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Characterization of fractional cuts of co-solvent enhanced lignocellulosic fractionation lignin isolated by sequential precipitation

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ABSTRACT

Lignin valorization is significantly hindered by the intrinsic heterogeneity of its complex structures and variability of biomass feedstocks. Fractionation of lignin can overcome these challenges by producing functionally distinct lignin cuts that can be further tailored to end products. Herein, lignin was extracted and depolymerized from poplar by the co-solvent enhanced lignocellulosic fractionation method with renewable THF to obtain CELF lignin. Several solvents were screened to separate soluble and insoluble fractions from the parent CELF lignin. The ethanol soluble portion was then fractionated into different molecular weight cuts via sequential precipitation of the lignin by reducing the concentration of THF. The physicochemical structures of different CELF lignin cuts were elucidated by GPC and NMR techniques. These results suggest that CELF lignin cuts with lower molecular weight contain progressively higher phenolic and carboxylic acid OH groups, which can be more suitable as green antioxidants than the parent lignin.

1. Introduction

Lignin represents one of the most abundant aromatic polymers on earth and can be derived from lignocellulosic biomass via pulping and biorefinery processing (Hu et al., 2018). It is currently underutilized in cellulosic ethanol plants with < 50% of the lignin generated being used to meet the internal energy requirements needed to generate 2nd generation ethanol which offers great opportunities for developing new value-added products from lignin (Ragauskas et al., 2014; Sannigrahi and Ragauskas, 2011). It has been reported that although lignin is crucial to plant growth, it poses a major impediment to biomass utilization and is considered as one of the most important factors responsible for biomass recalcitrance (Brosse et al., 2012).

Effective lignin valorization requires fine-tuning of upstream processes such as lignin isolation/purification and downstream processes including lignin depolymerization and upgrading (Fan et al., 2017; Li et al., 2017; Long et al., 2014; Rinaldi et al., 2016). Currently, lignin-rich streams in lignocellulosic biorefineries are generated by either enzymatic hydrolysis of carbohydrate rich pretreated material to leave most of the lignin in the solid residue or pretreatment of lignocellulosic

biomass to extract lignin from the plant cell wall (Moon et al., 2011; Ragauskas et al., 2014). Over the past few years, several promising pretreatment techniques have been developed to directly target lignin to improve the overall utilization of biomass for conversion to fuels (Elgharawy et al., 2016; Liu et al., 2018; Schulze et al., 2016; Shuai et al., 2016). Among the new type of pretreatment protocols, the organosolv pretreatment originated from the pulping process is an efficient pretreatment method to extract lignin and hemicellulose from lignocellulosic feedstocks, resulting in relatively cellulose rich residues. In a typical organosolv pretreatment, lignocellulosic materials are treated in an organic or aqueous organic solvent mixture with an acid catalyst. A variety of organic solvents such as alcohols, ketones, esters and phenols have been used in organosolv pretreatment, and low boiling point solvents such as methanol and ethanol are widely used in organosolv pretreatment for economic purposes. Recently, a new generation “lignin-first” thermochemical approach that applies miscible solutions of THF-water with dilute acid, termed as co-solvent enhanced lignocellulosic fractionation (CELF), has attracted increasing attention due to its ability to isolate a clean low-molecular weight lignin product from raw biomass while also enabling high recovery of the biomass

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sugars for their conversion into ethanol or fuel precursors such as furfural and 5-hydroxymethylfurfural (Nguyen et al., 2017, 2016; Smith et al., 2018, 2016). During CELF pretreatment, 85%–90% of the lignin can be solubilized into the co-solvent mixture, and an ash-free and sugar-free product known as CELF lignin can be obtained by precipitation after boiling off the THF (Cai et al., 2014, 2013). Recent studies have found that CELF lignin drastically reduces molecular weight from native lignin while minimizing competing cross-condensation reactions. Thus, CELF process could serve as a unique tool to produce highly fractionated lignin (Meng et al., 2018; Seemala et al., 2018).

