate content of the larch sawdust was determined based to a multistep-method, comprising sawdust hydrolysis, reduction of released monosaccharides to the corresponding alditols, acetylation of the resulting polyols, liquid-liquid separation, and GC-FID analysis [27]. The results from the chemical characterization of larch sawdust are summarized in Table 1. Part of the organosolv lignin fraction was crudely purified for further analysis. In detail, 1.5 g of lignins was dissolved in 10 mL of HPLC-grade acetone and filtered through a 0.45 μm nylon syringe filter (Ø 33 mm, Carl Roth, Germany) before solvent removal via a rotary evaporator and drying in a vacuum desiccator.

2.4.2. Attenuated Total Reflection Fourier-Transformed Infrared Spectroscopy (ATR-FTIR). Structural features of the larch organosolv samples were analyzed using infrared spectroscopy on an FTIR spectrometer (Cary 630 FTIR, Agilent) equipped with the diamond ATR accessory running on MicroLab FTIR Software. 32 background scans and 64 sample scans were collected, at a resolution of 2 cm^{-1} from 4000 to 600 cm^{-1}. The spectra were further processed using SpectraGryph v1.2.10 software.

Table 1: Chemical composition of larch sawdust, reported as mean ($n = 3$) ± standard deviation.

Component	Content (% (w/w))
Ash	0.17 ± 0.02
Extractives (water + acetone)	12.02 ± 0.12
Klason lignin	26.90 ± 0.10
Acid soluble lignin	0.30 ± 0.02
Carbohydrates (as monomers)	
Glucose	51.15 ± 0.33
Arabinose	0.99 ± 0.02
Galactose	4.97 ± 0.25
Mannose	7.39 ± 0.07
Xylose	3.12 ± 0.14

2.4.3. Nuclear Magnetic Resonance (NMR) Analysis. The semiquantification of functional groups via ^{1}H NMR was performed based on analysis protocols published by Pan et al. [28] with minor modifications. In detail, 1 g of the purified organosolv lignin was acetylated using 20 mL of a 1 : 1 pyridine : acetic anhydride mixture, reacting for 84 h at room temperature in dark, under constant shaking in a incubating mini shaker (VWR, USA). Afterwards, the mixture was added dropwise to 200 mL of constantly stirred ice-cold water that was acidified with 2 mL of concentrated HCl. The acetylated lignin was isolated over a Büchner funnel glass frit (porosity 3), washed with distilled water until the filtrate was pH neutral, and dried in vacuum over $CaCl_2$. 50 mg of the acetylated organosolv lignins were dissolved in 600 μL DMSO-d_6, and 10 mg of p-nitrobenzaldehyde (Sigma Aldrich, USA) were added as internal

standard. All NMR spectra were recorded on a Bruker Avance III 300 MHz NMR spectrometer equipped with a 5 mm TXI probe at 298 K. For the 1D ^{1}H NMR spectra, 16 scans were acquired and multiplied with an exponential windows function with a line broadening of 0.3 Hz prior to Fourier transformation. The spectra were integrated in MestreNova 8. The content of hydroxyl groups (in mmol·g^{-1}) was calculated on the basis of hydrogen atoms contained in the internal standard compared to that of acetyl and methoxyl groups, respectively, and by integration of the following spectral regions: internal standard (8.45–7.98 ppm), aliphatic acetyl (2.17–1.70 ppm), phenolic acetyl (2.50–2.17 ppm), and methoxyl groups (4.10–3.10 ppm). Furthermore, the content of carboxylic acid groups (COOH) was determined via ^{31}P NMR; for a detailed description, see Supplementary Materials.

2.4.4. Molecular Weight Analysis. The molecular weight was estimated through high-performance size-exclusion chromatography (HPSEC) using a styrene-divinylbenzene PLgel column (Polymer Laboratories, 5 mm, 100 Å, 600 mm length, 7.5 mm inner diameter) with a photodiode array detector (Dionex Ultimate 3000 UV/vis detector) set to 280 nm UV and tetrahydrofuran (1 mL·min^{-1}) as eluent. A calibration curve based on polyethylene oxide standards (Igepal, Sigma Aldrich) and injection of pure coniferyl alcohol monomers and dimers was used for quantitative assessment. The organosolv lignins were dissolved in tetrahydrofuran and filtered through a 0.45 mm PTFE membrane filter before analysis via HPSEC.

