# Characterization of Two Novel Bio-based Materials from Pulping Process Side Streams: Ecohelix and CleanFlow Black Lignin

Anna Abbadessa,[a,*] Petri Oinonen,[a,b] and Gunnar Henriksson [a,*]

The characteristics of two novel types of technical lignin, namely Ecohelix (EH) and CleanFlow black lignin (CFBL), isolated from two different pulping process side streams, were analyzed. EH and CFBL were analyzed in terms of general composition, chemical functionalities, molar mass distribution, and thermal stability. For comparison, two relevant types of commercially available lignosulfonate and kraft lignin were used. The results showed that EH contains a large amount of sulfonated lignin, together with carbohydrates and ash. As such, it can be considered a lignin-carbohydrate hybrid molecule. CFBL was found to contain 91.5% Klason lignin and the lowest amount of carbohydrates (0.3%). EH showed the highest content of aliphatic OH groups (5.44 mmol/g) and CFBL a high content of phenols (4.73 mmol/g). EH had a molecular weight of 31.4 kDa and a sufficient thermal stability. CFBL had the lowest molecular weight ($M_w$ = 2.0 kDa) and thermal stability of all kraft lignins analyzed in this study. These properties highlighted that EH is a suitable building block for material development and that CFBL is a promising material for the production of biofuel and biochemicals.

*Keywords: Lignin characterization; Lignin-carbohydrate complexes; Laccase; Ultrafiltered lignin; Kraft lignin; Lignosulfonate*

*Contact information: a: Department of Fiber and Polymer Technology, School of Engineering Sciences in Chemistry, Biotechnology, and Health, Royal Institute of Technology, Teknikringen 56-58, SE-100 44 Stockholm, Sweden; b: Ecohelix AB, Teknikringen 38, SE-100 44 Stockholm, Sweden;*
** Corresponding authors: ghenrik@kth.se; abbadessaanna@gmail.com*

## INTRODUCTION

The dramatic environmental impact of petroleum-based technologies used for the procurement of fuels and plastics, as well as the limited supply of fossil raw materials, has led to an increasing interest in viable alternatives (Ghatak 2011). Biomass represents one of the most suitable options, since it is a renewable feedstock that can be employed to produce energy within a carbon-neutral cycle or that can be potentially processed into high-value products, *e.g.*, chemicals and polymers (McKendry 2002a,b,c; Sheldon 2014; Xu *et al.* 2014; Isikgor and Becer 2015; Oh *et al.* 2015). Among the different types, ligno-cellulosic biomass that is derived from the side streams of the pulp and paper industry is highly attractive because its procurement is independent from the food industry. Rather, it represents a clever and environment-friendly way to handle waste waters and side products from the pulp and paper industry and to convert common mills into more profitable "integrated forest bio-refineries" (Van Heiningen 2006; FitzPatrick *et al.* 2010).

Wood-derived biomass is mainly composed of cellulose, hemicellulose, and lignin. One of the most important applications of wood is chemical pulping for use in papers, boards, and cellulose derivatives. In these applications, the lignin is partly degraded,

chemically modified, and consequently solubilized in pulping liquors. Any technical lignin that is isolated from the pulping processes is generally used as a combustible material within the same pulp mill where it is produced. Nevertheless, a significant amount of technical lignin is also separately commercialized. Lignin is usually marketed as a solid bio-fuel, whereas lignosulfonate, *i.e.*, sulfonated lignin derived from sulfite pulping, has found use as a concrete additive, emulsifier, and ion-exchange resin (Tejado *et al.* 2007; Tomani *et al.* 2011). In addition to these low-value applications, lignin has gained significant attention as a bio-polymer for higher value applications, *e.g.*, in polymer and material science (Ragauskas *et al.* 2014; Ten and Vermerris 2015; Upton and Kasko 2016). Besides the obvious advantages derived from its biodegradability, its environment-friendly method of production, and its relevant availability, the scientific interest in lignin is imputable to its unique chemical structure, characterized by aromatic subunits linked *via* several types of ether or carbon-carbon bonds (Upton and Kasko 2016). Furthermore, the presence of phenolic and carboxyl functionalities generally present in all types of lignin, as well as sulfonic acid groups mainly present in lignosulfonates, render lignin-based polymers interesting for further chemical functionalization and/or material processing.

