

Correlation between Lignin–Carbohydrate Complex Content in Grass Lignins and Phenolic Aldehyde Production by Rapid Spray Ozonolysis

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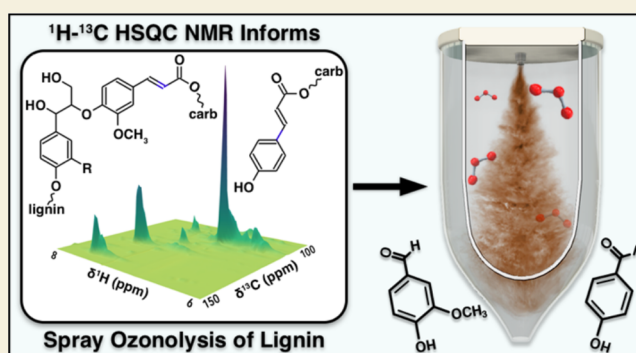
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ABSTRACT: We provide strong evidence that the amounts of phenolic aldehydes (vanillin and *p*-hydroxybenzaldehyde, *p*HB) selectively released during rapid ozonolysis of grass lignins are correlated with the unsubstituted aryl carbons of lignin–carbohydrate complexes present in these lignins. In the case of acetosolv lignin from corn stover, we observed a steady yield of vanillin and *p*HB (cumulatively ~5 wt % of the initial lignin). We demonstrate the continuous ozonolysis of the lignin in a spray reactor at ambient temperature and pressure. In sharp contrast, similar ozonolysis of acetosolv lignin from corn cobs resulted in a twofold increase in the combined yield (~10 wt %) of vanillin and *p*HB. Structural analysis with ^1H – ^{13}C heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance revealed that signals assigned to unsubstituted aryl carbons of lignin–carbohydrate complexes are quantitatively correlated to phenolic aldehyde production from spray ozonolysis. The ratios of the integrated peak volumes corresponding to coumarates and ferulates in the HSQC spectra of cob and corn stover lignins (SLs) are 2.4 and 2.0, respectively. These ratios are nearly identical to the observed 2.3-fold increase in *p*HB and 1.8-fold increase in vanillin production rates from corn cob lignin compared to corn SL. Considering that the annual U.S. lignin capacity from these grass lignin sources is ~60 million MT, the value creation potential from these flavoring agents is conservatively ~\$50 million annually from just 10% of the lignin. These new insights into structure/product correlation and spray reactor characteristics provide rational guidance for developing viable technologies to valorize grass lignins.

KEYWORDS: grass lignin structure, HSQC-NMR, valorization, spray ozonolysis, flavorings



INTRODUCTION

The corn harvesting industry in the U.S. generates approximately 350 million MT of field residues (leaves, stalks, and cobs).^{1–3} The corn residues represent a vastly underutilized renewable source of cellulosic fibers and aromatics in the lignin biopolymer. Fractionation of the corn residues would result in over 60 million MT of corn-based lignins annually.^{3–5} Lignin fractions from corn residues display a rich phenylpropanoid structure similar to those derived from traditional wood sources. However, lignins from corn residues also possess unique inter-fraction linkages known as lignin–carbohydrate complexes (LCCs).^{6–11} Valorization of these lignins is a challenging problem.^{12–17} In general, most lignin depolymerization technologies result in numerous monomeric compounds that pose separation challenges. Reported selective depolymerization strategies include lignin-first catalytic reduction strategies that produce just a few monomers^{17–21} and a non-catalytic fast ozonolysis process for grass lignins that produces phenolic aldehydes, vanillin and *p*-hydroxybenzaldehyde (*p*HB).^{22,23} As sustainable flavoring agents, these phenolic aldehydes command significantly more value than

commodity chemicals. They may also be applied as phenol substitutes to make renewable resins from lignin.^{22,24} Hence, methods that can maximize the recovery of these value-added components will facilitate the complete utilization of lignin and lead to profitable biorefineries.

