

Lignin Carboxymethylation: Probing Fundamental Insights into Structure–Reactivity Relationships

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Cite This: <https://doi.org/10.1021/acssuschemeng.3c07385>

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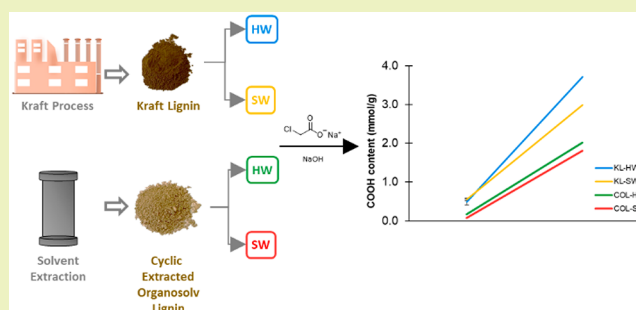


Supporting Information

ABSTRACT: Amidst declining fossil-based resources and environmental challenges, the focus on biobased materials has intensified. Carboxymethylation is one way to introduce reactive functionality to enhance the reactivity of lignin for a specified application. This research investigates the carboxymethylation of four lignin sources: eucalyptus kraft lignin, spruce kraft lignin, birch cyclic extracted organosolv lignin, and spruce cyclic extracted organosolv lignin. Our aim is to elucidate the role of the lignin structure in its reactivity. Using the advanced analytical techniques NMR spectroscopy, Fourier transform infrared spectroscopy, density functional theory, and size-exclusion chromatography, we provide a comprehensive characterization of the modified lignin.

The findings offer valuable insights into how the chemical and physical properties of molecular lignin affect the selectivity and efficiency of the carboxymethylation reaction. These fundamental findings hold great potential for guiding considerations on the selection of lignin sources for specific applications based on their molecular properties.

KEYWORDS: kraft lignin, cyclic extracted organosolv lignin, carboxymethylation, reactivity, sustainability



INTRODUCTION

The decreasing supply of fossil fuels and environmental concerns highlight the need for materials from renewable sources, especially in making polymers, which usually depend on oil-based ingredients.^{1,2} Lignin, a natural resource, is a promising alternative. By incorporating lignin, we can lessen our reliance on nonrenewable resources, supporting a move toward an ecofriendlier approach in materials science. Lignin stands out due to its abundance as a renewable aromatic structure combined with robust mechanical properties and thermal stability, positioning it as an ideal candidate for thermoset materials.^{3–10}

Lignin is a complex biopolymer predominantly comprised of three aromatic monolignols: *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol.^{11–13} These are characterized by their methoxy group substitutions at meta positions, forming *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units in the lignin structure.^{11–13} The composition of these units within lignin is intrinsically influenced by its botanical origin. For instance, hardwood-derived lignin primarily encompasses S and G units with minimal H units, whereas softwood-derived lignin predominantly contains G units and traces of H units. This inherent structural difference significantly influences molecular properties such as solubility, reactivity, and mechanical and thermal stability.^{14,15} Advancements in thermoset materials, particularly those derived from lignin, increasingly emphasize their recyclability and degradability.

Such attributes can promote a circular lifecycle, addressing postuse concerns. Although degradability/recyclability depends on certain environmental conditions, this particular property can be attained by designing cleavable linkages in the molecular structure of the material itself.¹⁶

One approach of designing degradable/recyclable lignin-based thermoset materials is the introduction of cleavable functionalities such as ester, ether, or amide linkages to the lignin structure. Among the various chemical modification techniques available to modify lignin functionalities, carboxymethylation stands out due to its ability to incorporate the lignin structure with a high content of carboxylic acid groups.¹⁷ These newly incorporated carboxylic groups can act as pivotal chemical moieties, facilitating cross-linking reactions essential for the development of lignin-based thermoset materials. Carboxymethylation procedures are well established for cellulose. Some studies have proven that carboxymethylation worked for chemical modification of lignin for various applications, including stabilizing agents,^{18,19} dispersants,^{20,21} retarder additives,²² and heavy-metal absorption.^{23–25} How-