The main challenge associated with the use of lignin as a building block for high-value applications is related to the heterogeneity of its physico-chemical properties. The source (softwood *versus* hardwood, plant species, *etc.*), type of pulping process (mechanical, thermo-mechanical, sulfite, kraft, soda, *etc.*), and type of lignin isolation process (ultrafiltration, precipitation, *etc.*) are only three examples of parameters that may affect the chemical structure of lignin and consequently its physico-chemical behavior (Upton and Kasko 2016). Among the several reactions that lignin may undergo during pulping based on the type of pulping process, the cleavage of α-aryl and β-aryl ether bonds leads to an increase in phenolic functionalities, a decrease in the average molecular weight, and an increase in polydispersity (Chakar and Ragauskas 2004; Upton and Kasko 2016). On the other hand, the formation of new C-C bonds, for instance *via* radical coupling reactions, can also occur, leading to significant molecular changes (Chakar and Ragauskas 2004). Finally, sulfonation occurring during sulfite pulping introduces negative charges on lignin and leads to a consequent high water-solubility, typical of the obtained lignosulfonates. Furthermore, the content of carbohydrate, ash, and extractives may differ for different types of lignin and consequently affect the polymer behavior as well as the properties of any final product, *e.g.*, film, foam, *etc.*, which is subsequently made (Nadif *et al.* 2002; Boeriu *et al.* 2004; Mansouri and Salvadó 2006; Tamaki and Mazza 2010; Monteil-Rivera *et al.* 2013).

All these aspects point out a general need for advanced characterization of each novel lignin-based material. The aim of this study was to characterize two novel lignin-based materials, *i.e.*, Ecohelix and CleanFlow black lignin, here abbreviated as EH and CFBL, respectively, and highlight their advantageous characteristics in comparison to well-known lignin-based materials of a similar category.

EH is a lignin-based material that is extracted from the pre-hydrolysis mixture during the early stage of a pulping process. The related patent (WO2012/071004) describes the extraction and purification processes of EH. Briefly, the pre-hydrolysis mixture, which contains wooden materials and cooking chemicals, undergoes micro-filtration to remove micro-sized (and bigger) particles, and subsequent ultra-filtration to remove chemicals and small molecular weight polymeric fractions, as well as to concentrate the retentate solution. The polymer present in the retentate is subsequently cross-linked *via* an oxidative coupling reaction catalyzed by the enzyme laccase, as inspired by the natural polymerizing capacity

of this enzyme toward lignin aromatic residues (Nakamura and Go 2005). The obtained polymer is further purified *via* ultra-filtration, and finally recovered and stored in the obtained highly concentrated aqueous solution. The EH polymer that is used in this study was obtained from a pilot plant coupled to a sulfite pulp mill belonging to Domsjö Fabriker AB, Sweden.

Laccase is a fungal oxidative enzyme that is naturally involved in the degradation of lignocellulosic biomass, as well as in wood biosynthesis, by catalyzing the formation of phenoxy radicals from phenolic hydroxyl groups (Widsten and Kandelbauer 2008). The subsequent uncatalyzed chemistry that phenoxy radicals undergo can lead to the formation of degradation products. The lignin-degrading capacity of laccase, often in combination with a mediator, has been suggested to be utilized in the pulp and paper industry *via* the enzymatic treatments of lignocellulosic starting materials and pulps, such as during bio-pulping and bio-bleaching (Widsten and Kandelbauer 2008). Nevertheless, phenoxy radicals that are generated from laccase activity can also couple to each other, resulting in cross-linking products. This phenomenon has been the leading principle for the use of laccase to catalyze the grafting of small phenolic compounds onto ligno-cellulosic materials, as well as to catalyze cross-linking reactions (Felby *et al.* 2002; Garcia-Ubasart *et al.* 2011; Garcia-Ubasart *et al.* 2012). The use of laccase to catalyze cross-linking reactions in lignin-containing materials has been extensively applied in our group, and it has been identified as an efficient method to increase the average molecular weight of commercially available lignosulfonates, as well as hemicellulose-lignin hybrid molecules derived from thermo-mechanical pulping (TMP) process waters and pre-hydrolysis extracts (Areskogh *et al.* 2010a,b,c,d; Areskogh and Henriksson 2011; Oinonen *et al.* 2013, 2015). Lately, this principle has been industrially applied within the start-up company Ecohelix, which resulted in the lignin-based material described in this study, *i.e.*, EH.