Production of phenolic aldehydes from ozonolysis of lignin depends upon the retention of LCCs in lignin after pretreatment. LCCs are composed of connecting ester and ether bonds with aromatic centers (e.g., phenyl–glycoside, coumarate–ester, ferulate–ester, etc.).^{10,11} Grass-type plants, such as corn, contain more LCCs including coumarate–ester and ferulate–ester LCCs while their presence is scarce in woody plants.^{6–8,11} Further, because of their instability during

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alkaline treatment conditions, lignins from either the Kraft process or sulfite pulping are not amenable to valorization by rapid ozonolysis.^{6,11} However, treatments like the acetosolv process (a variant of organosolv) produce high-quality cellulose and sulfur-free lignin that retains the LCCs.^{25–27}

Spray ozonolysis of lignin affords phenolic aldehydes through the selective cleavage of C=C bonds present in the LCCs by ozone in the presence of a protic solvent.^{28,29} Concentrations of ferulate LCCs are estimated to be 1–2 wt % in corn stover lignin (SL) and 2–3 wt % in corn cob lignin (CL).^{8,30,31} The corresponding weight fractions of coumarate LCCs are 3–5 and 5–8 wt % in SL and CL, respectively.^{30–33}

In this work, we demonstrate for the first time a direct correlation between the coumarate and ferulate contents in grass-type acetosolv lignin and the production of their phenolic aldehyde counterparts by ozonolysis. A continuous ozonolysis reactor is deployed to maximize the production of the phenolic aldehydes from the grass lignin sources. The corn CL and SL were analyzed by 2D nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC) to gain insights on the lignin's structure before and after ozonolysis. Our work demonstrates a convenient method to screen various grass lignins for their potential to produce valuable phenolic aldehydes.

EXPERIMENTAL MATERIALS

Ozone was produced on-site from dry oxygen using an Atlas Ozone generator. Glacial acetic acid, formic acid (88%), and methanol [high-performance liquid chromatography (HPLC) grade] were purchased from Fisher Chemical. Quantification standards for vanillin and pHB were purchased from Sigma Aldrich. Archer Daniels Midland (ADM) Company supplied acetosolv lignins as benchmarks to compare against the lignins produced in this work. Lignin samples were prepared by an acetosolv extraction using corn stover and corn cobs obtained from a local farm in Lawrence, KS, and corn cobs were supplied by a local processor, J-Six Enterprises of Seneca, KS.

EXPERIMENTAL METHODS

Spray Ozonolysis

Figure 1 illustrates the schematic of the continuous spray ozonolysis reactor, which was significantly modified from the previous version to

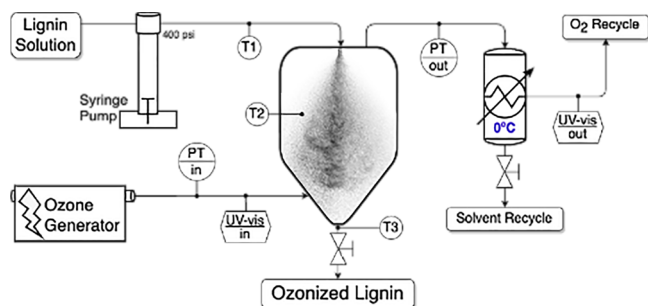


Figure 1. Schematic of spray ozonolysis reactor.

aid systematic evaluation. Specifically, the reactor was redesigned such that the lignin solution is sprayed from the top of the reactor to meet a countercurrent gas stream containing ozone. The gas stream exits at the top of the reactor to a condenser where the entrained acetic acid is condensed and recovered. Provisions were also made for measuring the ozone concentrations at the reactor inlet and outlet.

Feedstock solutions were prepared by dissolving 11.0 g (1.0 wt %) or 22.0 g (2.0 wt %) of acetosolv lignin derived from either corn stover or corn cob in 1.0 L of protic solvent. Glacial acetic acid and

88% aqueous formic acid were combined in a volumetric ratio of 75% acetic acid, 22% formic acid, and 3% water to generate the protic solvent. For the continuous spray ozonolysis experiments, up to 5.0 L of the lignin solution was prepared for a given sample.