The fractionation and structural heterogeneity of different lignin sources have been illustrated by various techniques in several studies including membrane ultrafiltration, solvent extraction, selective precipitation, and flash chromatography. Zikeli and coworkers fractionated wheat straw dioxane lignin by flash chromatography in N,N-dimethylformamide, and illustrated that the eluted high-mass fractions contained more *p*-hydroxyphenyl units while the low-mass fractions contained exclusively tricin units (Zikeli et al., 2016). Lignin fractionation in organic solvents has been developed over the decades, most of which are based on sequential dissolution of lignin fractions using a set of different organic solvents (Jaaskelainen et al., 2017). An et al. fractionated corn straw lignin via a sequential extraction method using dichloromethane, acetic ether, and butyl alcohol to four lignin fractions with different molecular weight and some of the fractions showed remarkable enhanced antioxidant performance (An et al., 2017). Similarly, *Eucalyptus* Kraft lignin was sequentially fractionated by ethyl ether, methane chloride, *n*-propanol, ethanol, methanol, and dioxane to six fractions with molecular weight ranging from 650 to 7800 g/mol, and the thermal stability of the lignin fractions was found to increase with the increasing of molecular weight (Yuan et al., 2009). In general, the molecular weight of lignin fractions isolated by solvent extraction increases with increase of hydrogen bonding capacity and polarity of the solvents (Boeriu et al., 2014). However, the combination of different organic solvents makes the fractionation process complicated and difficult to scale up in industry especially when chemical recycling needs to be considered (Jääskeläinen et al., 2017). On the other hand, precipitation fractionation techniques using two miscible solvents – a lignin solvent and an anti-solvent were introduced quite recently (Sadeghifar et al., 2017). Through tuning the solvent/anti-solvent ratio via adding anti-solvent into lignin solution or evaporating lignin solvent from lignin solution, lignin fractions with desired molecular weight and physicochemical properties could be obtained (Wang et al., 2018). Lignin fractions produced by these techniques have been tested for applications such as adhesives, antioxidants, dispersants, and polyurethanes (Delgado et al., 2018; Sadeghifar et al., 2017; Solt et al., 2018; Vanderlaan and Thring, 1998).

Lignin valorization is critical for economic viability of future integrated biorefineries, and it is crucial to develop efficient technologies to tune lignin properties for the utilization of lignin in various applications. In this study, a CELF process was first applied to a native poplar sample to extract and depolymerize lignin from biomass yielding a refined technical grade lignin product, termed as CELF lignin which was further subjected to organic solvent screening for extracting the high molecular weight fraction. A facile sequential precipitation method using different proportions of THF in water was then applied to the “low” molecular weight part to obtain a set of lignin fractions with unique properties. Finally, the molecular weight homogeneity, functional groups and interlinkages of lignin fractions were examined by GPC and NMR, respectively.

2. Materials and methods

2.1. Materials

Baseline *Populus (trichocarpa x deltoides)* was harvested from Oak

Ridge National Laboratory (ORNL) and shipped to National Renewable Energy Laboratory (NREL) for drying, debarking, and size-reduction. Extractives were removed by an ethanol/toluene Soxhlet extraction, and the composition of the poplar was measured to be ~45% glucan, ~15% xylan, and ~22% Klason lignin via a NREL procedure (Seemala et al., 2018; Sluiter et al., 2008). Hydrated ferric chloride was purchased from Sigma Aldrich (St. Louis, MO). Gasoline and diesel were obtained from a local gas station. Other organic solvents such as THF, toluene, and acetone were all purchased from Fisher Chemicals.

2.2. Lignin fractionation by biomass pretreatment

CELF pretreatment was performed in a 1 L Hastelloy Parr reactor (236HC Series) equipped with a double stacked pitch blade impeller rotated at 200 rpm driven by an electric motor (Meng et al., 2018). Poplar and acid catalyst (FeCl_3) loadings were 5 wt% and 1 wt% based on the dry weight of the total mass of the reaction mixture and anhydrous mass of the THF-water co-solvent, respectively. The pretreatment condition applied in this work was 180 °C, 20 min, and 4:1 THF/water ratio, which is the optimized condition for producing furfural and 5-hydroxymethylfurfural from xylan and glucan, respectively (Seemala et al., 2018). The CELF liquor was first neutralized to pH 7 by adding slightly more than stoichiometric amounts of $\text{Ca}(\text{OH})_2$. The lignin fraction solubilized in THF, CELF lignin, was then precipitated by boiling the hydrolyzate (~50 g) at 80 °C for 6 h inside a fume hood. ~15 g/L of THF was present in the flask after 6 h, and the flask was left overnight in the fume hood for 15 h to allow the remaining THF to evaporate at room temperature. To remove non-lignin impurities, the precipitated dark resinous lignin was then washed with an extensive amount of room temperature DI water. The purified lignin was then dried in a vacuum oven at 45 °C overnight.