Received: November 10, 2023

Revised: December 19, 2023

Accepted: December 20, 2023

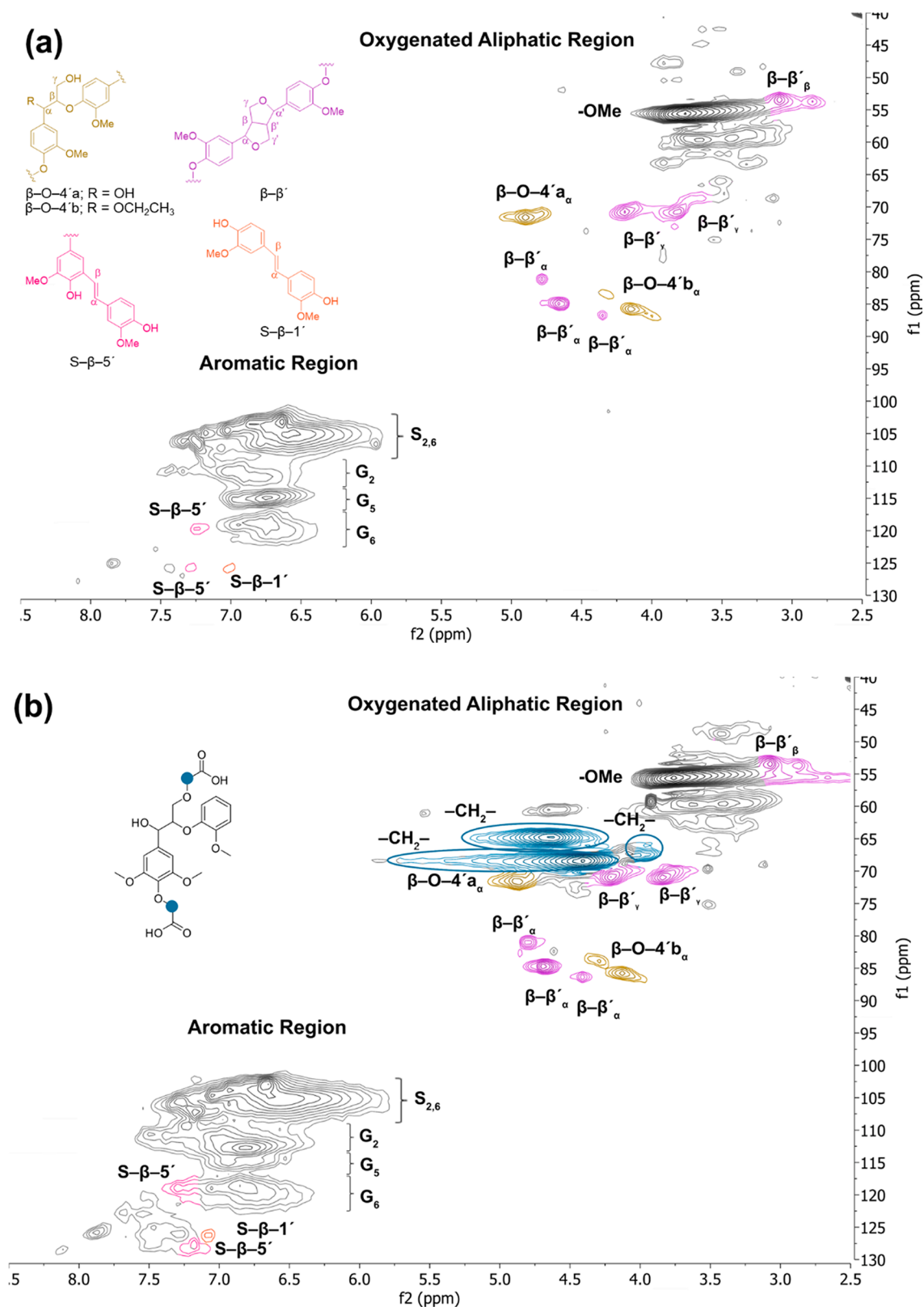


Figure 1. 2D HSQC NMR spectra of (a) reference (EKL) and (b) carboxymethylated eucalyptus Kraft lignin (CEKL_110_6), with f1 for ^{13}C and f2 for ^1H .

ever, most of the literature in the fields focuses on the solubility and charge density properties of carboxymethylated lignin. In the interest of tailoring the lignin properties for specific applications, fundamental insights into the reactivity and

selectivity of the reactions of its hydroxyl groups is essential. Therefore, to envisage applications of lignin as a precursor for biobased materials, the structure and reactivity relationships need to be elucidated.

However, harnessing lignin as a raw material is not straightforward. In its pristine state, lignin exhibits a profound heterogeneity, with its three main monolignols interconnecting in different patterns and their proportions changing based on the source plant species.²⁶ The extraction methodologies further complicate its characterization, introducing notable changes in the lignin structure. One lignin extraction procedure is the kraft process used in pulp production, wherein lignin is separated by intense chemical treatment.³ Subjecting lignin to this treatment induces significant alterations in its molecular structure, which deviates considerably from its native structure. Positively, however, the large production of technical lignin assures an extensive supply, paving the way for innovative applications.³ Among the advancements, the Lignoboost technique stands out, effectively extracting lignin from the kraft black liquor.²⁷

To overcome the challenge regarding the unknown structure of technical lignin, the use of a cyclic extracted organosolv lignin, obtained from our recently developed biorefinery process, was considered because the native lignin interunit linkages are highly preserved.^{28–30}

In this work, the carboxymethylation procedure, aimed at optimizing conditions and selectivity, was performed on four different lignin sources, eucalyptus kraft lignin, spruce kraft lignin, birch cyclic extracted organosolv lignin, and spruce cyclic extracted organosolv lignin, providing an opportunity to study the carboxymethylation efficiencies as a function of the lignin structure and source. We have examined the pivotal role of the lignin structure in determining the reactivity of its aliphatic and phenolic hydroxyl groups. Utilizing state-of-the-art NMR, Fourier transform infrared spectroscopy (FT-IR), density functional theory (DFT), and size-exclusion chromatography (SEC) techniques, we characterized the derived lignin samples to determine their chemical and physical attributes.