CFBL is a kraft lignin extracted by continuous ultrafiltration of black liquor derived from kraft pulping processes, as previously described (Keyoumu *et al.* 2004; Helander *et al.* 2013; Giummarella *et al.* 2016). Briefly, the ultrafiltration of black liquor is performed using a ceramic membrane with a low molecular weight cut-off (*e.g.*, 5 kDa). The pH of the permeated solution is subsequently adjusted to slightly acidic conditions, and lignin is further isolated by decantation.

Generally speaking, kraft lignin is isolated from an alkaline pulping process in the presence of sulfur-containing chemicals, a process known as kraft pulping, and it is the most abundantly produced type of lignin worldwide (Tejado *et al.* 2007). Kraft lignin has been extensively studied in the last decades, and scientific advances aiming at the detailed knowledge of its chemical structure, as well as at the identification of novel high-value applications, are continuously emerging (Azadi *et al.* 2013; Duval and Lawoko 2014; Xu *et al.* 2014; Li *et al.* 2016; Gall *et al.* 2017; Gillet *et al.* 2017). Currently studied high-value applications of lignin involve its use as a polymer in material science or its conversion to liquid fuel, $H_2$, and aromatic chemicals. One of the main limitations for the industrial use of lignin as a polymer is its poor homogeneity in terms of structure and molecular weight distribution. To overcome this issue, several efforts directed at the fractionation of lignin, for instance *via* membrane filtration and selective precipitation, have been reported (Alekhina *et al.* 2015; Yan *et al.* 2015; Duval *et al.* 2016; Gillet *et al.* 2017). On the other side, the conversion of lignin into biochemicals and biofuels also presents several challenges (Azadi *et al.* 2013; Xu *et al.* 2014). For this particular application, lignin has to be efficiently depolymerized, and degradation products have to be separated.

One of the limitations here is related to the recalcitrance and chemical stability of lignin. In this context, low molecular weight lignin factions are considered more advantageous. CFBL can be considered the result of a scientific strategy that aimed at producing a selected low molecular weight lignin fraction with high yield, using an industrially translatable procedure for value-added applications of lignin in fuel and chemical production.

In this study, the characteristics of EH and CFBL in terms of carbohydrate, lignin, and ash content, as well as in terms of molecular weight distribution and thermal stability, are reported and compared with those of four other relevant types of lignin. Two different types of lignosulfonates, lignosulfonate sodium salt and calcium salt from two different suppliers, were chosen for direct comparison to EH. Sodium lignosulfonate from Domsjö Fabriker AB is the most suitable material for comparative purposes because it is produced within the same pulp mill where the EH pilot plant is located. On the other hand, two different types of kraft lignin, kraft lignin produced through LignoBoost technology (Tomani 2010) and kraft lignin from UPM Biochemicals, were chosen for direct comparison to CFBL.

## EXPERIMENTAL

### Materials

All chemicals and solvents were purchased from Sigma Aldrich (Stockholm, Sweden) unless stated otherwise. Tetra-*N*-butylammonium hydroxide (40% w/w aqueous solution) was obtained from Alfa Aesar (Heysham, United Kingdom) and $H_2SO_4$ 72% from AppliChem Panreac (Barcelona, Spain). Ecohelix (batch DH6F), herein abbreviated as EH, was kindly donated by Ecohelix AB (Stockholm, Sweden) as a concentrated aqueous solution (approx. 26% w/w) that was freeze-dried prior use. Sodium lignosulfonate and calcium lignosulfonate DP401, herein abbreviated as LSD and LSB, respectively, were kindly donated by Domsjö Fabriker AB (Örnsköldsvik, Sweden) and Borregaard LignoTech (Sarpsborg, Norway), respectively. Ultrafiltered kraft lignin, abbreviated as CFBL, and LignoBoost, abbreviated as LBoost, were kindly donated by CleanFlow AB (Forshaga, Sweden) and Rise Innventia AB (Stockholm, Sweden), respectively. A third type of kraft lignin was purchased from UPM Biochemicals (Helsinki, Finland) and is abbreviated as UPML. Abbreviations, suppliers, and woody sources are listed in Table 1.

