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Refining of Ethanol Biorefinery Residues to Isolate Value Added Lignins

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Supporting Information

ABSTRACT: The isolation of lignin coproducts from the residual solids of a hardwood based biocatalytic bioethanol process was examined, using extraction methods based on aqueous alkali or aqueous ethanol. This work focused on understanding how the structural features of raw lignin in the process residue influenced the refined lignin isolation yields, in addition to polymeric and structural characteristics. On the basis of this approach, the extraction based lignin refining could be optimized. Mild extraction conditions allowed for recovery of approximately 40 wt % of the lignin present in the starting material. This yield could be increased to about 76 wt %, by the application of base or acid catalyzed reactive extraction conditions liberating the bonded insoluble fractions of the residue. All isolated lignin products were characterized in terms of their functional groups, molecular weights and thermal properties. The lignins from mild alkali and ethanol extractions showed similarities in their chemical profiles. In contrast, the reactive extractions and degradative aqueous treatments produced structurally different lignins. As expected, the molecular weight and the thermal properties of the isolated lignins were affected by the isolation process and these differences are rationalized.

KEYWORDS: *Lignin, Biorefinery residue, Extraction, Thermal properties, Molecular weight*

INTRODUCTION

Legislation drives the biofuel industry toward the utilization of second generation cellulosic feedstocks to replace corn feedstocks as the primary source of bioethanol.[1] Relative to corn ethanol, the conversion of cellulosic biomass to ethanol is a more complex process, and it does not provide coproducts with well-defined markets, such as livestock feeds.[2] Therefore, establishing new valuable coproducts for second generation bioethanol processes will enhance the long-term commercial viability of these processes.

Current biorefinery technologies are based on lignocellulosic feedstocks (such as crop residues, energy crops or wood) and generate large quantities of lignin-rich solids as byproducts. This material is commonly perceived as a source of energy, either for the plant or as pellets for sale.[3−5] Recent techno-economic analyses have shown that with high efficiency conversion systems, these plants will be self-sufficient in terms of heat and power, or be more economically viable using natural gas for process heat and power, and thus they may have excess lignin-rich residues.[6,7] Possible value addition pathways for the underutilized lignin biopolymer include a large spectrum of applications from low-value commodities to lucrative dispersants, carbon fibers, and high-value polymer applications. High-value applications inherently impose stringent specifications on lignins quality, in terms of purity, functionality profiles, thermal performance, and molecular weight distributions.[8−11]

The overall isolation costs remain a substantial barrier to the production of high-quality lignins. A low-cost source of lignin is not enough; a commercially viable process also needs to be a low-cost process. Therefore, economically feasible processes need to be developed to facilitate the commercialization of lignin based products from a lignocellulose biorefinery.

The isolation of lignin has been investigated in conjunction with the feedstock pretreatments as part of an integrated lignocellulose biorefinery.[12,13] An alternative approach is the postbioconversion recovery of lignins from the residual materials after the enzymatic hydrolysis and fermentation processes. The recent literature contains examples where postbioconversion techniques have been examined, including extraction with aqueous sodium hydroxide (NaOH)[14] or organic solvents such as dimethylformamide (DMF).[15]

Notably, prebioconversion isolation protocols have received more attention over their postbioconversion counterparts. This is because lignin is a cellulase enzyme inhibiting moiety that may hinder the hydrolytic conversion process.[16] The current work in our laboratory has involved the sequential application

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of electron beam and steam explosion (EB-SE) pretreatments to facilitate the conversion of wood feedstocks without a need for removal of the lignin from the substrate prior to enzymatic bioconverison. Consequently, the enzymatic saccharification process provides lignin-rich residues relatively free of carbohydrates, and thus amenable to postbioconversion extractions.

The present study examines the fundamentals of lignin extraction techniques based on aqueous alkali and organic ethanol−water media, and their potential to refine the lignin-rich residues of EB-SE bioconversion protocols. Specifically, we are examining the application of (i) mild extractions, (ii) reactive extractions and (iii) degradative acid treatments prior to extractions; and rate their efficacy to liberate high-quality lignin streams. The recovered lignins were compared in terms of yield, purity, functionality profiles, molecular weights and thermal properties. Consequently, this effort also offers an insight into the characteristics of lignin products isolated by different dissolution mechanisms.

MATERIALS AND METHODS

Materials. The solvents and reagents used were purchased from Sigma-Aldrich and Fisher scientific and used without purification. The Norwegian Birch was received from BioOil AS (Norway). Electron beam irradiation of the wood material was conducted by a private partner, and steam explosion was performed at Norwegian University of Life Sciences (UMB), Norway.

Pretreatment of Birch Wood and Preparation of Hydrolysis Residue. The Birch sawdust was irradiated by electron beam at a total dosage of 100 kGy, at equilibrium moisture content under atmospheric conditions. The steam explosion was the carried out in a 20 l reactor[17] (Cambi, Norway) at 200 °C with 6 min retention time. The moist material was frozen without washing or draining of the hydrolysate. Before the enzymatic hydrolysis, the sample was thawed, dried and homogenized by passing through a 20 mesh sieve (<2 wt % retained).

Enzymatic hydrolysis was carried out at pH 4.9 acetate buffer at a 5 wt % consistency, by using 10 FPU/g of a Ctec2 enzyme cocktail (Novozymes, USA). The mixture was incubated at 50 °C for 72 h. After filtration of the hydrolysate, the solids were washed with deionized water (approximately 20 mL/g) and air-dried. Dry material was homogenized by 40 mesh sieve (<5 wt % retained). The recovered powdery residue corresponded to 33.6 wt % of the used EB-SE Birch. The material was dried in a 25 °C vacuum oven prior use.

Lignin Refining Processes. *Degradative Acid Treatment in Aqueous Media.* The dried residue (approximately 6 g) was weighed into a 250 mL flask, and 100 mL of aqueous 0.5 M HCl was added. The mixture was refluxed in an oil bath at 100 °C for 2 h, limiting the access of air by an oil lock. After the reaction, the hydrolysate was centrifuged. The residue was washed using pH 3 HCl solution and freeze-dried.