RESULTS AND DISCUSSION

Synthesis of Carboxymethylated Lignin. Carboxymethylation is a chemical process characterized by the reaction of hydroxyl (–OH) moieties with carboxymethyl (–CH₂–COOH) functionalities. The changes in the hydroxyl and carboxyl group content are intrinsically influenced by several critical parameters, including the alkaline medium concentration, carboxymethylation reagent concentration, reaction time, and temperatures.^{17,20}

Increases in the carboxylic acid group content within the lignin structure can be elucidated by contrasting the FT-IR spectra of the reference lignin and carboxymethylated lignin samples. As depicted in Figures S1–S11, the FT-IR spectra exhibit a pronounced peak intensity at 1720 cm^{–1}, attributable to the carbonyl group C=O, signifying an increase in the number of carboxylic acid groups within the lignin structure. Additionally, alterations in the stretching bands observed at 3400 and 3100 cm^{–1}, corresponding to aliphatic and phenolic hydroxyl groups, respectively, further indicated the occurrence of carboxymethylation on both aliphatic and phenolic hydroxyl sites.²⁰ Therefore, the FT-IR data confirmed the successful incorporation of carboxylic acid groups into the selected lignin samples. To further achieve a more comprehensive understanding of the structural modifications in both aliphatic and phenolic hydroxyl groups of the lignin samples, as well as the effects of the reaction conditions on lignin interunits,

characterization using ³¹P NMR and 2D NMR experiments was performed.

Structural Analysis. Advanced NMR techniques, including 2D heteronuclear single-quantum coherence (HSQC), 2D dept-ed-HSQC, and 2D heteronuclear multiple-bond correlation (HMBC) NMR, were employed to examine the structural modifications in the carboxymethylated lignin samples. The 2D HSQC NMR spectra in Figure 1 compare the reference and carboxymethylated eucalyptus kraft lignin samples, with peak assignments based on literature.^{29,31} Figure 1 highlights the spectral alterations postcarboxymethylation of eucalyptus kraft lignin, most notably the appearance of two new peak signals: ¹H/¹³C at $\delta = 4.02/67.6$ ppm corresponding to the methylene signals of the carboxymethyl groups inserted in the aliphatic region.³² The methylene signals corresponding to insertion of the carboxymethyl groups into the guaiacyl (G) units appeared at $\delta = 4.63/64.7$ ppm. Simultaneously, those aligned with syringyl (S) units appeared at $\delta = 4.40/68.3$ ppm.

Figures S18 and S24 show the appearance of two distinct carboxymethylation peaks associated with spruce kraft lignin and spruce cyclic extracted organosolv lignin. Specifically, the peaks were observed at ¹H/¹³C resonances of $\delta = 4.02/67.6$ and $4.66/64.7$ ppm and $\delta = 4.00/67.6$ and $4.61/64.7$ ppm for both lignin types, respectively. The two methylene signals attest to the carboxymethylation occurring on two distinct sites: aliphatic and phenolic hydroxyl.

The 2D dept-ed-HSQC and 2D HMBC techniques, as shown in Figures S25–S28, effectively confirm carboxymethylation in all samples. This is evidenced by the appearance of methylene protons in both the aliphatic and aromatic regions.

Influence of the Carboxymethylation Conditions on the Lignin Structure. To determine whether, and to what extent, the carboxymethylation reaction influenced interunit connections within the lignin structure, semiquantitative analysis of lignin interunit linkages, presented in Table S3, was derived from the 2D HSQC spectra of spruce cyclic extracted organosolv lignin samples, using both the reference and carboxymethylated lignin samples. The cyclic extracted organosolv lignin was chosen for this specific investigation due to its well-defined structure.

Within the scope of the established design parameters, it was observed that the β -O–4' content in carboxymethylated lignin samples was lower compared to that of the reference lignin samples. This reduction in β -O–4' linkages, ranging from approximately 14 to 32%, can be attributed to the carboxymethylation conditions, which may lead to the cleavage of sensitive linkages such as β -O–4'. Despite this decrease, the total β -O–4' content for all carboxymethylated lignin samples remained at or above 20 per 100 aromatic units (Ar).^{29,33} Such findings underscore the mildness of the carboxymethylation reaction conditions to retain the native structure of lignin. Lignin's intrinsic structure, exemplified by its β -O–4' linkages, is a critical aspect when assessing modification techniques because these structural components dictate its functionality, properties, and potential applications.