2.4.5. Thioacidolysis. Thioacidolysis followed by GC-MS analysis was conducted based on a method published by Aguié-Béghin et al. [11]. In detail, the lignin-derived thioacidolysis monomers, namely, guaiacyl (G), p-hydroxyphenyl (H), and syringyl (S) were analyzed as trimethylsilyl derivatives via GC-MS (Saturn 2100, Varian, Agilent, USA) equipped with a poly(dimethylsiloxane) column (30 m × 0.25 mm; PB-1, Supelco, Sigma Aldrich, USA) using 5 mg of sample and 0.20 mg heneicosane ($C_{21}H_{44}$, Fluka) as internal standard. The temperature program was 313 to 453 K at 30 K·min^{-1}, followed by 453 to 533 K at 2 K·min^{-1}. An ion trap was used as a mass spectrometer set to an ionization energy of 70 eV and positive detection mode. Quantitative determination of the G and vanillin monomers (no H and S units found) was performed from ion chromatograms reconstructed at *m/z* 269, as compared with the signal from the internal standard measured from the ion chromatogram reconstructed at *m/z* (57 + 71 + 85).

2.4.6. Determination of the Antioxidative Potential. The radical-scavenging capability of the of the organosolv lignins was assessed as an indicator for the antioxidative potential, based on a method published by Dizhbite et al. [29] and was applied with minor modifications. In detail, 800 μL of ethanolic $6{,}00 \times 10^{-5}$ mol/l 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) (Sigma Aldrich, USA) was mixed with 200 μL of a 9 : 1 aqueous dioxane solution containing varying lignin concentrations (1–250 μg/mL) in 1 mL single-use microBRAND® UV cuvettes (1 cm path length; Sigma Aldrich, USA). The reaction mixture was stored in darkness at room temperature under periodical shaking for 18 min, and the absorption was consequently determined at 517 nm (λ_{max}) with a Cary 60 UV/VIS spectrometer (Agilent, USA).

To determine the quenching percentage (Q), the sample absorption (A_0) was corrected by the absorption of both the diluted lignin samples in the corresponding concentration without DPPH (A_{ref}) and of the DPPH$^{\bullet}$ solution without lignin (A_0):

$$Q = \frac{A - (A_{ref} + A_0)}{A_0} \times 100. \quad (2)$$

By plotting the quenching percentage against the lignin concentration, the EC_{50} can be determined, which is defined as the value at 50% Q. The radical-scavenging capability of the organosolv lignin samples was assessed in terms of antiradical power (ARP), defined as the reciprocal value of EC_{50} [30]. The ARPs of 3,5-di-tert-butyl-4-hydroxytoluene (BHT) and 2/3-*t*-butyl-4-hydroxyanisole (BHA) were determined as reference. All samples were analyzed in triplicate, and the mean values ± standard deviation are reported.

3. Results and Discussion

3.1. Yield of Organosolv Fractionation Experiments. Ethanol organosolv pretreatment is known to be effective at separating cellulose from lignin as well as hemicellulose in multiple feedstocks, including woody biomass [31]. Results from the catalyzed and autocatalyzed organosolv fractionation experiments of larch sawdust are summarized in Table 2. The moisture content of the larch sawdust was 19.1 ± 0.5% for all experiments. No predrying was performed because high moisture content facilitates feedstock impregnation with pulping liquor and thus increases the delignification rate [32]. The pretreatment time was set to 0.5 h as preliminary experiments have shown that there is no significant difference in lignin yield between 0.5 and 1.0 h (not shown). The maximum yield of ethanol organosolv lignin (EOL), 21.82% (w/w), was achieved at 460 K with a catalyst loading of 1.65% (w/w). The results also indicate that an increase of both temperature (440 to 460 K) and catalyst loading (0.00% to 1.65%) lead to an increase of organosolv lignin yield as well as acid-soluble lignin (ASL) in the aqueous phase. The increase in lignin yield is due to more excessive ether bond cleavage at higher process severities [11]. Similarly, the increase in ASL is caused by further fragmentation of dissolved lignin polymers to more water-soluble species. The yield of solid substrate decreased with both increasing temperature and catalyst loading due to increased delignification as well as carbohydrate degradation. The Klason lignin content of the solid substrate first decreased with increasing temperature and catalyst loading, reaching a minimum at 455 K at 0.75% (w/w) H_2SO_4. However, with increasing process severity, the rate of