Alkali based Mild and Reactive Extractions. The dried residue (approximately 6 g) was weighed into a 250 mL flask, and 100 mL of aqueous 1.0 M sodium hydroxide (NaOH) solution was added. The mixture was incubated in an oil bath at 25 or 100 °C (low-severity alkali (LSA) and medium-severity alkali (MSA), respectively), using an air lock. The solvent was replaced after centrifugation at the end of each 1.5 h cycle, totaling 6 h of extraction (4 cycles). The combined supernatants were titrated to pH 2 using concentrated HCl, which allowed for the dissolved lignin to precipitate. After precipitation, the mixture was stirred for 3 h and centrifuged. The raw lignin was washed three times with pH 3 HCl solution, and the final product was recovered after freeze-drying.

The high-severity alkali (HSA) extraction followed similar procedures but the lignin isolation was done in a 300 mL Parr reactor at an elevated pressure at 140 °C. The heating ramp to the target temperature lasted 15 min, and the 2 h extraction time was the total time at 140 °C.

The relative yields between the isolation cycles were determined by UV/vis-spectrophotometric analyses. A portion of 100 μL was withdrawn from the extraction supernatant, diluted to 100 mL using 1.0 M NaOH and the absorbance of the solution was measured at 280 nm, in a quartz cuvette, using a Beckman DU 640 spectrophotometer. The lignin concentration was determined against a calibration curve prepared from MSA isolated lignin.

Ethanol based Mild and Reactive Isolated Lignins. The lignin isolation by the ethanol−water system (7/3 by weight) was carried out in an analogous manner to the alkali treatments, with a few alterations. The incubation temperature was 80 °C in all cases. In the case of the reactive acid catalyzed ethanol extraction (ACE), the 0.5 M HCl acid catalyst was added during only the first extraction cycle. The extracted lignin was precipitated from the solution by evaporation of the extraction solvent to approximately 50 mL, and after the addition of 100 mL of pH 3 HCl solution. After centrifugation, the washing and recovery was done as previously described.

Isolation of the Enzymatic Mild Acidolysis Lignin (EMAL) as Analytical Reference. Enzymatic mild acidolysis lignin (EMAL) was prepared from the residue as described by Wu and Argyropoulos (2003),[18] applying short ball milling and secondary enzymatic hydrolysis to the residue prior to the acidolysis step.

Characterization. *Compositional Analyses.* The contents of lignin and carbohydrates were determined by a slightly modified analytical procedure by NREL.[19] A Beckman DU 640 spectrophotometer and a quartz cuvette were used for the spectrophotometric determinations of lignin at 205 nm, using the standard extinction coefficient of 110 l/g cm.[20] For sugar determination, the filtrate was neutralized, filtered and injected into an Agilent 1200 HPLC system equipped with Shodex SP0810 8*300 mm column. Milli-Q water was used as the mobile phase with 0.5 mL/min flow at 80 °C. Calibration was carried out with six standard solutions of glucose, xylose, galactose, arabinose and mannose (0.1 to 20 mg/mL).

FT-IR Spectroscopy. The FT-IR analyses were conducted on dry samples (vacuum oven for 20 h at 50 °C) using a PerkinElmer Frontier spectrometer with attenuated total reflection (ATR) attachment. All spectra were collected by using 32 scans, and the background was collected prior each analysis. The spectrum was baseline corrected using the PerkinElmer Spectrum software.

The principal component analysis (PCA) analysis was carried out using the Unscambler 10.3 software from Camo. An average spectrum of two analyses was used, which was then normalized and centered using the average value of each variable (wavenumber). The spectral regions between 3700 to 2700 and 1800 to 650 cm^{-1} were used in the analysis. The obtained loadings of the PCA analysis are provided in the Supporting Information.

Acetylation of Isolated Lignins. The used procedure was adapted from Gellerstedt (1992).[21] As an additional step to the cited procedure, the final product was dissolved in dioxane and freeze-dried. The average weight gain percentage (WGP) of the acetylated product was 35%.

NMR Spectroscopy. ^{1}H NMR spectra were collected on acetylated lignins dissolved in $CDCl_3$ by acquiring 128 scans with a relaxation delay of 15 s. Pentafluorobenzaldehyde (PFB) was used as an internal standard. The ^{13}C NMR analyses was carried out with acetylated lignins following a similar procedure to that described by Capanema et al. (2004).[22] Quantification of the ^{13}C spectra was carried out on the basis of the phenolic acetyl signals at 168.5 and 168.1 ppm, where the integral was set to corresponding mmol/g value obtained from the quantitative ^{1}H analysis. Quantitative ^{31}P NMR analyses were carried out with nonderivatized lignins as described by Granata and Argyropoulos (1995)[23] and Sadeghifar et al. (2012).[24]

A Bruker 300 MHz instrument equipped with a Quad probe (^{31}P, ^{13}C, ^{19}F and ^{1}H) was used for all ^{1}H NMR and ^{31}P NMR analyses. A Bruker 500 MHz instrument with a dual broad band probe (^{13}C and ^{1}H) was used for the ^{13}C NMR acquisitions. Processing of all NMR spectra was done using MestReNova Lite and Spinworks software. Phase and baseline corrections were applied if needed before signal integration against the known quantities of added standards. The signals were identified according to published literature by Lundquist

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Table 1. Summary of Conditions Used for the Refining Treatments, and Abbreviations Used in the Text

refining methods	abbreviation	treatment conditions
alkali based extraction		
low-severity alkali extraction	LSA	aqueous 1.0 M NaOH; 24 °C; 6 h
medium-severity alkali extraction	MSA	aqueous 1.0 M NaOH; 100 °C; 6 h
high-severity alkali extraction	HSA	aqueous 1.0 M NaOH; 140 °C; 2 h
acid treatment and medium-severity alkali extraction	A-MSA	aqueous 0.5 M HCl; 100 °C; 2 h
		aqueous 1.0 M NaOH; 100 °C; 6 h
ethanol based extraction		
ethanol extraction	E	70% EtOH; 80 °C; 6 h
acid treatment and ethanol extraction	A-E	aqueous 0.5 M HCl; 100 °C; 2 h
		70% EtOH; 80 °C; 6 h
acid catalyzed ethanol extraction	ACE	70% EtOH, 0.5 M HCl; 100 °C ; 2 h
		70% EtOH; 100 °C; 4 h
analytical reference		
mild acidolysis enzymatic lignin	EMAL[18]	ball milling
		secondary enzymatic treatment
		85% dioxane, 0.01 M HCl; 100 °C; 2 h

(1979)[25] for ^{1}H spectra, Ralph (2004)[26] for ^{13}C spectra, and Wu and Argyropoulos (2003)[18] and Granata and Argyropoulos (1995)[23] for ^{31}P spectra. A partial least squares (PLS) analysis using the Unscrambler software from Camo was used based on the quantitative information provided by ^{1}H and ^{31}P analyses, and the thermal analyses (data not shown).