Reactivity Analysis. Effect of the Lignin Structure and Origin. To precisely quantify the carboxymethylation efficiency of the primary functional groups of lignin, we employed ³¹P NMR spectroscopy, following the methodology previously described in the literature.³⁴

The ³¹P NMR spectra of the studied samples are collected in Figures S29–S51. Figure 2 elucidates the hydroxy functionality content differentiating the reference and carboxymethylated

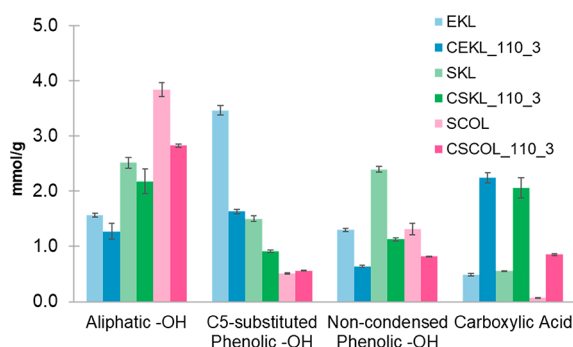


Figure 2. Comparison of the hydroxy functionalities between the reference and carboxymethylated lignin samples.

samples. Notably, the carboxymethylated samples, for eucalyptus kraft lignin, spruce kraft lignin, and spruce cyclic extracted organosolv lignin, designated as CEKL_110_3, CSKL_110_3, and CSCOL_110_3, respectively, were synthesized under specific conditions: a temperature of 110 °C and a ratio of 1:3 between the hydroxyl groups of lignin and sodium monochloroacetate. Within the context of eucalyptus and spruce kraft lignin samples, the phenolic hydroxyls demonstrated enhanced reactivity toward carboxymethylation compared to their aliphatic counterparts, likely a result of the phenolate anions generated from phenolic hydroxyls being better nucleophiles. Conversely, Figure 2 shows that spruce cyclic extracted organosolv lignin exhibited a heightened reactivity of aliphatic hydroxyls during the carboxymethylation reaction, exceeding that of phenolic hydroxyls. Upon analysis of spruce cyclic extracted organosolv lignin, conversion rates into ether methyl carboxylate groups were found to be 26% for aliphatic hydroxyls and 24% for phenolic hydroxyls, as detailed in Table S6. While phenolic hydroxyls are inherently more nucleophilic, the abundance of aliphatic hydroxyls in spruce cyclic extracted organosolv lignin results in a noticeable decrease postcarboxymethylation (Figure 2). This is contrasted with kraft lignin, which has fewer aliphatic hydroxyls. Thus, the apparent reactivity of aliphatic hydroxyls is primarily due to their higher quantity, not higher intrinsic reactivity compared to phenolic hydroxyls. Moreover, such a distinctive behavior might stem from the inherent accessibility of the aliphatic chain, combined with steric interference.³⁵

In a detailed examination of samples' postcarboxymethylation at 110 °C utilizing a 3 equimolar ratio of sodium monochloroacetate, a notable increase in the carboxylic acid content appeared. This increase was especially noteworthy when compared to the reference lignin samples, highlighting the profound chemical alterations imparted by the carboxymethylation process at high temperature and excess of sodium monochloroacetate. Interestingly, CEKL_110_3 was found to have the highest increase of the carboxylic acid content, approximately 1.8 mmol/g, followed closely by CSKL_110_3 at 1.5 mmol/g and CSCOL_110_3 at 0.8 mmol/g. This increase in the carboxylic acid content could be attributed predominantly to the effects of the lignin structure on its reactivity.

The observed reduction in aliphatic and phenolic hydroxyl groups during carboxymethylation did not correspond precisely to the increase in carboxylic acid groups. This variation might be attributed to the partial cleavage of β -O-4 linkages, which potentially leads to the formation of additional hydroxyl groups. However, this cleavage was not extensive

enough to significantly affect the molecular weight, as seen from the SEC data. Another contribution to the mismatch in consumed hydroxyls and formed carboxylic acid groups was possibly the association of the carboxylates with metal ions present in the lignin. These would evade the ³¹P NMR analysis because these formed associations prevented phosphonate derivatization, leading to an underestimation of the carboxylates.

In our review of the literature,²⁰ a variety of conditions were noted, which we initially replicated in our study. Subsequently, we explored the effects of elevated temperatures and increased the concentrations of the carboxymethylation reagent. This approach revealed an enhanced degree of substitution for carboxylic acid. The inherent wood origins dictated the distinct structural and chemical variations observed between hardwood and softwood kraft lignin. As illustrated in Figure 2, eucalyptus kraft lignin possessed a more abundant phenolic hydroxyl content compared to spruce kraft lignin. Moreover, the figure emphasizes the tendency of the carboxymethylation reaction to occur predominantly on phenolic hydroxyl sites for kraft lignin.