Table 2: Process parameters, mass flows of the organosolv extraction experiments, and lignin content of the solid substrate.

	Process parameters[a]			Mass flows (% w/w)			KL of substrate
	T^b (K)	C (wt%.)	CSF	S	EOL	ASL	(% w/w)
OS-A	440	1.10	1.65	47.49	16.36	4.62	18.16
OS-B	445	1.10	1.80	44.52	17.61	4.77	16.54
OS-C	450	1.10	1.95	38.56	19.12	5.40	14.16
OS-D	455	0.75	1.93	38.68	19.06	5.45	14.14
OS-E	455	1.10	2.10	34.14	20.01	5.64	16.05
OS-F	460	1.10	2.24	29.74	20.83	5.85	19.76
OS-G	460	1.65	2.42	22.42	21.82	6.14	29.24
AC-OS	460	0.00	—	80.11	6.47	3.05	24.59

[a]36 g of feedstock (on dry basis), 250 mL of 75% aq. ethanol, and 30 min residence time. [b]T, temperature; C, catalyst loading on dry wood basis; CSF, combined severity factor; S, solid substrate; EOL, ethanol organosolv lignin; ASL, acid-soluble lignin in the aqueous phase; KL, Klason lignin; AC-OS, autocatalyzed organosolv.

carbohydrate degradation surpassed that of delignification and the content of Klason lignin increased, to reach a maximum of 29.24%.

Nitsos et al. [5] reported a maximum lignin yield of 16.80% for acid-catalyzed organosolv pulping of spruce wood at a CSF of 2.23 (456 K, 60 min, 1% (on dry wood basis (odw)) H_2SO_4, 60% EtOH). A significantly higher yield was achieved in this study, 20.83%, at a CSF of 2.24. However, Pan et al. [19] reported a maximum lignin yield of 24.96% for lodgepole pine, at a CSF of 2.48 (453 K, 70 min, 1.10% (odw) H_2SO_4, 75% EtOH), surpassing this work by 3.14% at a comparable CSF of 2.42.

As the ethanol organosolv fractionations were performed at both low pH and high temperature, it is possible that pseudolignins were formed. They are hypothesized to form primarily from sugar degradation products, such as furfural and 5-hydroxymethylfurfural, via first rearrangement reactions followed by oxidation, polymerization, and condensation reactions to yield aromatic components [33, 34]. These aromatic components can increase the Klason lignin content of the pulp phase and furthermore reduce enzymatic digestibility by unproductive binding with enzymes. This phenomenon is most likely to have occurred in the high severity pulping runs, OS-F and OS-G, since significant amounts of carbohydrates could not be retained in the solid substrate phase.

Furthermore, the lignin yield of the autocatalyzed fractionation experiment was low (6.47% (w/w)). Lesar et al. [4] reported a lignin yield of 17.3% for softwood under autocatalyzed conditions. However, these results were achieved under more severe conditions (493 K, 196 min, 65% EtOH). Thus, with proper reaction optimization, higher removal efficiency is possible, but still inferior to the catalyzed organosolv experiments.

CSF analysis was used to be able to easily compare and correlate ethanol organosolv fractionation yields and physicochemical characteristics of the isolated larch lignins. However, CSF was not used to analyze the processing conditions of the autocatalyzed experiment, as no acidic catalyst was used.