Before each experiment, the spray chamber was rinsed with acetic acid. All experiments began after sufficient time (15–30 min) was allowed for the ozone concentrations to stabilize at identical values at the inlet and outlet of a 6 L glass spray chamber. The ozone generator was operated in the 1.0–5.0 SLPM range to produce a stream of 1.0–5.0 mol % ozone in dry oxygen. Using an HPLC pump, the 1.0 wt % lignin solution was pumped into the spray chamber through an MW085 Bete MicroWhirl nozzle at a constant flow rate between 3.5 and 7.5 LPH, generating a back-pressure of approximately 400 psig. The nozzle, located at the top of the reactor, aerosolizes the lignin solution into a countercurrent stream of dilute ozone. The ozonized liquid mixture exits the reactor at the bottom forming a liquid seal. The average reactor residence time of the liquid can be estimated by,

$$t_R = \frac{h_L}{Q_L}$$

where h_L is the liquid hold up in the reactor and Q_L is the liquid flow rate. The holdup ranges from 6 to 8 mL; thus during typical sprays at 4.5 LPH, the residence is ca. 5–7 s.

The gas stream, largely depleted of ozone, exits the top of the reactor (Figure 1), where it is fed to a downstream condenser to remove any entrained acid vapors. Ozone concentrations at the gas inlet and gas outlet of the reactor were continuously monitored using UV detectors. The reactor temperature and pressure were also continuously monitored. Periodically, the ozonized liquid stream exiting the reactor was sampled to analyze the phenolic aldehyde yields. Product yields are expressed in weight percent relative to the mass of lignin in the feed stream.

$$Y_p (\text{wt } \%) = \frac{m_{\text{product}}}{m_{\text{lignin}}} \times 100$$

ANALYTICAL METHODS

Gas Chromatography/Flame Ionization Detector

Liquid samples from the reactor effluent were analyzed by a gas chromatograph (GC) equipped with a flame ionization detector (FID). Samples of starting solutions and ozonized product solutions were collected in 300 μL aliquots and combined with 1.0 mL of methanol. An Agilent 7890A GC equipped with a 30 m HP-INNOWAX column was used. Samples were administered in 1 μL injections with an injector temperature of 250 $^{\circ}\text{C}$ and helium (2 SCCM) as carrier gas. The column oven temperature was held at 40 $^{\circ}\text{C}$ for 5 min and then ramped to 220 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$ and held at 220 $^{\circ}\text{C}$ for 20 min. External standards were used to calibrate the product quantities (see the Supplementary Information).

^1H – ^{13}C Heteronuclear Single Quantum Coherence NMR

The lignin samples were analyzed by NMR spectroscopy using a Bruker AVIII 500 MHz spectrometer with a multinuclear BFFO cryoprobe. Two-dimensional ^1H – ^{13}C heteronuclear single quantum coherence (HSQC) spectra were obtained with a standard hsqcedetgpcisp.2.2 pulse sequence. A typical experiment used the following parameters: 16 scans, 2 second relaxation delay, 256 increments in the ^{13}C dimension, and spectral widths of 10 ppm for ^1H dimension and 210 ppm for ^{13}C dimension. Samples were prepared by dissolving approximately 13.5 mg of lignin with 500 μL of dimethylsulfoxide- d_6 (DMSO- d_6). Chemical shifts were calibrated against the central DMSO- d_6 peak ($\delta_{\text{H}}/\delta_{\text{C}}$ 2.50/39.5). Signals were assigned based on published data.^{32,34,35} Analytical spectral processing was carried out using MestreNova software. Signal contours were automatically integrated after standard data processing including solvent shifting and baseline correction.