**Table 1.** List of Lignin-based Materials Analyzed in this Study with Supplier, Abbreviation, and Woody Source

| Material | Supplier | Abbreviation | Source |
|---|---|---|---|
| Sodium lignosulfonate-carbohydrate | Ecohelix AB | EH | softwood |
| Sodium lignosulfonate | Domsjö Fabriker AB | LSD | softwood |
| Calcium lignosulfonate | Borregaard LignoTech | LSB | hardwood |
| Ultrafiltered kraft lignin | CleanFlow AB | CFBL | softwood |
| Kraft lignin | UPM Biochemicals | UPML | softwood |
| Kraft lignin (LignoBoost) | Rise Innventia AB | LBoost | softwood |

## Methods

*Neutral sugars, lignin, and ash content*

To quantify the content of the expected neutral monosaccharidic units, *i.e.*, arabinose, rhamnose, galactose, glucose, xylose, and mannose, in lignin samples, a method based on acidic degradation (according to SCAN-CM 71:09, 2009) followed by analytical ion exchange chromatography was used. Briefly, 200 mg of sample was mixed with 3 mL $H_2SO_4$ (72% w/w, aqueous solution), and the mixture was placed in a desiccator for 1 h. After proper mixing, the mixture was placed in the desiccator for additional 20 minutes and finally diluted with MilliQ water to a volume of 84 mL. Hydrolysis was carried out in an autoclave for 60 minutes at 125 °C. Next, filtration under vacuum was performed to recover the insoluble residue, which was subsequently dried overnight at 105 °C and weighed to estimate the Klason lignin content. On the other side, the volume of the permeate solution was adjusted to 100 mL. The obtained solution was filtered through 0.2 µm filters and injected in a high-performance anion-exchange chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a pulsed amperometric detector (HPAEC-PAD) and a CarboPac PA-1 column, using water, 300 mM NaOH aqueous solution, and 200 mM NaOH/170 mM sodium acetate aqueous solution as eluents in a multi-step gradient mode. A 10-fold dilution of the permeate prior to the analysis was performed only for EH samples where a much higher sugar content was expected. Chromatograms were processed using a Chromeleon 7.1 software, and correction for water content was done using 0.9 and 0.88 as correction factors for hexoses and pentoses, respectively. Solutions of the abovementioned neutral monosaccharides at known concentrations, which underwent similar hydrolysis conditions as for the samples, were used as standards. After proper dilution, the permeate solutions of CFBL, UPML, and LBoost were also used to measure the acid-soluble lignin (ASL) content according to a previously described Ultraviolet (UV) spectrophotometric method. An extinction coefficient of 128 l $g^{-1}cm^{-1}$ was used (Dence 1992). A quantitative determination of the sulfonated lignin (herein abbreviated as LS) was performed for EH, LSD, and LSB according to a UV spectrophotometric method reported earlier (Lin 1992). The ash content was determined as the residual solid material after the combustion of lignin samples according to the ASTM method D1102-84 (2013). In brief, known amounts of sample were placed into dried ceramic crucibles and incubated overnight at 105 °C. Next, the dry weight of each sample was measured, and samples were subsequently incubated in a furnace heated to 600 °C overnight. The weight of the ash was measured after complete cooling to room temperature in a desiccator. The measurements were performed in duplicate.