3.2. Influence of Process Parameters on Characteristics of Larch Organosolv Lignin. FT-IR analysis is a commonly used technique to analyze structural features of lignin, due to its speed and simplicity. In Figure 2, five IR spectra are shown, including organosolv lignin from low (OS-A), mid (OS-D), and high severity (OS-G) fractionation as well as autocatalyzed organosolv lignin (AC-OS) and milled wood lignin (MWL). All spectra look similar, albeit small differences, indicating comparable chemical structures. The absorption bands of the lignin samples were assigned based on published studies [35, 36], which are summarized in Table S1 in the Supplementary Materials. The strong broad signals at 3420 cm^{-1} are ascribed to hydroxyl bond (O-H) stretching. The signals at 2930 and 3855 cm^{-1} correspond to C-H bond stretching in methyl- and methylene groups. The band at 1700 cm^{-1} is ascribed to C=O bond stretching in unconjugated systems. Three peaks associated with aromatic skeletal vibrations are also found, at 1600, 1510, and 1420 cm^{-1}. A relative decrease of all three peaks in the organosolv lignins compared to MWL indicates that structural degradation of the lignin structure occurred during the fractionation process. The band at 1360 cm^{-1} is assigned to phenolic hydroxyl vibrations. The peak at 1264 cm^{-1} is characteristic for ring vibrations of guaiacyl monomers. Four bands correspond to aromatic C-H bond vibrations typically found in guaiacyl monomers, 1140 cm^{-1}, 1030 cm^{-1}, 850 cm^{-1}, and 813 cm^{-1}. The lack of characteristic syringyl (S) vibrations at 1330 and 1120 cm^{-1} and of p-hydroxyphenyl (H) vibrations at 1168 and 833 cm^{-1} in all IR spectra prove that lignin in European larch is indeed G-type. The peaks at 1215 and 1126 cm^{-1} can both be ascribed to C=O stretching vibrations, whereas the former also includes C-C and C-H bond stretching vibrations in CH_3.

High-performance size-exclusion chromatography was performed, to assess the impact of treatment severity on molecular mass distribution of the resulting organosolv lignin fraction. Although average molecular mass estimation based on a polystyrene calibration is by no means able to produce accurate absolute masses, it is indeed a valid tool for comparative purposes. Results listed in Table 3 indicate that the differences between number average molecular weights (M_n) of organosolv lignins from low (440 K, 1.10 % H_2SO_4) and high severity (460 K, 1.65% H_2SO_4) fractionation experiments are insignificant, 1058 g·mol^{-1} and 932 g·mol^{-1}, respectively. Weight average molecular weights (M_w) decrease with increasing process severity, from 2501 g·mol^{-1} (OS-A) to 1980 g·mol^{-1} (OS-G). As expected, larch organosolv lignin from the autocatalyzed pulping run exhibits the lowest M_w (1655 g·mol^{-1}) as it is less efficient at removing lignin from the feedstock. The average molecular weights and their variance between low and high severity fractionation experiments are significantly smaller compared to previously published data with similar fractionation conditions, with M_w ranging from 3.2×10^3 to 6.5×10^3 [37] and 2,240 to 4,600 [20], respectively.