In contrast, originating from the cyclic biorefinery process of spruce sawdust, spruce cyclic extracted organosolv lignin retains more of the native lignin structure. Given this context, the aliphatic hydroxyl groups intrinsic to SCOL appeared to be more accessible as nucleophiles during carboxymethylation, compared to eucalyptus and spruce kraft lignin.

Effect of the Temperature and Concentration of the Reagent. Figure 3a shows the increase of the carboxylic acid

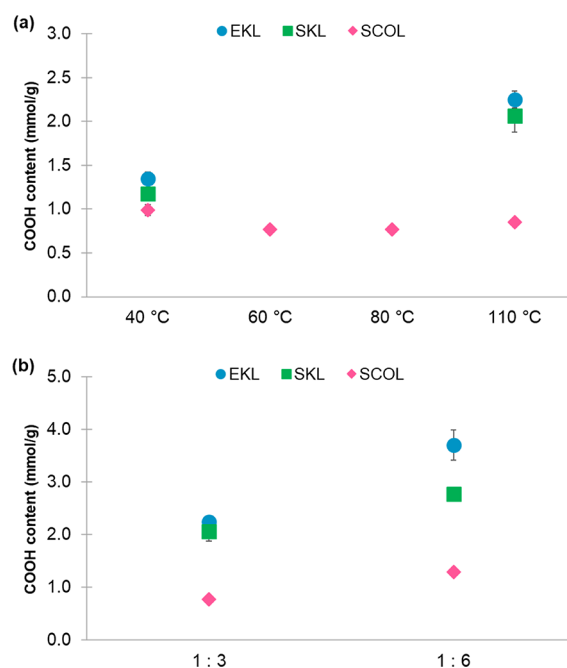


Figure 3. (a) Effect of the temperature on the carboxylic acid content of carboxymethylated lignin samples. (b) Effect of the concentration of sodium monochloroacetate on the carboxylic acid content of carboxymethylated lignin samples.

content in carboxymethylated lignin as influenced by temperature variations. For kraft lignin samples, elevating the reaction temperature from 40 to 110 °C markedly increased the carboxylic acid content from 1.3 to 2.2 mmol/g for carboxymethylated eucalyptus kraft lignin and likewise from 1.2 to 2.1 mmol/g for its carboxymethylated spruce kraft

Table 1. Functional Groups and Yields of Both Soluble and Insoluble Fractions of the Carboxymethylated Lignin Samples

sample ^a	aliphatic –OH (mmol/g) ^b	C5-substituted phenolic –OH (mmol/g) ^b	noncondensed phenolic –OH (mmol/g) ^b	carboxylic acid (mmol/g) ^b	yield (%) ^c	conversion (%) ^d
CEKL_110_6_sol	1.1	0.7	0.3	3.7	60	50
CEKL_110_6_ins	1.3	0.9	0.4	2.8	35	36
CSKL_110_6_sol	2.1	0.8	0.7	3.0	96	37
CSKL_110_6_ins	1.6	0.7	0.6	1.6	7	16
CBCOL_80_6_sol	2.7	0.5	0.2	2.0	65	35
CBCOL_80_6_ins	3.5	0.3	0.2	1.0	35	16
CSCOL_80_6_sol	3.2	0.3	0.4	1.8	39	31
CSCOL_80_6_ins	3.1	0.4	0.4	1.1	58	19

^aThe carboxymethylation reactions were performed at the optimum conditions for the respective lignin types. ^bThe content of functional groups in lignin was obtained from the ³¹P NMR results on the carboxymethylated lignin samples. ^cThe yields of both soluble and insoluble fractions were calculated based on the dry mass of soluble and insoluble fractions after the carboxymethylation reaction, compared to the initial mass of lignin before the reaction (corrected by 0.08 g/mmol as a net increase in mass for each carboxymethyl groups attached in the lignin structure).³⁶ ^dThe conversion was calculated based on the amount of COOH formed in carboxymethylated lignin divided with the initial OH groups in lignin.

lignin. In contrast, for spruce cyclic extracted organosolv lignin, an increase in the temperature resulted in a reduction of the carboxylic acid content in the lignin structure, albeit the change was relatively subtle.

Figure 3b shows the influence of the molar ratio between the hydroxyl group in lignin and sodium monochloroacetate on the carboxylic acid content of carboxymethylated lignin. When subjected to the highest temperature conditions (110 °C), a significant increase in the carboxylic acid content was observed with the 6 equimolar ratio of sodium monochloroacetate, provided that all other variables remained constant. Notably, cyclic extracted organosolv lignin diverged from this pattern, precipitating immediately upon reaction initiation, thereby halting the process.