Molecular Weight Determination by Size-Exclusion Chromatography (SEC). Acetylated lignins were dissolved in DMF at a concentration of 0.3 mg/mL and filtered through a 0.22 μm PTFE filter. An injection volume of 50 μL was used via a manual injector. The SEC system consisted of a Waters 515 pump connected to Waters HT6E, HT6 and HT2 styragel columns in series with a Waters 484 UV-absorbance detector. Sample elution was detected using wavelength of 285 nm. The mobile phase used was DMF with 0.03 M of lithium bromide (LiBr). Calibrations were carried out using benzoylated dextran and model sugars ranging in molecular weights from 1360 to 0.18 kDa. The Empower software was used for controlling the system operation and for the preparation of the calibration curves.

Thermal Analysis. Thermogravimetric analyses (TGA) were carried out after drying the lignin samples in a 25 °C vacuum oven overnight. Samples of 10 ± 0.5 mg were degraded on a platinum pan using a TA-Instrument model Q500. The temperature program and other conditions used were as follows. The sample was equilibrated at 40 °C under a nitrogen atmosphere, ramped to 105 °C with 10 °C/min rate, and held isothermally for 20 min. The weight loss was recorded during a 10 °C/min ramp to 800 °C. After 800 °C was reached, the atmosphere was changed to oxygen and the charred sample was oxidized for 10 min. Residual mass was recorded as ash content present in the sample. The data was processed using Microsoft Excel software, where the weight after the isothermal step at 105 °C was used to correspond to 100 wt %.

Differential scanning calorimetry (DSC) was carried out after the lignin sample was dried in a 60 °C vacuum oven for 6 h. A sample of 5 ± 0.5 mg was loaded into a hermetic aluminum pan and the lid was punctured. The analysis was carried out using a TA-Instrument model Q100. The temperature program used was as follows. Equilibration at 40 °C, removal of the thermal history by a heating ramp of 10 °C/min to 160 °C, and cooling back to 40 °C at a rate of 10 °C/min. Prior actual data collection the sample was equilibrated at 40 °C and then the thermogram was recorded while heating to 200 °C at 10 °C/min. The glass transition temperature (T_g) was determined from the thermograms by determining the step transition using a TA Universal analysis software.

RESULTS AND DISCUSSION

Preparation and Characteristics of the Lignin-Rich Model Residue from the Enzymatic Hydrolysis of EB-SE Wood. A starting material for the refining work was produced by hydrolytic enzymes from an EB-SE pretreated birch sawdust (100 kGy irradiation + 6 min SE treatment at 200 °C). Efficient hydrolysis of the carbohydrates in the wood substrate yielded 33 wt % recovery as a solid residue, having lignin content of 83 wt %. This served as a model for a lignocellulose fermentation process residue. About 82 wt % of the lignin in the original wood substrate was available for recovery as a purified lignin product.

The lignin-rich residue contained minor proportions of carbohydrates, mainly as incompletely digested cellulose, but also as residual hemicelluloses (see Table 2). The amount of protein impurities in our work was significantly lower when compared to an actual real process residue. This is because in this work, no fermentation was applied. As such, the approximate 1 wt % enzyme loading that was used is not anticipated to have a significant influence on the lignin isolation process.

Unlike the highly soluble analytical lignin preparations, the hydrolysis residue was not completely soluble in common lignin solvents, including aqueous alkaline solutions (1.0 M NaOH), organic solvents (dioxane−water, ethanol−water, dimethyl sulfoxide, pyridine), and an ionic liquid (1-allyl-3-methylimidazolium chloride). Only partial solubility of the process residues creates a challenge for the isolation of uniform lignin materials, in high yields. The structural features of the insoluble fractions and methods to convert them into isolable lignin are discussed in the following sections of this paper.

Description of the Approaches Examined for Lignin Refining. Two commercially practical isolation methods were examined in this paper: 1.0 M aqueous NaOH and/or 70% solutions of aqueous ethanol. These solvent systems were used under mild temperatures (mild extractions), or under elevated temperatures in the presence of hydrolyzing catalysts (reactive extractions), or in combination with acidic treatments (degradative acid treatments). The experimental details and abbreviations of the various approaches are summarized in Table 1 and are further described in the following sections.

Refining via Mild Extractions: Alkaline or Ethanol Solvent Systems. Initial experiments of this effort included mild

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Table 2. Composition of the Used Pretreated Wood Substrate, Lignin-Rich Residue after Enzymatic Hydrolysis, and the Isolated Lignins[a]

	carbohydrates[b] (wt %)					lignin (wt %)			ash (wt %)	lignin yield[c] (wt %)
starting materials	Glu	Xyl	Gal	Man	total	acid soluble	acid insoluble	total		
EB-SE Birch	33.4	16.3	1.5	2.6	53.8	3.1	30.8	33.9	n.d.	
hydrolysis residue	9.6	2.4	1.7	2.4	16.1	2.0	81.1	83.1	1.1	
lignin products	Glu	Xyl	Gal	Man	total	acid soluble	acid insoluble	total		
LSA	2.3	1.3		0.8	4.4	2.8	90.5	93.3	2.0	39.7
MSA	0.4	0.8		0.4	1.6	4.0	87.9	91.9	0	56.8
HSA	0.4	0.3			0.7	3.6	91.0	94.6	1.4	75.9
A-MSA	0.3	0.5		0.2	1.0	3.9	90.6	94.9	0.7	40.5
E	0.4	1.1		0.4	1.9	3.5	91.7	95.2	0.3	40.1
A-E	0.1	0.4		0.3	0.8	3.1	93.1	96.2	0	21.1
ACE	0.5	0.8		0.3	1.6	3.7	90.4	94.1	0.2	45.5
EMAL	0.5	0.7		0.2	1.4	2.1	92.2	94.3	1.2	35.4

[a]Average standard deviation of the determined total lignin contents is 0.5%. Glu = glucose; Xyl = xylose; Gal = galactose; Man = mannose. [b]Presented values as the weight fractions of the anhydrosugars. [c]Calculated on the basis of the 83.1 wt % lignin content in the hydrolysis residue.

extractions with aqueous base or alcohol so as to estimate the quantity of lignin that can be readily dissolved without a significant energy input or reactive processing. The conditions during low-severity alkali (LSA) and aqueous ethanol (E) extractions were not expected to be able to alter the polymeric structure of lignin.