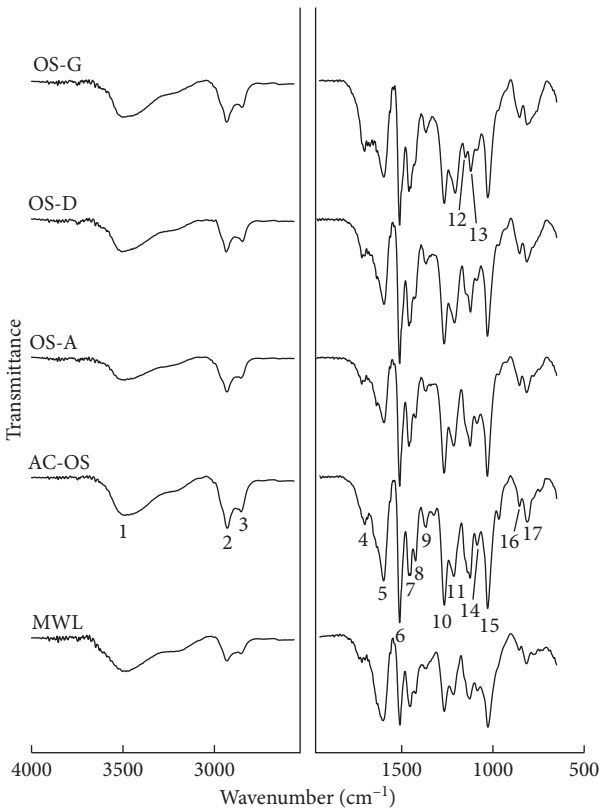

Figure 2: ATR-FTIR spectra of larch lignin samples; for peak identification, see Supplementary Materials.

Table 3: Results from molecular weight analysis of larch organosolv samples.

	CSF	M_n (g·mol^{-1})	M_w (g·mol^{-1})	M_w/M_n
OS-A	1.65	1058	2501	2.36
OS-B	1.80	1096	2296	2.09
OS-C	1.95	1117	2403	2.15
OS-D	1.93	1063	2281	2.15
OS-E	2.10	1064	2223	2.09
OS-F	2.24	970	2022	2.08
OS-G	2.42	932	1981	2.13
AC-OS	—	998	1655	1.66

Proton NMR analysis is known to be a reliable tool to semiquantify the content of functional groups of acetylated lignin samples, specifically phenolic (PhOH) and aliphatic hydroxyl groups (AlkOH) as well as methoxyl groups (MeO) [38, 39]. The result shown in Table 4 indicates a direct correlation ($r = 0.9894$) between process severity and content of phenolic hydroxyl groups, increasing from 3.15 mmol·g^{-1} (OS-A) to 4.15 mmol·g^{-1} (OS-G). This increase can be attributed to a more efficient cleavage of α- and β-aryl ether bonds at higher temperatures and catalyst loading [40]. These results are in good accordance with the literature, where PhOH contents for organosolv lignins from 3.01 to 4.25 mmol·g^{-1} for lodgepole pine [20], 2.34 to 4.04 mmol·g^{-1} for *Miscanthus x giganteus* [37], and 2.21 to 4.38 mmol·g^{-1} for hybrid poplar [28] were reported.

On the contrary, the content of aliphatic hydroxyl groups is decreasing with increasing severity, from 4.15 to 3.25 mmol·g^{-1}. It was proposed by McDonough [41] that this phenomenon is also associated with acid-catalyzed β-aryl ether cleavage. In detail, a γ-hydroxymethyl group is released as formaldehyde, forming an enol ether bond on the lignin side chain that is consequently broken [42]. Thus, the same mechanisms leading to an increase of PhOH groups during the organosolv fractionation process led to a decrease of AlkOH groups. Additionally, during organosolv pulping, the whole side chain can be cleaved off, reducing the AlkOH content further. The determined concentration range for aliphatic hydroxyl groups corresponds well to literature values, ranging from 3.77 to 4.72 mmol·g^{-1} for lodgepole pine [20], 1.07 to 3.11 mmol·g^{-1} for *Miscanthus x giganteus* [37], and 2.73 to 5.01 mmol·g^{-1} for hybrid poplar [28].

As can be seen in Table 4, the total amount of free hydroxyl groups does indeed not change significantly between all the acid-catalyzed fractionation experiments, just a slight increase from OS-C to OS-G can be observed. Both the MWL and the autocatalyzed organosolv lignin contain a significantly higher amount of total hydroxyl groups, 9.12 mmol/g and 8.65 mmol/g, respectively, which is in good accordance with the literature [43]. Furthermore, the content of free carboxylic acid groups was low in all larch organosolv fractions, ranging from 0.07 to 0.2 mmol·g^{-1}. The highest content was achieved in the autocatalyzed sample because of its comparably least severe method of extraction. However, no significant difference can be observed within the acid-catalyzed organosolv samples.