With respect to kraft lignin, doubling the sodium monochloroacetate concentration from a 3 to 6 equimolar ratio resulted in an increase of the carboxylic acid content from 2.2 to 3.7 mmol/g in carboxymethylated eucalyptus kraft lignin and from 2.1 to 2.8 mmol/g for carboxymethylated spruce kraft lignin. Given the incompatibility of the reaction conditions of 110 °C and $[\text{OH}]/[\text{ClCH}_2\text{COONa}] = 1:6$ with spruce cyclic extracted organosolv lignin, the investigation concerning the reagent's concentration effect was undertaken at a temperature of 80 °C. At a reaction temperature of 80 °C, an increased concentration of sodium monochloroacetate yielded an increase in the carboxylic acid content from 0.8 to 1.3 mmol/g, a change that, while observable, remained relatively modest.

It is evident that the differences in the chemical properties of lignin are responsible for the observed differences in the carboxymethylation efficiencies and selectivity. For instance, under specific conditions, the carboxymethylation efficiency of eucalyptus kraft lignin was superior to that of spruce kraft lignin. Spruce cyclic extracted organosolv lignin, which is superior to kraft lignin with respect to the abundance of native bonds, was the least prone to the carboxymethylation reaction. Interestingly, complete carboxymethylation of the hydroxyl groups was not achieved for either lignin type. This incomplete conversion may be attributed to the varying solubility of each lignin type in alkaline solutions and the effects of the lignin heterogeneity on its reactivity. To determine the influence of these factors on the efficiency of the carboxymethylation reaction, additional sets of carboxymethylation experiments were conducted on eucalyptus kraft lignin, spruce kraft lignin, birch cyclic extracted organosolv lignin, and spruce cyclic extracted organosolv lignin (Table 1). In these experiments,

the alkaline soluble and insoluble fractions were separated upon completion of the carboxymethylation and analyzed separately.

Table 1 shows that, even when lignin was entirely soluble in an alkaline solution, a complete conversion was not achieved. A notable observation from the data is the variance in the carboxylic acid content between soluble and insoluble lignin fractions. Specifically, the lignin fraction that was soluble exhibited a more pronounced presence of carboxylic acid relative to the insoluble fraction. For instance, the sample CEKL_110_06_sol exhibited a conversion rate of 50%, which was significantly compared to its insoluble counterpart, CEKL_110_06_ins, with a conversion rate of 36%. This pattern of enhanced performance in the soluble fraction was further evident in the comparison of CSKL_110_06_sol and CSKL_110_06_ins, where the soluble fraction showed a conversion of 37%, surpassing the insoluble fraction (16% conversion) by 21%. Similar trends were observed in CBCOL_80_6_sol (35% conversion) versus CBCOL_80_6_ins (16% conversion) and CSCOL_80_6_sol (31% conversion) compared to CSCOL_80_6_ins (19% conversion), although the difference in the latter pair was less marked. These results suggested that solubility affected the carboxymethylation efficiency and reactivity but was not the sole determinant. Lignin's heterogeneity, which made some fractions more prone to carboxymethylation, along with structural and compositional variations in different lignin fractions, could also play a significant role in reactivity.

Another intriguing trend observed relates to the source and processing methods of lignin. Hardwood lignin, regardless of whether it was obtained from the kraft process or the cyclic extraction organosolv method, displayed a superior carboxymethylation efficiency compared to softwood lignin extracted through the same methods. To better understand this phenomenon, we used DFT calculations to explore the distinct behaviors of hardwood and softwood lignin. Our focus was on their carboxymethylation reaction energetics, charge density, and molecular conformation in aqueous solutions.

DFT Calculations on Lignin Oligomers and Reactivity Relationships. Using β -aryl ethers, the most common interunit in native lignin, the energetics of the carboxymethylation reaction on phenolic hydroxyl was determined using DFT methods. Figure S52 shows the Gibbs free energetics of the reaction for the carboxymethylation of phenolic anions of both stereoisomers (*erythro* and *threo*). The results show that the carboxylation of phenolic hydroxyl in *erythro*-aryl ether

isomers of the syringyl dimer was more exothermic than that of the corresponding guaiacyl unit, while among the *threo* stereoisomers, this trend was reversed. It is also worth noting that the *erythro:threo* ratio of β -aryl ethers in wood lignin is reported at about 2.8.³⁷ Given this stereochemical preference and the greater exergonicity of the *erythro* stereoisomer, these results are consistent with the experimentally observed results, which showed that hardwood lignin, which is dominated by S units, was more prone to carboxymethylation than softwood lignin, which is dominated by G units.

Next, we investigated the accessibility of the hydroxyl groups. Such accessibility is influenced by the molecular conformation of lignin in specific media. Lignin molecules exhibit rigid and flexible bonds; notably, the β -O-4' bond is flexible, permitting rotation and folding and facilitating intermolecular π - π stacking.^{33,38} Therefore, these molecular conformations could possibly play an important role in the carboxymethylation reaction. To examine this, DFT modeling experiments were initiated on β -O-4' hexamers of hardwood lignin (comprising four S units and two G units) and softwood lignin (six G units) (Figure 4). We recently verified the

their differential reactivities in the carboxymethylation reaction. Specifically, additional methoxy groups in S units provided greater external exposure of hydroxyl groups in the hardwood configuration, which further suggested higher accessibility. Conversely, the softwood hexamer's inward-facing hydroxyl groups, confined within the spiral, were surrounded by a hydrophobic environment, which, we reason, to be inaccessible to the hydrophilic reactants. Thus, the inherent structural differences between these hexamers could help to explain the observed heightened reactivity of hardwoods in carboxymethylation reactions compared to softwoods.