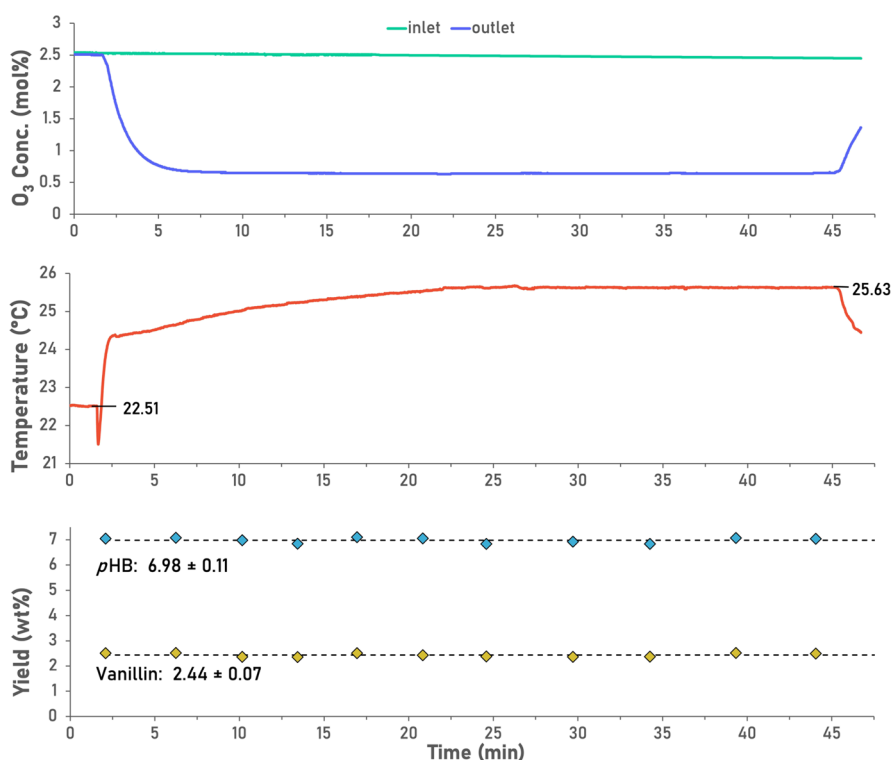


Figure 2. Temporal concentration and temperature profiles during continuous spray ozonolysis of cob lignin. Lignin content in feed stream: 1.0 wt %; feed flow rate: 4.5 L/h; O₃ concentration in feed gas: 2.50 mol % of O₃; and gas flow rate: 3.0 SLPM.

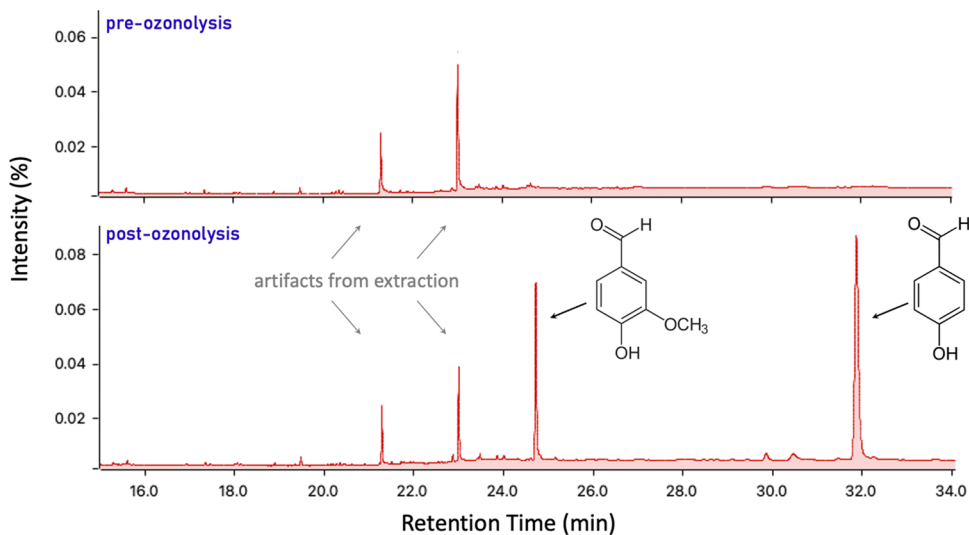


Figure 3. Bottom: typical phenolic aldehyde products in ozonized lignin mixture, detected by GC/FID analysis; vanillin (24.71 min) and *p*HB (31.88 min). The extraneous major peaks (21.28 and 23.00 min) are carryovers from the acetosolv extraction process, which is shown in the pre-ozonolysis spectra (top).

Gel Permeation Chromatography

GPC analysis was performed on an Agilent 1260 Infinity GPC system with a refractive index detector. Spectra were obtained by eluting the pre- and post-ozonolysis samples through two columns in series, a 300 mm Polargel-M followed by a 300 mm Polargel-L, and held at 40 °C. The samples eluted in a non-gradient mobile phase of dimethylformamide stabilized with 0.1 wt % of tetrabutyl-ammonium bromide at 1.0 mL/min. Chromatograms were calibrated against poly(methyl methacrylate) standards. Samples were prepared by evaporating the reaction solvent to recover ca. 40 mg of lignin or ozonized lignin and then redissolved in 1.0 mL of mobile phase.