Methoxyl group content ranges from 5.77 to 6.27 mmol·g^{-1}. Pearson correlation analysis indicates that the content of MeO in larch organosolv lignin is independent of the pulping conditions ($r = -0.4644$). These observations are consistent with data published by Pan et al. [28] on the correlation of organosolv fractionation conditions to physicochemical lignin properties of hybrid poplar and Gilarranz et al. [40] on autocatalyzed methanol pulping of Tasmanian blue gum. The MWL sample exhibits the lowest content of PhOH and the highest content of AlkOH. This is well documented in the literature, as the Björkman method [44] is the most benign lignin extraction process, conserving most of its natural structural features. Additionally, the content of methoxyl groups is significantly lower in both MWL and the autocatalyzed organosolv lignin compared to the sulfuric acid catalyzed organosolv samples. This can be explained by nucleophilic addition of ethanol on the benzylic position during acid catalyst pulping and a general higher lignin extraction efficiency [45]. The high content of AlkOH in the autocatalyzed organosolv sample despite its comparably high content of PhOH can be explained due to the absence of an acidic catalyst as described above. Of the eight different organosolv lignins investigated in this study, OS-G probably has the highest applicability as it contains the highest amount of phenolic hydroxyl groups. A higher content of free hydroxyl groups correlates increased chemical reactivity, thus making OS-G an excellent candidate for use as an antioxidant or as a monomer for biopolymer production.

Table 4: Functional group content of the larch organosolv samples. PhOH-, AlkOH-, and MeO contents were determined via ^{1}H NMR and COOH content via ^{31}P NMR.

	CSF	PhOH[a] ($mmol \cdot g^{-1}$)	AlkOH ($mmol \cdot g^{-1}$)	Total OH ($mmol \cdot g^{-1}$)	PhOH/AlkOH ratio	MeO ($mmol \cdot g^{-1}$)	COOH ($mmol \cdot g^{-1}$)
OS-A	1.65	3.15	4.15	7.30	0.76	6.27	0.09
OS-B	1.80	3.45	3.91	7.36	0.88	6.05	0.07
OS-C	1.95	3.50	3.72	7.22	0.94	5.92	0.07
OS-D	1.93	3.47	3.73	7.20	0.93	5.77	0.07
OS-E	2.10	3.74	3.60	7.34	1.04	6.09	0.09
OS-F	2.24	3.91	3.51	7.42	1.11	5.71	0.09
OS-G	2.42	4.15	3.25	7.40	1.28	6.01	0.1
AC-OS	—	3.60	5.05	8.65	0.71	4.78	0.2
MWL	—	3.08	6.04	9.12	0.51	4.16	—[b]

[a]PhOH, phenolic hydroxyl groups; AlkOH, aliphatic hydroxyl groups; MeO, methoxyl groups; COOH, carboxylic acid groups. [b]Not analyzed via ^{31}P NMR.

To get a more complete view on the influence of pulping conditions on changes in the lignin structure, thioacidolysis of the larch organosolv samples was performed, and the results are summarized in Table 5. Thioacidolysis is based on acid-catalyzed depolymerization of lignins with a high specificity to aryl ether bonds. A high content of releasable monomers therefore indicates more benign extraction conditions and consequently a less altered lignin structure [46]. Additionally, a high content of releasable monomers correlates to a low content of phenolic hydroxyl groups, as they are primarily formed during β-ether bond cleavage. Only guaiacyl (G) thioacidolysis monomers and vanillin, an oxidized guaiacyl derivate, were detected, neither p-hydroxyphenyl-(H) nor syringyl (S) monomers. This can be explained by the limited natural occurrence of both monomers in conifers of maximum 8 % and 2 %, respectively [47], in addition to a higher liability of syringyl units towards ether cleavage. The content of releasable G-units and vanillin both decreases with increasing processing severity from 171 $\mu mol \cdot g^{-1}$ to 242 $\mu mol \cdot g^{-1}$ (OS-B) to 11 $\mu mol \cdot g^{-1}$ and 99 $\mu mol \cdot g^{-1}$ (OS-G), respectively. This trend is in good accordance with the results of ^{1}H NMR analysis, and the maximum content of releasable monomers correlates to the minimum of PhOH hydroxyl groups and vice versa. The lower thioacidolysis yields of OS-A compared to OS-B could be explained due to the less-efficient lignin extraction in the presence of the same sulfuric acid loading. As expected, the content of releasable monomers is highest in the autocatalyzed lignin sample due to the relatively benign extraction conditions.