Both isolation processes showed adequate solvation capacity to extract lignin. The yields of the recovered lignins were 40 wt % with alkali (LSA) and 40 wt % with ethanol−water system (E) (see Table 2). In terms of the solubility limits, the alkali system seemed to extract lignin more efficiently, although the ethanol system obtained similar yields after multiple cycles (i.e., larger applied solvent volume). The lignin solvation in the alkali system was most prominent during the very first extraction cycle (cycle #1 in Figure 1), whereas in the ethanol system the

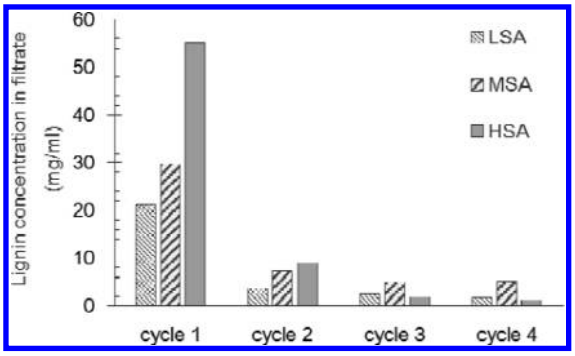

Figure 1. Concentration of the extracted lignin in the four cycles of batch wise extractions using 1.0 NaOH solution at various temperatures. Time and the applied temperature for each cycle were as follows. 24 °C and 1.5 h for LSA, 100 °C and 1.5 h for MSA, 140 °C and 0.5 h for HSA.

appearance of the recovered solvent (intensity of the brown coloration) indicated lignin to be extracted more evenly during all four cycles. These results could be anticipated regarding the strong ionization driven solvation of under alkaline conditions, and weaker electrostatic interactions that govern the ethanol based system.[27]

Unexpectedly, the two mild isolation processes produced lignins that were similar in terms of the yields and chemical features (see the Characterization section). This could be rationalized by factors related to the lignin structure itself as present in the residue, rather than the solvating interactions in the two media. Minor differences were observed between the LSA and E in the molecular weight distributions and the product recovery yields (see Table S1 of the Supporting Information for more details), but overall the total proportions of isolated lignin were similar.

The extraction yield by mild extractions could be rationalized as being limited by both the molecular weight of the lignin and the presence of cross-linked network segments within the lignin. This is strongly supported by the insolubility of any additional lignin from the residue using "more polar" solvents (see the residue preparation section). Because these macromolecular characteristics may prevent the efficient solvation under these mild conditions, reactive isolation processes were used to liberate the higher molecular weight fraction of lignin.

Alkaline Extraction under Reactive Conditions. A high-temperature alkaline isolation system enables base catalyzed hydrolysis of covalent linkages of lignin.[28] Consequently, the insoluble cross-linked fraction and high molecular weight lignin polymers will depolymerize into more soluble form, as an analogue to the typical pulping operations.

As anticipated, the lignin yields from the medium (MSA)- and high (HSA)-severity alkali extractions were higher in comparison to the LSA treatment (24 °C). The MSA that was carried out at atmospheric pressure and at 100 °C resulted in a yield of 57 wt %, whereas the HSA carried out under a 7 bar pressure at 140 °C reached up to 76 wt % yield (the highest yield attained in this study). The relative extraction efficiencies for the three temperatures of alkali systems were also compared on a cycle-to-cycle basis (see Figure 1). Application of a higher extraction temperature increased the proportion of extracted lignin, especially during the first cycle, which then dropped drastically during cycles 2 to 4. This may signify a temperature induced formation of soluble lignin fragments, over the direct temperature driven solvation effects. Besides the targeted lignin products, additional water-soluble fractions were recovered and quantified (see Table SI1 in the Supporting Information). At high temperatures, there was a significant loss of material as water-soluble, low molecular weight byproducts. The HSA treatment resulted in an 80 wt % total recovery of the starting material, whereas in contrast, 97 wt % of the starting material was recovered from the mild ethanol isolation process.

The positive yield influence of the MSA and HSA treatments is evidently largely due to the base catalyzed cleavage of the aryl