RESULTS AND DISCUSSION

Continuous Spray Ozonolysis

Figure 2 shows the temperature and concentration profiles during an extended ozonolysis run for 45 min. The run was performed at ambient temperature (22 °C) and pressure. Details of the experimental variables such as the flow rate of the lignin solution, the solvent used, the lignin content in the feed, the gas flow rate, and the ozone concentration in the gas stream are included in the figure caption. Within 10 s of initiating the spray, the yields of both primary phenolic

aldehydes reached a steady state. This reflects the almost-instantaneous nature of the ozonolysis reaction wherein achieving maximum production rates will depend upon minimizing mass transport limitations across the gas–liquid interface. The spray reactor design chosen for this work serves to increase the contact area between the aerosolized substrate and ozone, thereby minimizing transport limitations. In addition, the spray reactor limits the ozonized liquid phase from excessive exposure to ozone. As the ozonized droplets coalesce, the contact area between the liquid and gas phases decreases significantly, thereby avoiding undesired oxidation of the ozonolysis products. The approximate residence time of the liquid phase in the reactor (defined as the liquid phase holdup divided by the liquid phase flow rate) is on the order of a few seconds.

In contrast to the liquid phase product profiles, the ozone concentration profile takes longer (~5 min) to reach a steady state. This reflects the different dynamics associated with mixing and simultaneous ozone depletion in the gas phase of the reactor. The bulk reactor temperature initially spikes to 24.5 °C (a rise of ~2 °C) and then gradually reaches a steady state of 25.6 °C after 20 min. Beyond this time, the temperature remains stable for the remaining duration. The initial spike reflects the dissipation of heat from the liquid phase that absorbs the reaction exotherm upon ozonolysis. The gradual attainment of a steady bulk phase temperature is attributed to the poor thermal conductivity in the gas phase in dissipating the heat generated in the liquid droplets.

A distinct lack of signals attributed to carboxylic acids or other monomers in the GC/FID spectra of an ozonized product (Figure 3) suggests that the spray ozonolysis of lignin is selective and non-destructive toward the phenolic aldehyde products even in the presence of excess ozone. The lack of carboxylic acid byproducts indicates a Dussault ozonolysis mechanism as expected in a protic solvent. This mechanism avoids the formation of energetic intermediates and thus enhances safety.^{22,28,29}

The foregoing results and analysis show that ozonolysis of grass lignins to extract valuable aldehydes can be performed in a continuous reactor at mild conditions and in a safe manner.

Production of Phenolic Aldehydes from Spray Ozonolysis of Lignin from Corn Residues

The combined yields of the two phenolic aldehydes (vanillin and *p*HB) produced from grass lignins derived from various sources are shown in Figure 4. The observed yields were nearly equal among the various samples of either corn CL or corn SL obtained from ADM and extracted in house. The combined yield of vanillin and *p*HB (approximately 5 wt %) is also consistent with the value previously reported.^{22,23} Most interestingly, corn CL samples produced a combined yield of up to ca. 10 wt % at identical ozonolysis conditions. This is consistent with the generally higher concentration of LCCs reported for corn CLs compared to corn SLs.

A few experiments were conducted to understand how the ozone and lignin throughput affect the yields of the phenolic aldehydes. When changing inlet ozone concentration and gas flow rate such that the ozone throughput is 75 SCCM, the product yields (and hence their production rates) remained roughly constant (Figure 5). When both the concentration of lignin in the feed stream (2.0 wt %) and ozone concentration in the inlet gas (5.0 mol %) were doubled, the vanillin and *p*HB yields remained constant, implying that their production

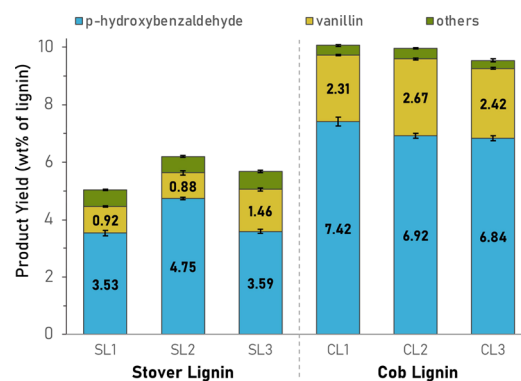


Figure 4. Phenolic aldehyde yields from various lignin sources after spray ozonolysis treatment at ambient pressure and temperature. Lignin content in feed stream: 1.0 wt %; feed flow rate: 4.5 L/h; O₃ concentration in feed gas: 2.50 mol % of O₃; gas flow rate: 3.0 SLPM.