3.3. Correlation between Process Parameters and Antiradical Potential. As parameter to assess the reactivity of isolated larch organosolv lignins, their respective antiradical potential (ARP) was chosen. The ARP was analyzed using DPPH$^{\bullet}$ as a radical source in aqueous dioxane and the loss of absorption at 517 nm as indicator for the scavenging capability. As can be seen in Figure 3, the pulping conditions do have a significant impact on the ARP of the resulting organosolv lignin fractions. Larch sawdust extracted under the most benign conditions (440 K, 1.10% H_2SO_4) yielded in organosolv lignin with the smallest ARP of 66.1, corresponding to the highest EC50 of 15.6 $\mu g \cdot mL^{-1}$. With increasing process severity, the radical scavenging capability also rises, up to a maximum of 100.7 (460 K, 1.10 wt% H_2OS_4), equal to an EC50 of 9.9 $\mu g \cdot mL^{-1}$. The autocatalyzed organosolv lignin sample (AC-OS) achieved an ARP of 82.0, comparable to the samples isolated at 455 K at 0.75 (OS-D) and 1.10% (w/w) H_2SO_4 (OS-E), respectively. Statistical analysis of the relationship between process severity and ARP demonstrate a strong direct correlation ($r = 0.9976$). This finding is in good accordance with previously published data on organosolv lignins of *Miscanthus x giganteus* [37], hybrid polar [28], and aspen, spruce, and birch wood [29].

Table 5: Yields from thioacidolysis and GC-MS of the thioethylated guaiacyl (G) monomer derivatives (mean of two independent analyses).

	CSF	G-units ($\mu mol \cdot g^{-1}$)	Vanillin·($\mu mol\ g^{-1}$)
OS-A	1.65	136	298
OS-B	1.80	171	242
OS-C	1.95	156	209
OS-D	1.93	116	232
OS-E	2.10	58	175
OS-F	2.24	24	127
OS-G	2.42	11	99
AC-OS	—	344	233

The content of nonetherified phenolic hydroxyl groups is primarily responsible for the antioxidative potential of lignin [29, 48]. Indeed, as can be seen in Figure 3, the organosolv sample with the lowest content of PhOH exhibits the lowest antioxidative potential and vice versa (66.1/3.15 $mmol \cdot g^{-1}$ vs. 100.7/4.15 $mmol \cdot g^{-1}$). As expected, the content of aliphatic hydroxyl groups is indirectly correlated ($r = -0.9844$) to ARP because of their indirect relationship to PhOH. Results further indicate that the contribution of methoxyl group to ARP is negligible. However, more recent findings by Ponomarenko et al. [48] indicate that o-methoxyl groups indeed have a positive effect on DPPH$^{\bullet}$ scavenging capacity. This discrepancy can be explained by the large variety of lignins used in the aforementioned study, including grass, hardwood, and softwood lignins from different technical processes.

Furthermore, results from this study show that average molecular weights and antioxidant activity have a weak to very weak indirect correlation ($r(\mathrm{Mn}) = -0.7564$,