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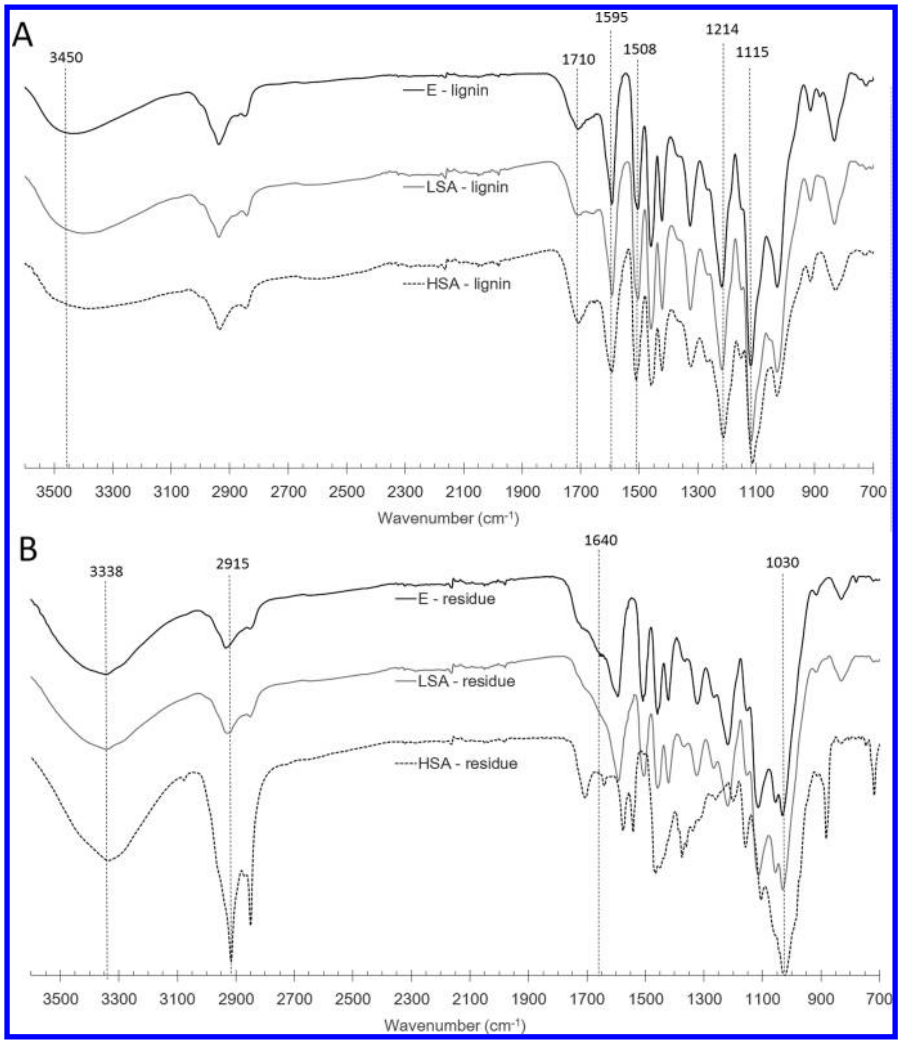

Figure 2. FT-IR spectra of the isolated lignins and the solid treatment residues resulting from mild and severe refining treatments. (A) Isolated lignin products from E, LSA and HSA treatments. (B) Recovered residual solids from E, LSA and HSA treatments.

ether bonds between the lignin units.[28] The reduced amount of the especially labile β-O-4 linkages was apparent in the NMR analyses. Hydrolysis of lignin in alkaline media, essentially traditional "soda pulping", seems to be a commercially practical way to increase the yield of recovered lignin. The adverse effects caused by excessive cleavage of β-O-4 linkages and formation of water-soluble byproducts should be minimized by controlling the isolation conditions (optimization of the temperature-reaction time ratio).

Reactive Extraction in Acidic Ethanol Media. Acid catalyzed hydrolysis in ethanol (ACE) was also investigated as a reactive extraction method and is analogous to organosolv pulping.[29] In this work, 0.5 M HCl was applied as the operational catalyst during the first extraction cycle whereas the following three cycles were carried out without the added acid. The ACE extraction provided minor yield improvements in comparison to the mild E extraction, up to 46 wt %. The chemical functionality of the isolated lignin did not change significantly in comparison to the noncatalyzed extraction, but there was a noticeable reduction in the average molecular weight. These results suggest that the ACE treatment could induce a minor degree of hydrolysis to liberate part of the otherwise insoluble high molecular weight lignin moieties.

Degradative Acid Treatments Combined with Extraction. Acid catalyzed hydrolysis in aqueous media was investigated as an alternative means of increasing the yield of purified lignin. This pretreatment was targeted to eliminate carbohydrate impurities, and any remaining lignin−carbohydrate complexes,[30] but also to induce limited depolymerization of the lignin into more soluble fragments.[28] A degradative treatment in aqueous 0.5 M hydrochloric acid (HCl) solutions was applied as a preparative processing step prior to extraction by ethanol (E) or by medium-severity alkali (MSA).

On the basis of the data of Table 2, the acid treatment resulted in minor benefits in terms of reduced carbohydrate impurities, although with a reduction in the yield of isolated lignin. This was reduced to 21 wt % after the ethanol extraction, and to 41 wt % after the alkaline (MSA) extraction. The reason for this reduction in yield is likely the occurrence of acid catalyzed condensation reactions[28] that bound some of the potentially soluble lignin moieties to the insoluble component, creating larger and more insoluble structures. At the same time, the average molecular weight of the isolated soluble fraction was effectively decreased in both A-MSA and A-E systems (see the Size Exclusion Chromatography section), which may point to the presence of concurrent depolymerization and condensation reactions.

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Characterization. *FT-IR Spectroscopy.* The FT-IR spectroscopic analyses of the isolated lignins (Figure 2A) confirmed the predominance of the most typical lignin bands.[31] Furthermore, no indications related to enzyme impurities were apparent based on the absence of characteristic amide bands in the spectra. Comparison between lignins from mild extractions and EMAL lignin is provided in Figure SI3 of the Supporting Information. Major differences were observed in the FT-IR spectra of the isolated lignins, and the corresponding residual solids (Figures 2 and 3). These differences were further