Parts b and d of Figure 4 provide detailed insights into the role of β -O-4' in the intramolecular π - π stacking between phenolic rings.³³ Two distinct conformations are evident: one with sandwiched stacked phenolic rings and the other with T-shaped stacking phenolic rings. For hardwood β -O-4' hexamers (Figure 4b), the sandwiched stacks showed an inter-ring distance of 3.8 Å, while the T-shaped stacks measured 6.2 Å apart. In contrast, the softwood β -O-4' hexamers (Figure 4d) exhibited only sandwiched stacking: one top-to-top parallel stacking ($\sim$ 3.8 Å) and the other parallel-displaced stacking measuring 6.1 Å.

These observations highlight the influence of substituents on the phenolic rings on their molecular shapes and, consequently, volume. Furthermore, the combination of flexible interunits and intramolecular π - π illustrates the role of the molecular structure in the reactivity of lignin. Specifically, the S units in hardwood β -O-4' hexamers afforded a greater molecular volume compared to their softwood counterparts, which appeared to be more dense molecules. This expanded volume in hardwood was indicative of the increased accessibility of hydroxyl groups relative to softwood. The difference in the molecular volume and hydroxyl group accessibility between hardwood and softwood can be attributed to the specific substituent patterns on their phenolic rings. As a direct consequence of this higher accessibility, hardwood exhibits enhanced reactivity in carboxymethylation reactions. This distinction in reactivity is important when considering the applicability of these materials in specific chemical modification.

Molecular Weight and Dispersity. The results on the molecular weight and dispersity of reference and carboxymethylated lignin samples are presented in Table 2 and Figures

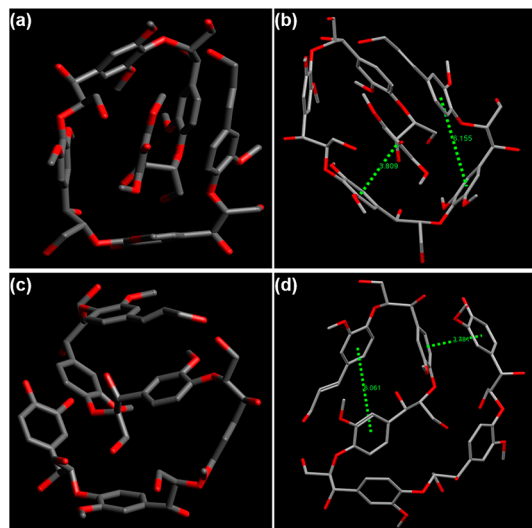


Figure 4. DFT models of β -O-4' hexamers of (a and b) hardwood lignin (comprising four S units and two G units) and (c and d) softwood lignin (comprising six G units).

presence of structures consisting only of β -O-4' linkages in native spruce lignin by combining HSQC with MALDI-ToF analysis.³³ Thus, the modeled structural motifs represent structural populations in cyclic extracted organosolv lignin.

Figure 4a illustrates the molecular conformation of the hardwood β -O-4' hexamer, which is comprised of four S units and two G units. Interestingly, the molecule formed an e-shaped conformation. Conversely, Figure 4c depicts the softwood β -O-4' hexamer, entirely composed of six G units, which adopted a spiral (s-shaped) conformation. In the e-shaped conformation of the hardwood β -O-4' hexamer, the hydroxyl groups predominantly projected outward, except for those situated in the inward curve of the formation. In contrast, the spiral conformation of the softwood β -O-4' hexamer exposed some hydroxyl groups externally, yet some regions confined the hydroxyl groups within the spiral structure.

The contrasting molecular conformations between hardwood and softwood β -O-4' hexamers may offer insights into

Table 2. Molecular Weight and Dispersity of Soluble and Insoluble Fractions of Carboxymethylated Lignin Samples

sample	M_n^a	M_w^a	$\bar{D}^a$
EKL	824	2083	2.53
CEKL_110_6_sol	506	1183	2.34
CEKL_110_6_ins	706	2180	3.09
SKL	1600	7370	4.61
CSKL_110_6_sol	1127	5023	4.46
CSKL_110_6_ins	956	4877	5.10
BCOL	1201	9622	8.01
CBCOL_80_6_sol	NA	NA	5.46
CBCOL_80_6_ins	NA	NA	23.29
SCOL	1669	4180	2.50
CSCOL_80_6_sol	NA	NA	15.55
CSCOL_80_6_ins	NA	NA	18.68

^aData were obtained by SEC in DMSO. NA = not applicable due to aggregate formation.