2.3. CELF lignin fractionation by solvent extraction

Water and several organic solvents including acetone, ethanol, THF, toluene, gasoline, and diesel were selected and screened to dissolve CELF lignin at room temperature for 24 h. The undissolved part was isolated by centrifugation and the soluble fraction was then obtained via rotary evaporation under reduced pressure. Lignin fractionation yields were calculated based on the dry weight of the recovered fractions.

2.4. Lignin fractionation by sequential precipitation

The ethanol soluble CELF lignin was fractionated according to the scheme presented in Fig. 1. Ethanol soluble CELF lignin obtained in the previous step was dispersed into 40% THF under stirring for 12 h at room temperature, and the solubility test indicated that it was completely dissolved in 40% THF. Water was then added to the THF solution to reduce the THF concentration by 5 wt% at a time. The solution was centrifuged and the insoluble fractions if appeared were separated from the supernatant after 60 min of mixing. The first insoluble fraction appeared after the 100% THF was diluted to 35% and was termed as Fraction F_{35} . After centrifugation, the supernatant was collected and the THF concentration was further decreased to 30% with addition of water, and the insoluble fraction was again separated by centrifugation (referred to as fraction F_{30}). This procedure was repeated in the foregoing step, and the reduction of THF concentration was continued to 25 wt% (F_{25}) and 20 wt% (F_{20}). The remaining soluble lignin in 20% THF ($F_{<20}$) was finally separated by rotary evaporation. The insoluble fractions obtained after each precipitation were dried in a vacuum oven at 45 °C for 24 h, and the fraction yield was calculated based on the starting dry weight of lignin.

Fig. 1. Flowcharts of CELF lignin fractionation by solvent extraction and sequential precipitation.

2.5. Molecular weight analysis of CELF lignin fractions

Lignin samples (~5 mg) were dried in a VWR 1400E vacuum oven and then dissolved in pure THF (~5 mL) overnight and the solutions were filtered through a 0.45 µm PTFE filter prior to GPC analysis. The molecular weight analysis was determined by an Agilent GPC SECurity 1200 system equipped with a UV detector (270 nm). THF was used as the mobile phase at a flow rate of 1.0 mL/min, and polystyrene narrow standards were used to prepare the calibration curve.

2.6. NMR analysis of CELF fractions

2.6.1. HSQC NMR analysis

Two dimensional ^{13}C – ^1H heteronuclear single quantum coherence (HSQC) was acquired on a Bruker Avance III HD 500-MHz spectrometer. ~40 mg dry lignin samples were dissolved in 0.5 mL of DMSO- d_6 . HSQC experiments were carried out with a Bruker pulse sequence (hsqctgpsi2.2) using a N_2 cryoprobe (BBO ^1H & ^{19}F -5 mm) under the following conditions: 166 ppm spectra width in F1 (^{13}C) dimension with 256 data points (acquisition time 6.1 ms), 12 ppm spectra width in F2 (^1H) dimension with 1024 data points (acquisition time 85.2 ms), a 1.0 s delay, a $J_{\text{C-H}}$ of 145 Hz, and 128 scans. The relative abundance of lignin interunit linkage and compositional subunits were estimated using volume integration of contours in the spectra. The semi-quantitative analysis for S, G, and PB units was conducted by integrating the peaks of $\text{S}_{2/6}$, oxidized $\text{S}_{2/6}$, G_2 , and $\text{PB}_{2/6}$, and the α position of β -O-4, β -5, β - β was used to estimate the relative abundance of

lignin interunit linkages.

2.6.2. ^{31}P NMR analysis

The ^{31}P NMR spectra were obtained after derivatization of lignin with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) (Pu et al., 2011). The spectra of phosphitylated lignin were acquired using an inverse-gated decoupling pulse sequence (Waltz-16), 90° pulse, 25 s pulse delay with 64 scans. N-hydroxy-5-norbornene-2,3-dicarboximide was used as the internal standard.

3. Results and discussion

3.1. CELF lignin fractionation by organic solvent extraction

Poplar was first subjected to CELF pretreatment, and a refined technical grade lignin product, termed as CELF lignin was recovered from the process. Water and several common industrial organic solvents including acetone, ethanol, toluene, THF, gasoline, and diesel were screened for their ability to partially solubilize CELF lignin. Polar aprotic solvents such as N,N-dimethylformamide and dimethyl sulfoxide were not selected due to their ability to fully solubilize even highly crosslinked lignin samples, and solvents such as dichloromethane and dioxane were not chosen either because of environmental and regulatory concerns (Duval et al., 2016). The molecular weight distributions and yields of the soluble and insoluble fractions in different solvents are presented in Table 1. THF was found to fully dissolve the CELF lignin as expected, while gasoline and diesel

Table 1

Yield and molecular weight of water and several organic solvents soluble and insoluble lignin fractions.