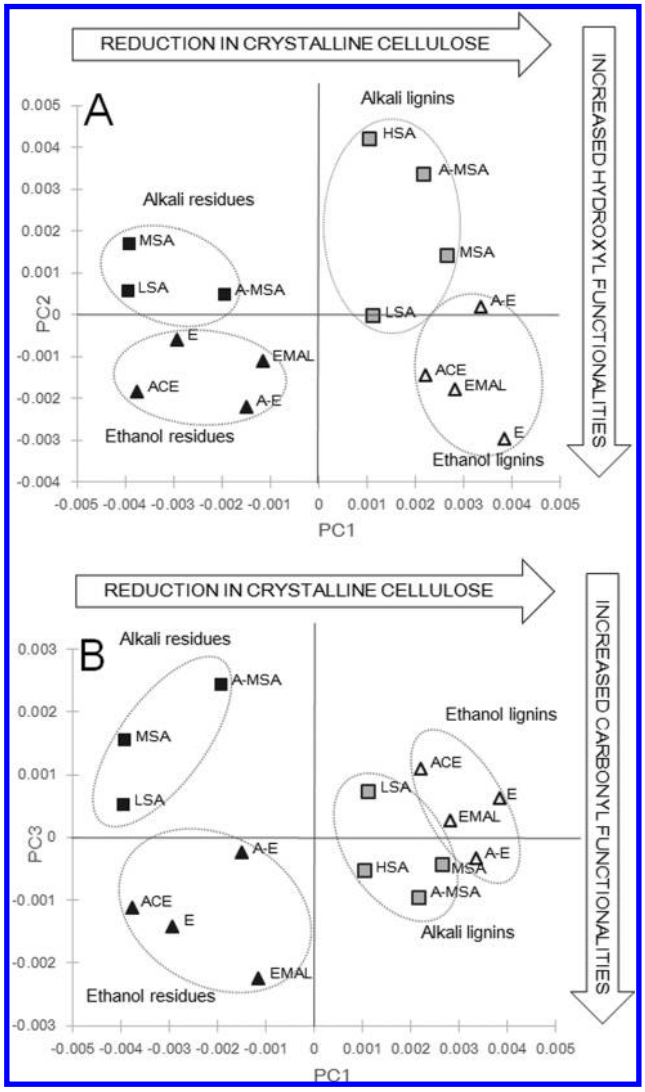

Figure 3. Score plots from the PCA analyses. (A) PC1 as a function of PC2. (B) PC2 as a function of PC3. The residue from HSA was not included, as this was an outlier.

investigated using principal component analysis (PCA). Although subtle differences were found in the hydroxyl and aliphatic C−H regions between 3700 and 2700 cm^{-1}, the principal component analysis scores were mainly caused by differences within the fingerprint region between 1800 and 650 cm^{-1}.

As expected the isolated lignins were easily differentiated from the carbohydrate-rich residues along the PC1 axis, which accounts for 54% of the spectral variation (Figure 3A). The chemical features (PCA loadings shown in Figure SI2 of the Supporting Information) for PC1 include bands at 1115 and 1214 cm^{-1} associated with the syringyl units in lignin.[32] The bands at 996, 1056, 3290 and 3338 cm^{-1} are attributed to cellulose with intermolecular hydrogen bonds, i.e., its crystalline form.[33]

Differences along PC2 (28% of the spectral variation) clearly separate the lignins isolated with alkaline and ethanol extraction processes. The loadings (see the Supporting Information) indicated that this separation is attributed to hydroxyl bands at 1030 and 1124 cm^{-1}.[33] Subtle differences in these primary hydroxyl bands are to be expected because the extraction mode, e.g., ionization of hydroxyls vs simple dissolution, are fundamentally different.

Separation along PC3 accounts for 9% of the spectral variations (Figure 3B). The loadings plots (see the Supporting Information) are governed by the bands at 1720 and 1640 cm^{-1}. These are likely arising from the carboxylic acids and confirmed by the quantitative ^{31}P NMR analyses (see the following section). The PC3 loading at 1710 cm^{-1} is quite broad, suggesting the presence of complex hydrogen bonding patterns. Carbonyl groups (such as ketones) may also influence the PC3 scores. Additionally, separation along PC3 can be attributed to aromatic and methoxyl vibrations at 1595, 1508 and 1420 cm^{-1}.[33]

Cellulose enrichment was anticipated in the residual solids as a result of successful lignin extractions and this was seen in the case of the HSA residue (Figure 2B). Furthermore, for the HSA residue some deposited (or possibly esterified) hydrophobic alkyl-rich moieties could be observed (band 2915 cm^{-1}), likely originating from wood extractives.

NMR Spectroscopy. Quantitative analysis of the functionalities present in the isolated lignins was carried out using various NMR techniques. Figure 4 presents the abundancies of the hydroxyl functionalities determined by quantitative ^{31}P NMR. The observed reduction in the aliphatic hydroxyl contents is dramatic between the mild LSA and E extractions and the most severe reactive HSA extraction. Furthermore, the changes observed in the amount of phenolic groups corresponds with the creation of new chain ends produced by depolymerization reactions (see the following section). At the same time, the formation of condensed structures, revealed here as condensed phenolic OH, has evidently taken place especially in the case of aqueous acidic treatments. These are expected lignin reactions in acidic environments.[28] For the case of HSA lignin, the condensed functionalities may partially originate from the original model residue. Such sparingly soluble polymer structures were most likely converted to soluble fragments via alkaline hydrolysis of the β-O-4 linkages during the HSA treatment.

As expected, the lignins isolated with an alkaline extraction process are rich in carboxylic acid groups. The observed carboxylic acids in the lignins may be partially due to the presence of fatty acid impurities.[34] However, the content of saturated aliphatic carbons and COOH functionalities do not seem to correlate. Aliphatic carbons were seen to be abundant in the ethanol lignins (Table 3) whereas COOH groups were abundant in the products of the reactive alkaline extractions. The specific mechanism for formation of COOH functionalities in lignin, while yet unidentified, could be due to auto-oxidation reactions by the initially dissolved oxygen in the media.[35] The COOH functionalities may have also formed in the starting material during the irradiation treatment.[36] Presumably, these structures have been fragmented and dissolved by the HSA

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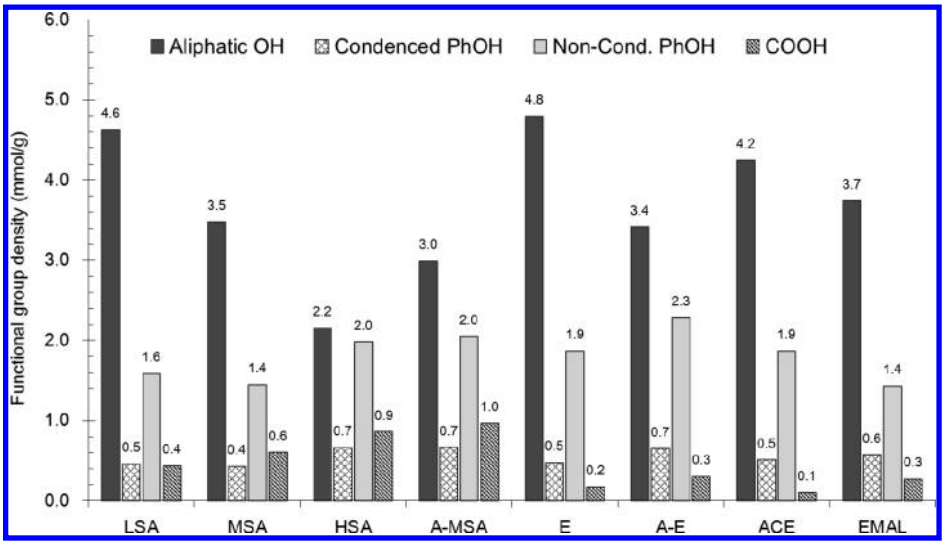

Figure 4. Hydroxyl bearing functional groups present in the isolated lignins determined by quantitative ^{31}P NMR.