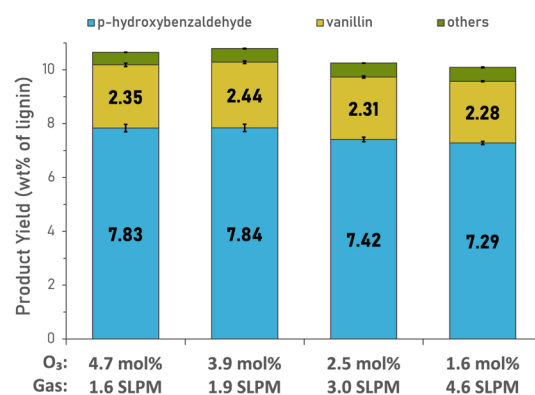


Figure 5. Combined effect of ozone concentration and total gas flow rate on phenolic aldehyde yields from spray ozonolysis. Ozone throughput was held constant at 75 SCCM for all runs. Other operating conditions are same as in Figure 3.

rates doubled (Figure 6). Not only does this demonstrate easy process scalability but also that the phenolic aldehyde production rates are governed by the ozone-to-liquid mass transfer rates. At the investigated operating conditions, ozone transfer rates are not adversely affected. As discussed in the following section, analysis of the ozonized lignin suggests

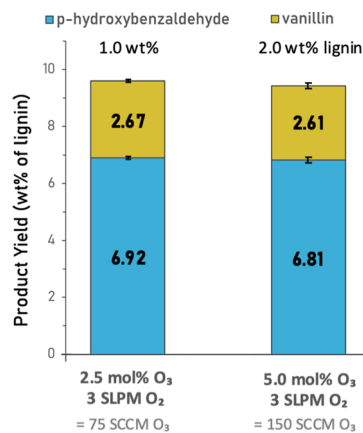


Figure 6. Phenolic aldehyde yields from spray ozonolysis when doubling lignin and ozone throughputs. Other operating conditions are same as in Figure 3.

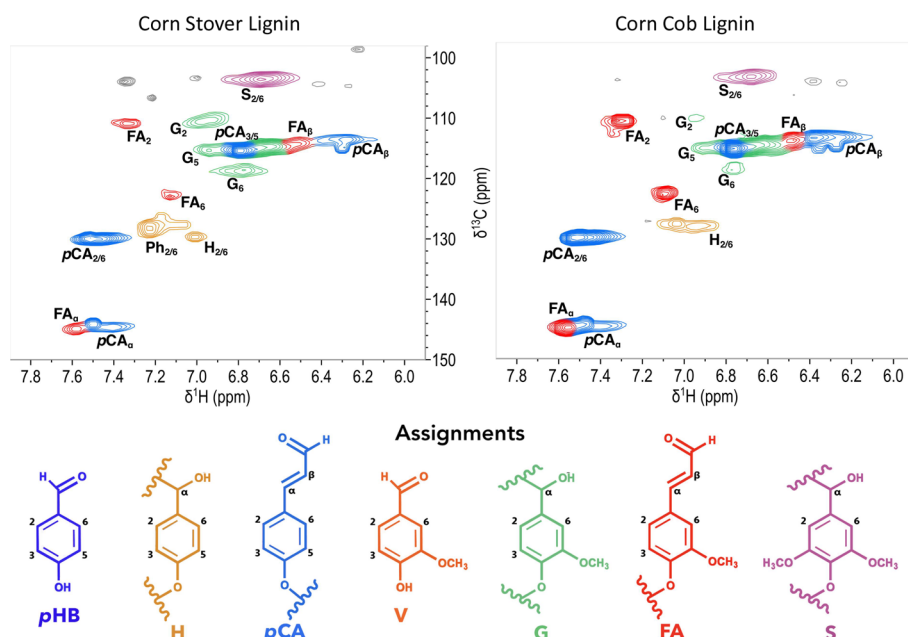


Figure 7. Aromatic regions of the ^1H – ^{13}C HSQC NMR spectra of untreated stover lignin (SL1) and cob lignin (CL1).

complete conversion of the ferulates and coumarates at the investigated operating conditions.