Substrate		M _w (g/mol)	M _n (g/mol)	PDI	Yield (%)
Parent CELF lignin		1814 ± 20	1017 ± 19	1.8	N/A
Water	Soluble	529 ± 24	203 ± 5	2.6	3.1
	Insoluble	1902 ± 25	944 ± 8	2.0	93.1
Acetone	Soluble	1275 ± 56	841 ± 25	1.5	74.7
	Insoluble	2004 ± 78	1063 ± 16	1.8	24.6
Ethanol	Soluble	1195 ± 55	832 ± 25	1.4	61.2
	Insoluble	2779 ± 156	1403 ± 60	2.0	39.8
Toluene	Soluble	1006 ± 12	667 ± 52	1.5	6.6
	Insoluble	1783 ± 53	879 ± 7	2.0	96.7
Gasoline	Soluble	N/A	N/A	N/A	0
	Insoluble	1834 ± 41	959 ± 42	1.9	100
Diesel	Soluble	N/A	N/A	N/A	0
	Insoluble	1849 ± 33	951 ± 75	1.9	100
THF	Soluble	1868 ± 41	1036 ± 48	1.8	100
	Insoluble	N/A	N/A	N/A	0

N/A- not applicable.

were unable to dissolve CELF lignin presumably due to their low hydrogen bonding capacity. The majority of parent CELF lignin was insoluble in water (~93%) and toluene (~97%), while ~75% and ~60% of CELF lignin were dissolved in acetone and ethanol, respectively. Duval et al. also reported that the solubility of kraft lignin was higher in acetone than that in ethanol due to acetone's higher Hansen polar solubility parameter despite its poor hydrogen bonding capacity (Duval et al., 2016). In general, the organic solvent soluble lignin fraction all have lower molecular weight and polydispersity index (PDI) compared to its parent CELF lignin. The dissolution of lignin fractions with higher weight-average molecular weight is also accompanied by the higher yields of soluble fragments according to Table 1. All the insoluble fractions have significantly higher molecular weights compared to soluble fractions, which could be explained by the fact that it is more difficult for the solvent to penetrate the highly longer and branched macromolecular chains (Passoni et al., 2016). This is in accordance with the results obtained by Sarkanen et al. and Jääskeläinen et al. (Jääskeläinen et al., 2017; Sarkanen et al., 1984). Among these tested solvents, based on the yields and molecular weight distribution analysis, ethanol proved to be the most effective solvent to separate high molecular weight (ethanol insoluble – EI) fraction from low molecular weight (ethanol soluble – ES) fraction. Ethanol is also considered as one of the cheapest and readily available green solvents that can be produced from lignocellulosic biomass.

3.2. Lignin fractionation by sequential precipitation

Ethanol soluble (ES) CELF lignin was subjected to a sequential precipitation method to further obtain lignin fractions with different chemical structures and molecular weights. ES CELF lignin was first dissolved in THF, and then water was added to the THF solution stepwise. THF was selected instead of ethanol because of two reasons: (1) aqueous ethanol solvent precipitation has been successfully applied on lignin fractionation in a recent study and (2) it has been reported that aqueous THF solvent is a great “theta” solvent, in which lignin adopts extended coil configurations and does not aggregate (Jääskeläinen et al., 2017; Smith et al., 2016). Results indicated that ~2 g of CELF lignin could be completely dissolved in 50 mL 40% of THF solution (60% H₂O). Lignin usually exhibits maximum solubility in solvents with the solubility parameter (δ) close to its own. Addition of water did not cause immediate precipitation of lignin because it has been reported that the solubility of lignin in aqueous organic solvents at a specific organic solvent/water ratio was found to be the best with δ values of the mixture close to that of lignin which is in the range of 13 to 14.2 (cal/cm³)^{1/2} (Ni and Hu, 1995; Ye et al., 2014). The δ value for

Table 2

Yield and molecular weight of ethanol soluble (ES) CELF lignin and its fractions.