Table 3. Quantities of Different Functional Groups Determined by ^{1}H and ^{13}C NMR Analyses for Acetylated Lignins

			quantity of units (mmol/g)*				
structure in acetylated lignin	corresponding nuclei ^{13}C NMR	chemical shift (ppm)	LSA	MSA	HSA	E	A-E
β-O-4 (both α and γ OH)	Cβ	80.1	0.9	0.7	0.3	1.1	0.8
α−OH	Cα	74.3	0.7	0.5	0.2	0.8	0.5
methoxyl	−OMe	55.8	5.4	4.7	4.8	6.4	5.3
	^{1}H NMR						
saturated aliphatics	C−H	1.8−0.6	1.5	1.8	1.6	3.1	7.2
syringyl ring	arom. H (2 per unit)	6.8−6.3	2.4	2.0	2.2	2.5	2.2
guaiacyl ring	arom. H (3 per unit)	6.8−7.2	0.9	0.8	1.1	1.0	0.9
S/G ratio			2.6	2.5	2.1	2.6	2.3

Quantification based on the integrals of the phenolic acetyl signals, where the known value from the quantitative ^{1}H NMR analyses was set as a standard.

extraction, but remained intact in the solid residues of the mild extractions (Figure 3B).

The ^{13}C NMR analyses of selected lignin samples provided more evidence about the structure altering character of the reactive extraction method. Only several discrete signals were resolved well enough for reliable quantification. Progressively diminishing amounts of β-O-4 linkages in lignin were evident, based on the data presented in Table 3, as anticipated for the increasingly severe hydrolysis reactions. Another indicator of chemical alterations occurring during the HSA treatment is the appearance of signals at 123.9, 127.1, 129.7 and 140.2 ppm in the ^{13}C NMR spectra of these lignins, and elimination of signals at 133.1 and 134.9 (see Figure SI4 in the Supporting Information). Reliable assignment of these peaks is difficult because a variety of carbon nuclei common in altered lignins may possess similar chemical shifts,[26] including structures with unsaturated side chains, α-carbonyls and/or various condensed structures.[28]

Proton and ^{13}C NMR analyses revealed the presence of aliphatic moieties most likely arising from extractives. These compounds could be coprecipitating with the lignin especially in the case of the ethanol extractions. Such hydrophobic extractives can conceivably be retained on the residual solids after the alkali extractions (as implied by the FT-IR data of the HSA residue). The presence of these aliphatic compounds could also have an influence on the thermal properties of the isolated lignins (see the Thermal Analyses section).

Size Exclusion Chromatography. Molecular weight distributions and the corresponding weight averages of the lignin products were determined after acetylation (Table 4). The data

Table 4. Determined Average Molecular Weights of the Isolated Lignins after Acetylation

extraction conditions	number-average (M_n) (Da)	weight-average (M_w) (Da)	main peak (MP) (Da)	polydispersity index (M_w/M_n)
LSA	2000	27 000	2500	13.5
MSA	2000	37 800	2700	18.9
HSA	1400	6200	1800	4.4
A-MSA	1600	13 500	2000	8.4
E	1600	11 000	1700	6.9
A-E	1300	2500	1600	1.9
ACE	1600	6000	2000	3.8
EMAL	2800	17 000	2100	6.1

shows that the alkali processes (Figure 5A) were able to recover higher molecular weight fraction relative to the ethanol processes (Figure 5B). These larger lignin structures can be distinguished as visible shoulders in the elution profiles of the SEC analyses. The elution profiles in Figure 5 indicate the size of the polymers below approximately 22 mL elution volume to represent a limit where larger structures lack the capability for a stable solvated state. The solubility of lignin within the size range from 19 to 25 mL is favorable mainly in strongly

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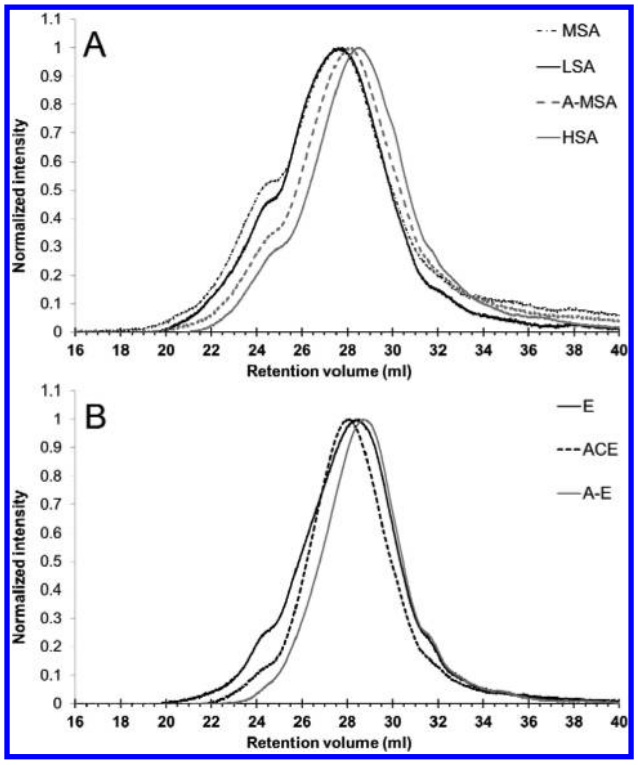

Figure 5. SEC chromatograms of isolated lignins. (A) Alkali based extractions. (B) Ethanol based extractions.

solvating media, such as alkali. The expectedly weaker solvation capacity of ethanol thus explains the products of lower M_w and of narrower polydispersity from the mild ethanol (E) extraction in comparison to the alkali (LSA), while still offering very similar functionality profiles (see Figures 3 and 4, and Table 3). These observations suggest that the ethanol isolated lignin fraction is maybe is more linear, which could be beneficial for specific applications.[37]

The reactive extraction methods HSA or ACE were seen to offer lignin products of reduced polydispersity, by depolymerizing the structures of higher degree of polymerization. The creation of phenolic hydroxyl groups (most likely present as chain ends) is obvious from the plot shown in Figure 6. Therefore, the reactive extraction procedures HSA and ACE can be used to control the average molecular weight of the recovered lignin in order to obtain somewhat lower polydispersities, while obtaining high yields of products. Alternatively, mild- or medium-severity alkaline extraction procedures (LSA or MSA) in combination with fractional precipitation protocols[10,38] could be used to recover lignins of higher molecular weight to the detriment of yield.