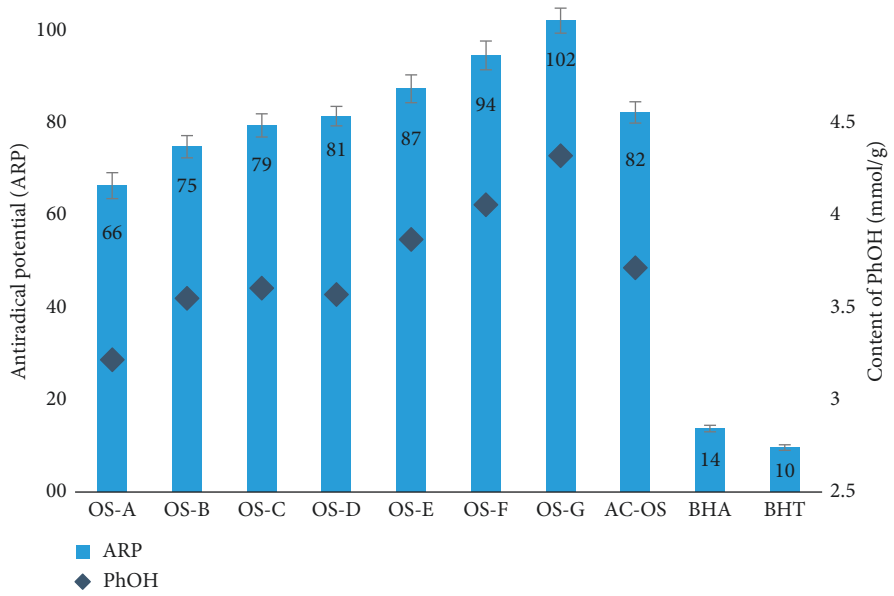

Figure 3: Relationship between antiradical potential and content of phenolic hydroxyl groups; ARP is reported as mean ($n = 3$), and the error bars represent the standard deviation.

$r(\mathrm{Mw}) = -0.5722$). The difference in average molecular weights between low and high severity pulping conditions is quite low, as discussed above, and significantly smaller compared to previously reported results [28, 37, 49]. Thus, the average molecular weights cannot be used as proper indicator for the potential antioxidative potential of larch organosolv lignins.

However, a direct comparison of antioxidative potential is typically complicated by distinct differences in the DPPH$^{\bullet}$ analysis conditions, such as radical concentration, reaction time, and solvent ratio/volume. The results by Pan et al. [28] were achieved under quite similar conditions. Their results for EC_{50} of hybrid poplar organosolv lignins range from 8.2 to 80.0 $\mu g \cdot mL^{-1}$, compared to 9.9 to 15.6 $\mu g \cdot mL^{-1}$ for the European larch in this study. This parity indicates that under similar pulping conditions, organosolv lignin from the European larch can achieve antioxidative potentials similar to that of hardwood lignins.

4. Conclusion

In this study, for the first time, the relationship between physicochemical characteristics and pulping conditions of ethanol organosolv lignins from the European larch are investigated. This study should serve as the initial step for possible future applications of larch organosolv lignins as antioxidant or feedstock for biopolymer production. ATR-FTIR, ^{1}H NMR, ^{31}P NMR, HPSEC, thioacidolysis, and an antioxidative assay are used as analytical tools. The harshest reaction conditions (OS-G), 460 K and 1.10 % (w/w) H_2SO_4, resulted in the highest yield of organosolv lignin (21.82%). Additionally, OS-G has the highest content of phenolic hydroxyl groups (4.15 $mmol \cdot g^{-1}$) and antiradical potential as well as the lowest content of releasable guaiacyl monomers by thioacidolysis (11 $\mu mol \cdot g^{-1}$). OS-A shows the lowest PhOH content (3.15 $mmol \cdot g^{-1}$), lowest antiradical potential, and highest content of releasable guaiacyl monomers (484 $\mu mol \cdot g^{-1}$). The autocatalyzed organosolv lignin exhibited the highest aliphatic hydroxyl group (5.05 $mmol \cdot g^{-1}$) content and lowest weight average molecular weight. Both the high antiradical potential and content of free phenolic hydroxyl groups indicate that larch organosolv lignin indeed has potential for further valorization.

Further investigations are planned, to properly assess potential future applicability of the material. These include method optimization for the organosolv pulping process, alternative fractionation methods, such as Kraft, Organocell, and γ-valerolactone pulping, and additional analysis techniques, such as zeta potential, 2D HSQC NMR, and thermal analyses.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supplementary Materials

The supplementary materials contain a detailed description of milled wood lignin isolation and purification, ^{31}P NMR analysis, and a table containing a detailed assignment of the

absorption bands from the IR analysis of the lignin samples. (*Supplementary Materials*)

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