Substrate	M _w (g/mol)	M _n (g/mol)	PDI	Yield (%)
ES CELF lignin	1195 ± 55	832 ± 25	1.4	N/A
F ₃₅	1366 ± 38	880 ± 27	1.6	18.0
F ₃₀	1168 ± 15	656 ± 28	1.8	51.0
F ₂₅	750 ± 17	578 ± 13	1.3	17.5
F ₂₀	681 ± 13	426 ± 10	1.6	3.2
F < 20	560 ± 10	314 ± 2	1.8	7.5

N/A- not applicable.

water and THF are 23.4 and 9.5 (cal/cm³)^{1/2}, respectively (Ye et al., 2014). Water could also serve as a highly efficient plasticizer due to its small molecular size, facilitating the diffusion of organic solvent molecules into the compact associated lignin complexes and subsequently increasing the solubility of lignin in aqueous organic solvent (Wang et al., 2018). Thus, the CELF lignin solubility actually increased in THF/water mixture with the addition of water at the beginning as the δ value approaches that of lignin. As the THF concentration decreased from 40% to 35%, the first insoluble fraction appeared termed as F₃₅. The yields of different lignin fractions are shown in Table 2. The maximum fraction yield (~51%) occurred in 30% THF, and no obvious relationship was found between fraction yield and the THF concentration.

3.3. Lignin characterization

3.3.1. Molecular weight analysis of lignin fractions by GPC

All the lignin fractions could be fully dissolved in THF without further acetylation, making molecular weight analysis via GPC easier. The molecular weights and polydispersity estimated using polystyrene standard as well as the yields of the parent lignin (ES CELF lignin) and its fractions are presented in Table 2. Results indicated that both M_w and M_n of the individual fractions obtained via sequential precipitation method decreased as the THF concentration decreased from 35% to 20%. However, no obvious trend was obtained for the PDI change as all the fractions demonstrated relatively low polydispersity as shown in Table 2. Lignin fraction F₃₅ had a higher molecular weight than its parent ES CELF lignin, while the remaining fractions all had lower molecular weight. It was reported that such low molecular weight lignin fractions are generally oligomers or lignin chain fragments which actually are not generated by the fractionation process but are already present in their parent lignin before precipitation (Passoni et al., 2016; Saito et al., 2014). Around 7.5% ES CELF lignin remained dissolved in 20% THF solution, which contained mainly phenolic fragments up to trimers. Our study clearly showed that a series of well-defined lignin fractions with desired molar mass could be obtained by adjusting the volumes of water into a THF solution of CELF lignin appropriately.

3.3.2. Chemical structural analysis of lignin fractions by HSQC and ³¹P NMR

In an effort to evaluate the potential of these lignin streams obtained after ethanol extraction and sequential precipitation, a detailed characterization of these lignin fractions was performed. A previous study has shown that the aryl ether interunit linkages of CELF lignin were significantly cleaved under acid conditions in THF, resulting in a dramatic decrease in lignin molecular weight and increase in phenolic hydroxyl groups (Meng et al., 2018). The CELF lignin obtained in this study had a ~85% decrease in weight-average molecular weight and a 4-fold increase in content of phenolic hydroxyl groups compared to poplar native lignin (Meng et al., 2018). Specifically, no β -O-4 and β -5 interunit linkages were detected while only trace amount of β - β linkages were present in the parent CELF lignin. The clean anomeric correlation regions (δ_C/δ_H 90–105/3.9–5.4 ppm), which are mainly assigned to the lignin-carbohydrate complex (LCC), also revealed that LCC bonds were significantly cleaved during the CELF process.

Table 3

Semi-quantitative information (%) for lignin subunits in lignin fractions obtained through CELF process, ethanol extraction, and sequential precipitation.

	CELF lignin	EI CELF	ES CELF	F ₃₅	F ₃₀	F ₂₅	F _{<20}
S	66.3	63.1	75.1	66.7	61.0	70.3	77.1
G	33.7	36.9	24.9	33.2	39.0	29.7	22.9
PB	23.5	18.3	29.6	25.4	20.0	17.4	39.7
S/G	2.0	1.7	3.0	2.0	1.6	2.4	3.4

EI- ethanol insoluble; ES- ethanol soluble.