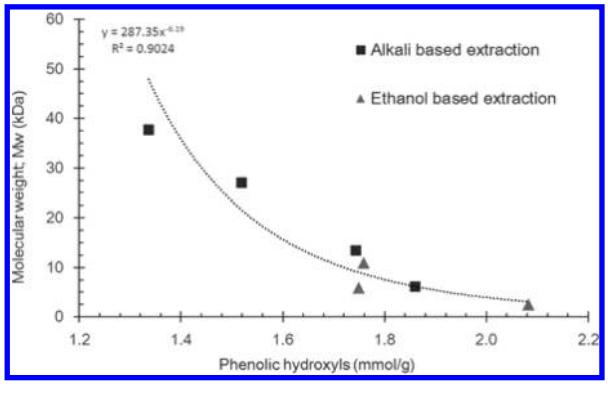

Figure 6. Weight-average molecular weight as a function of phenolic hydroxyl group content, showing a relationship between depolymerization and the creation of phenolic chain ends. The presented regression line corresponds to the whole sample population.

Thermal Analyses. The thermal properties of the isolated lignins were within the range of what is expected for biorefinery lignins[15] (see Table 5), although there were interesting trends related to the isolation medium. On average, the ethanol lignins showed glass transitionx temperatures (T_g) of approximately 30 °C lower in comparison to the alkali products. To address the chemical factors behind these observations, PLS analysis was done using the NMR data, T_g, and the wt % of the residue after 800 °C (data not shown). The solvent effect was largely determined by the larger amounts of aliphatic impurities, lower amounts of carboxylic acids and higher phenolic OH content (i.e., lower molecular weight) in the ethanol lignins. These similar attributes have been observed with low T_g organosolv lignins by Huijgen et al. (2014).[12] Illustrations of the correlations between these variables and the T_g are provided in the Figure SI6 of the Supporting Information.

Table 5. Thermal Properties of the Isolated Lignins Determined by the TGA and DSC Analyses

extraction conditions	glass transition (T_g) (°C)	5% weight loss (°C)	degradation point (T_d) (°C)	residue at 800 °C (wt %)
LSA	157	230	326	39
MSA	154	228	328	45
HSA	159	239	346	46
A-MSA	156	226	335	45
E	132	240	342	32
A-E	123	234	340	31
ACE	126	223	343	32
EMAL	151	233	342	31
hydrolysis residue	155	245	347	29

Thermogravimetric analyses carried out for all extracted lignins showed the following trends. The temperature where a 5% weight loss occurred was practically the same for all lignins (Table 5). This was in agreement with our expectations because there were no chemical foundations to cause augmented or reduced thermal stabilities. Furthermore, the molecular weight ranges of the isolated examined lignins were not that far apart to offer variations in the thermal stability of the lignins.

The amounts of charred residues remaining at 800 °C were significantly lower for the ethanol lignins (Table 5). This is in agreement with the work of García et al. (2012)[39] who concluded that the char residues are influenced by the isolation medium. The PLS analysis correlated the low carbon residues to the presence of aliphatic impurities and the abundancy of the oxygen bearing functionalities. The extractive compounds could be expected to be more readily volatile in comparison to the lignin, and the presence of hydroxyls (especially phenolic OH) has been connected to thermal instability,[24] and possibly leading to volatile degradation products. The amount of inorganic residues that remained after switching to an oxygen atmosphere were lower than 2 wt % for all samples (see Table 2).

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CONCLUSIONS

Alkali and ethanol based lignin isolation processes were found to be comparable in terms of their ability for extracting the freely soluble proportion of lignin from biorefinery residues. The extraction yields offered by simple mild extractions (LSA and E) are mainly limited by their capacity to dissolve the larger more complex polymeric lignin and lignin−carbohydrate structures. Consequently, the application of reactive (higher temperature) alkaline extraction conditions (MSA and HSA) efficiently increased the yield of the isolated lignin since it was shown to cause the cleavage of β-O-4 ether linkages. Acid catalyzed aqueous treatments provided lower extraction yields than the aforementioned reactive alkali extractions. Finally, acid catalyzed reactive extractions (ACE) in ethanol provided some yield improvements, and should be optimized further to estimate their true potential.

The mild extraction conditions used in both alkali and ethanol media seem to offer lignins that retained most of the structural features of native lignins. Significant structural alterations were observed by the application of alkali or acid catalyzed reactive extractions (MSA, HSA and ACE) and degradative acid treatments (A-MSA and A-E) because these treatments showed fewer hydroxyl groups, increased carboxylic acids and lower molecular weights.

Overall, the isolation of lignin from the solid residues of bioethanol production, using simple low-energy extraction systems, was found practical. Lignin products of relatively low carbohydrate impurities and of good solubility in a variety of organic solvents were obtained. Reactive conditions can be integrated into an extraction process so as to influence the functionality and the molecular weights of the lignin products in a certain direction as per the data obtained during this effort. The conclusions of this work point to the need of focused future efforts that aim at minimizing solvent volumes, specifying the affiliated solvation efficiencies of the alkali and ethanol systems and optimizing the precipitation and recovery protocols.

ASSOCIATED CONTENT

Supporting Information

(SI1) Scheme and table about total mass balance of the extraction. (SI2) Loading plots from the PCA analysis. (SI3) Additional FT-IR spectra and PCA analysis. (SI4) ^{13}C NMR spectra of alkali lignins. (SI5) DSC thermograms. (SI6) Graphics describing correlations among lignin T_g, molecular weight and functionalities. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.5b00337.

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Notes

The authors declare no competing financial interest.

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