Characterization of Lignins from Corn Residues

To gain additional insights into the correlation between lignin type and phenolic aldehydes production, the chemical structures of the lignins were probed using NMR techniques. Structural characterization of the untreated stover and CLs with ^1H – ^{13}C HSQC NMR indicate similar structures (Figure 7). As expected, both lignins contain signals corresponding to the ferulate (FA) and coumarate (*p*CA) LCCs. The signals at ($\delta_{\text{H}}/\delta_{\text{C}}$ 7.34/110.8) and ($\delta_{\text{H}}/\delta_{\text{C}}$ 7.10/123.2) were assigned to the C_2 and C_6 , respectively, of the ferulate LCCs, which were present in both lignins. Coumarate LCCs were also present and the C_2/C_6 signals were assigned to ($\delta_{\text{H}}/\delta_{\text{C}}$ 7.46/130.2). Because the signals assigned to C_2 and C_6 are isolated from signals of other moieties, these signals provide a basis for comparison. We recognize that these signals may overestimate the absolute concentration of their respective moieties,³² but it is reasonable to assume the signals of similar but separate samples are identically overestimated. Thus, we can compare the integrated peak volumes to obtain a quantitative relationship.

As summarized in Table 1, the calculated volumes of the peaks assigned to ferulates in CL are ca. 2.4-fold greater than the volume of the corresponding peaks in the SL spectra (Rows 1 & 2, Table 1). Average vanillin yields from CL were approximately 2.3-fold greater those of SL (Figure 3).

Table 1. Assignments and Normalized Peak Volumes of Ferulate and Coumarate Signals Identified in the ^1H – ^{13}C HSQC NMR Spectra of Stover and Cob Lignins

assignment	δ_{H} (ppm)	δ_{C} (ppm)	stover lignin (SL) peak volume	cob lignin (CL) peak volume	CL/SL
FA ₂	7.34	110.8	16.10	38.23	2.38
FA ₆	7.10	123.2	13.99	34.31	2.44
<i>p</i> CA ₂ + <i>p</i> CA ₆	7.46	130.2	110.35	217.75	1.97

Similarly, the CL/SL volume ratio for coumarate peaks was ca. 2.0, while the ratio of *p*HB yields from CL and SL lignins averaged ca. 1.8 (Row 3, Table 1). Within a given lignin sample (SL or CL), the ratios of the coumarates to ferulates approximate the yields of *p*HB to vanillin (Table 2). After

Table 2. Ratios of LCCs in CL Compared to SL Determined by ^1H – ^{13}C HSQC Compared to the Ratio of Their Respective Ozonolysis Product Yield (Phenolic Aldehydes)

LCC—product	CL/SL ratio from HSQC ^a	ratio of ozonolysis yields ^b
coumarate— <i>p</i> HB	2.4	2.3
ferulate—vanillin	2.0	1.8

^aPredicted by peak volumes provided in Table 1. ^bDetermined by the average of yields as shown in Figure 4.

ozonolysis (Figure 8), the coumarate and ferulate peaks are either slightly shifted ($\text{C}_{2/6}$ and $\text{C}_{3/5}$) to the environment of the respective phenolic aldehyde or starkly absent (C_β). Much of the aliphatic sidechains remain intact. This observation is supported by GPC analysis (Figure 9), which indicates that the bulk of the lignin's structure (i.e., molecular weight distribution above 10^4 Da) is preserved following ozonolysis. Cumulatively, these findings strongly suggest that the ferulate and coumarate moieties in lignin are selectively and completely converted to valuable phenolic aldehydes and their concentrations in the grass lignins are directly proportional to the observed yields of vanillin and *p*HB.