A semi-quantitative analysis using volume integration of contours in HSQC spectra was performed, and the monolignol composition of each lignin fractions obtained in this study is shown in Table 3. Results show that ethanol is preferred for dissolving CELF lignin with high S/G ratio and high content of p-hydroxybenzoate (PB) units. Li and coworkers also reported that the methanol soluble lignin fractions gave a higher S/G ratio than the original and methanol insoluble lignin fractions (Li and McDonald, 2014). As the THF concentration decreases, the content of PB units in precipitated lignin fractions decreases in the following order: F₂₅ < F₃₀ < F₃₅. Interestingly, the relative amount of PB units in F_{<20} actually was the highest among these fractions. In terms of the relationship between molecular weight and lignin subunits, no clear correlation between S/G ratio or PB content and molecular weight was obtained in this study. A few studies have reported that S/G ratio of lignin fractions gradually decreased with increased molecular weight due to the fact that guaiacyl compounds had a reactive site on the phenyl ring to participate in the condensation reactions, forming higher molecular weight fragments (Moerck et al., 1988; Moerck et al., 1986; Van der Klashorst and Strauss, 1987). However, Park et al. also reported that there was no noticeable trend for S/G ratios between lignin fractions obtained by sequential organic solvents extraction of organosolv lignin and milled wood lignin (Park et al., 2018). The different conclusions obtained here and in literature were probably due to the fact that the aryl ether interlinkages are completely cleaved during the CELF process (Wang et al., 2010).

Hydroxyl groups represent an important characteristic associated with the properties of lignin. Quantitative ³¹P NMR analysis was used to determine the amount of various types of hydroxyl groups in lignin, including aliphatic, guaiacyl, C₅ substituted, p-hydroxyphenyl, and carboxylic hydroxyl group. The content of hydroxyl groups in lignin fractions obtained via ethanol extraction and sequential precipitation

method are shown in Figs. 2 and 3, respectively. Unlike native poplar lignin which has aliphatic OH groups as its dominant OH type, the parent CELF lignin as well as its different fractions all have phenolic OH groups as their dominant hydroxyl group ranging from ~67% to ~76% of total OH groups due to the extensive cleavage of aryl ether bonds (Meng et al., 2018). Specifically, C₅ substituted OH was the most prominent OH type among various phenolic OH groups, followed by guaiacyl and p-hydroxyphenyl OH. ES CELF lignin has ~25% more phenolic OH, ~74% more carboxylic acid OH, and ~20% more total OH group than EI CELF lignin. The content of guaiacyl and p-hydroxyphenyl OH group in ES CELF lignin increased compared to its parent CELF lignin, whereas EI CELF lignin showed a slight decrease.

Lignin fractions obtained via sequential precipitation of ES CELF lignin show comparable aliphatic OH content. This is in accordance with the results obtained by Domínguez-Robles et al., showing that no clear trend is observed in the content of aliphatic OH groups according to the molecular weight of acetone fractionated Kraft and organosolv lignin (Domínguez-Robles et al., 2018). Meanwhile, the low molecular weight lignin fractions collected from lower concentrations of THF exhibit higher phenolic OH and carboxylic acid OH content. This could be due to the fact that degradation of highly cross-linked lignin into small molecular weight fractions promotes the formation of new phenolic OH groups which enhances the hydrophilicity of CELF lignin (Cui et al., 2014; Wang et al., 2018). Similar results have been reported for Kraft lignin and organosolv lignin (Cui et al., 2014; Sadeghifar et al., 2017). These variations in OH content especially in phenolic and carboxylic acid OH group yield an overall increase of total OH groups in lignin fractions with decreasing molecular weight. It has been reported that free phenolic OH groups are essential for the antioxidant activity of lignin as well as the reactivity of lignin towards formaldehyde, thus these lignin fractions could have potential for applications such as antioxidants and phenolic resins (Mansouri and Salvadó, 2006; Pan et al., 2006).

4. Conclusions

Poplar lignin was fractionated into a series of lignin fractions with different molecular weights and chemical structures. GPC analysis indicated that lignin fractions with lower molecular weight were more tolerant to decreasing concentrations of THF in co-solvent mixture. NMR analysis demonstrated that the obtained lower molecular weight lignin fraction possessed more phenolic and carboxylic acid OH groups.

Fig. 2. Quantification of different hydroxyl group content (mmol/g) of CELF lignin fraction before and after ethanol extraction.

Fig. 3. Quantification of different hydroxyl group content (mmol/g) of lignin fraction obtained by sequential precipitation.

Our results indicated that the sequential precipitation method used in this study along with CELF pretreatment is a novel and facile way for the reduction of lignin's heterogeneity and isolation of lignins free of aryl ether linkages from the plant cell wall.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2018.09.130>.

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