S53–S56. Before carboxymethylation, eucalyptus and softwood kraft lignin samples were completely soluble in dimethyl sulfoxide (DMSO); postcarboxymethylation, they remained soluble but required more time to dissolve. Birch and spruce cyclic extracted organosolv lignin samples, both before and after carboxymethylation, displayed initial solubility challenges in DMSO, but they were soluble in DMSO. The insoluble fractions of all carboxymethylated lignin samples were difficult to dissolve in DMSO, yet eventually did so. For the alkaline soluble fraction of carboxymethylated kraft lignin, a lower molecular weight was observed in contrast to the alkaline insoluble fraction of carboxymethylated kraft lignin. This showed that the solubility was affected not only by the carboxymethylation reaction but more by the intrinsic heterogeneity of the pristine lignin, which was also shown in the molecular weight data. In the case of the cyclic extracted organosolv carboxymethylated lignins (BCOL and SCOL), on the other hand, an anomaly was observed where the SEC chromatograms displayed an extensive broadening, likely due to the formation of aggregates in the DMSO solvent, as illustrated in Figures S55 and S56. This formation of nanoparticles led to artifacts in molecular weight determinations.

CONCLUSIONS

We have systematically examined the reactivity patterns of various lignin types, specifically eucalyptus kraft lignin, spruce kraft lignin, birch cyclic extracted organosolv lignin, and spruce cyclic extracted organosolv lignin, during their carboxymethylation in an alkaline environment. Across all evaluated lignin sources, a notable preference for the reactivity of phenolic hydroxyl over that of its aliphatic counterpart was observed. However, an intriguing deviation was seen for cyclic extracted organosolv lignin, wherein the aliphatic hydroxyl displayed a higher conversion rate, attributable to its abundant presence in this specific lignin type.

The chemical composition and structural characteristics of each lignin type played important roles in dictating the efficiency of their carboxymethylation. Notably, hardwood lignin, irrespective of its origin from the kraft process or the cyclic extraction organosolv technique, exhibited enhanced reactivity during carboxymethylation as opposed to softwood lignin, which was processed similarly. This phenomenon, supported by DFT simulations, reveals that phenolic hydroxyl in *erythro*-aryl ether isomers of the syringyl dimer was more exothermic during the carboxymethylation reaction compared to the corresponding guaiacyl unit. The other reason was explained by the presence of two methoxy substituents in the phenolic rings of syringyl units in hardwood lignin, rendering the lignin structure less sterically encumbered and favoring the transformation from hydroxyl to carboxymethyl groups. Specifically, the molecular conformation of hardwood lignin offered greater exposure and accessibility of its hydroxyl groups, whereas the hydroxyl entities in softwood lignin were more hindered in a denser molecular conformation. In essence, these comprehensive insights underline the potential of carboxymethylation as a strategic intervention to incorporate carboxylic acid into lignin matrixes, paving the way for their further valorization to biomaterials. Although carboxymethylation was the subject of this study, the fundamental concepts apply to any lignin valorization attempts that target the functionalization of its hydroxyls.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssuschemeng.3c07385>.

FT-IR results, assignment of 2D HSQC NMR chemical shifts, semiquantification of interunit linkages of spruce cyclic extracted organosolv lignin, 2D HSQC NMR spectra, 2D dept-ed-HSQC NMR spectra, 2D HMBC NMR spectra, assignment of ^{31}P NMR chemical shifts, quantification of lignin functional groups, ^{31}P NMR spectra, DFT calculation, and SEC results (PDF)

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Author Contributions

F.A.: investigation, methodology, data acquisition and analysis, and writing—original draft. M.K.: writing review and editing. T.E.: DFT methodology and analysis, writing review, and editing. M.L.: conceptualization, supervision, funding acquisition, writing review, and editing.

Notes

The authors declare the following competing financial interest(s): M.L. and M.K. are cofounders of a company, Prologreen AB, that produces lignin.

ACKNOWLEDGMENTS

The Swedish Research Council (Vetenskapsrådet; Grant 2021-05739) is acknowledged for financial support. This work was made possible in part by a grant for high-performance computing resources and technical support from the Alabama Supercomputer Authority. This research was supported in part by the U.S. Department of Agriculture, Forest Service.

ABBREVIATIONS

BCOL, birch cyclic extracted organosolv lignin; CBCOL, carboxymethylated birch cyclic extracted organosolv lignin (with different reaction temperatures and concentrations of the reagent); CEKL, carboxymethylated eucalyptus kraft lignin (with different reaction temperatures and concentrations of the

reagent); CSKL, carboxymethylated spruce kraft lignin (with different reaction temperatures and concentrations of the reagent); CSCOL, carboxymethylated spruce cyclic extracted organosolv lignin (with different reaction temperatures and concentrations of the reagent); EKL, eucalyptus kraft lignin; SCOL, spruce cyclic extracted organosolv lignin; SKL, spruce kraft lignin

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