CONCLUSIONS

This work establishes a direct correlation between the concentrations of lignin–carbohydrate complexes in grass lignins and the phenolic aldehydes (vanillin and *p*HB) produced from them by ozonolysis. ^1H – ^{13}C HSQC NMR spectroscopy reveals that the ferulate and coumarate concentrations are greater in corn cob lignin compared to corn stover lignin. The yields of vanillin and *p*HB from corn cob lignin are correspondingly higher compared to corn stover lignin. The combined yield of these two phenolic aldehydes

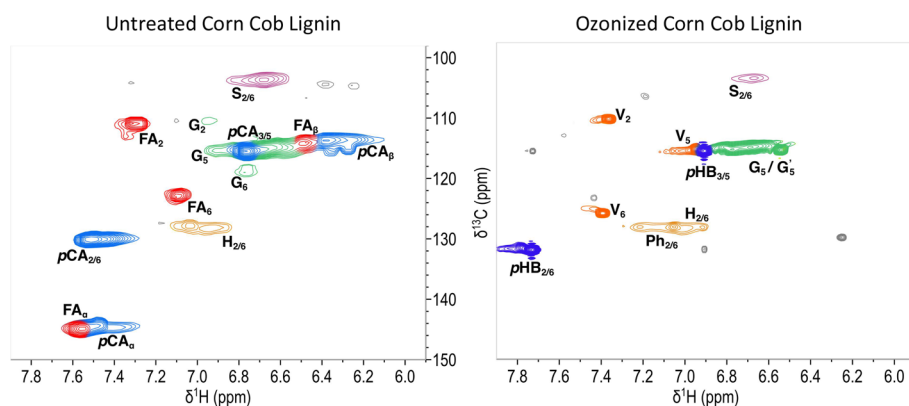


Figure 8. Aromatic region of the ^1H - ^{13}C HSQC NMR spectra of untreated corn cob lignin (CL1) and ozonized cob lignin (OZCL1).

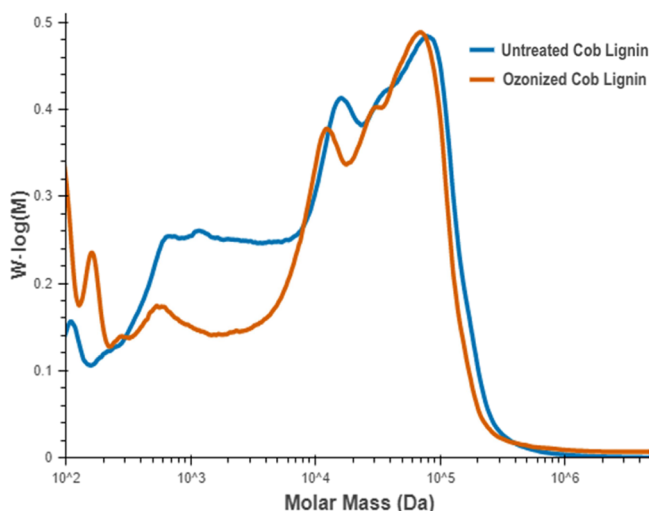


Figure 9. GPC spectra for untreated corn cob lignin (blue) and ozonized cob lignin (orange). Formation of monomer products is shown in the peaks around 10^2 Da.

(~ 10 wt %) is approximately twofold greater in the case of corn cob lignin. The ozonolysis to selectively release the phenolic aldehydes from grass lignins was performed safely in a continuous spray reactor, achieving complete conversion of the ferulates and coumarates to phenolic aldehydes while leaving the remaining lignin's macrostructure largely intact. During an extended 45-min run, steady phenolic aldehyde production, characterized by steady ozone consumption and temperature profile, was demonstrated. The maximum temperature rise was just a few degrees above ambient. Doubling both the lignin concentration in the feed solvent and the ozone concentration in the feed gas commensurately increased the aldehyde production rates, demonstrating potential scalability of the process. These new insights and enhancements to spray ozonolysis guide the development of commercially viable strategies to valorize grass lignins.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acseengineeringau.2c00041>.

GC/FID calibrations as referenced in the analytical methods (PDF)

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Author Contributions

CRediT: **Steffan Green** conceptualization (supporting), data curation (lead), formal analysis (lead), investigation (lead), methodology (lead), validation (lead), writing-original draft (lead); **Thomas Binder** investigation (supporting), methodology (supporting), resources (supporting), supervision (supporting), writing-review & editing (supporting); **Erik Hagberg** conceptualization (supporting), funding acquisition (supporting), project administration (supporting), resources (supporting), supervision (supporting), writing-review & editing (supporting); **Bala Subramaniam** conceptualization (equal), funding acquisition (lead), investigation (supporting), methodology (supporting), project administration (lead), supervision (lead), writing-review & editing (lead).

Notes

The authors declare no competing financial interest.

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