*Uronic acids content*

For the determination of uronic acids, *i.e.*, glucuronic acid and galacturonic acid, a less aggressive acidic treatment, as described elsewhere, was used, with some modifications (Albersheim *et al.* 1967; De Ruiter *et al.* 1992). Samples were mixed with 2M trifluoroacetic acid (TFA) aqueous solution at a concentration of 1 mg/mL and incubated at 120 °C for 3 hours. Subsequently, 1.5 mL of the reaction mixture was taken and dried overnight under mild $N_2$ flow. Only for EH samples was a smaller amount, *i.e.* 0.1 mL, taken considering the expected higher sugar content. The residue was further re-dissolved in 1 mL of MilliQ water, filtered through 0.2 µm filters, and injected into the same anion-exchange chromatography system as described above, using water, 300 mM NaOH aqueous solution, 200 mM NaOH/170 mM sodium acetate aqueous solution, and 1 M sodium acetate aqueous solution as eluents, in a multi-step gradient mode. The solutions

with known amounts of glucuronic acid and galacturonic acid that underwent the same acidic treatment were used as standards. With the aim of comparing the neutral sugar content determination according to $H_2SO_4$-based hydrolysis and TFA-based hydrolysis, known amounts of the neutral sugars arabinose, rhamnose, galactose, glucose, xylose, and mannose were also included in the standard solutions of this analysis.

*Chemical functionalities*

To determine the content of chemical functionalities, *i.e.*, aliphatic and phenolic hydroxyl groups, as well as carboxyl groups, a method based on $^{31}$P-Nuclear Magnetic Resonance ($^{31}$P-NMR) was used as previously described by Argyropoulos (1994). Briefly, 30 mg of lignin sample was dissolved in 200 µL of a dimethylformamide (DMF):pyridine (Pyr) (1:1 v/v ratio) mixture for 30 minutes at 50 °C. After complete dissolution, 100 µL of internal standard solution (Endo-*N*-Hydroxy-5-norbornene-2,3-dicarboximide, eHNDI in Pyr at a concentration of 60 mg/mL in presence of chromium(III) acetylacetonate at a concentration of 5 mg/mL) was added, and the resulting solution was stirred for 30 minutes at room temperature. A volume ranging between 175 and 250 µL of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (CTMDP) was quickly added, followed by drop-wise addition of 450 µL of deuterated chloroform ($CDCl_3$). The reaction mixture was stirred for 1 hour at room temperature. $^{31}$P-NMR spectra were recorded using a Bruker Spectrospin 400 Ultra Shield™, a number of scans of 256, and a time delay of 5 s. Spectra were processed using a MestReNova 9.0 software, and chemical shifts were referred to the peak at 152.0 ppm, which is representative of the P present on the product derived from the reaction of CTMDP with the internal standard eHNDI. For EH, LSD, and LSB, which are not soluble in the DMF:Pyr mixture, a prior step of ion exchange was performed. In brief, 250 mg of polymer was dissolved in 3 mL of demineralized water, and 2.5 g of Dowex 50W8 100 mesh ion exchange resin ($H^+$ form) was added. The mixture was vigorously stirred for 10 minutes, and subsequently the resin was removed by filtration under vacuum. The resulting clear solution was titrated to pH 7 by slow addition of tetrabutylammonium-hydroxide (TBA-OH) aqueous solution (40% w/w) and freeze-dried. TBA-coupled lignin samples (hereafter referred to as EH-TBA, LSD-TBA and LSB-TBA) were weighed after freeze-drying to determine the TBA-polymer ratio and further used for $^{31}$P-NMR analysis as described above. Measurements were performed in duplicate.

*Molar mass distribution*

Aqueous and organic Size Exclusion Chromatography (SEC) were used to determine the molar mass distribution and polydispersity index (PDI) of lignosulfonates and kraft lignin samples, respectively (Oinonen *et al.* 2015; Duval *et al.* 2016). In brief, EH, LSD, and LSB were dissolved in 10 mM NaOH aqueous solution at a concentration of 2 mg/mL. The resulting solutions were filtered through 0.2 µm filters and injected into an SEC system (UltiMate 3000, Dionex, Sunnyvale, CA, USA) equipped with a guard column (50 × 8 mm, 10 µm particle size) and a series of three Polymer Standard Service, PSS suprema columns (300 × 8 mm, 10 µm particle size, with a pore size of 30, 1000, and 1000 Å). The system was coupled with a DAD-3000 UV/VIS detector (Dionex, Sunnyvale, CA, USA) and a Waters-410 refractive index (RI) detector (Waters, Milford, MA, USA). The eluent used was 10 mM NaOH aqueous solution at a flow rate of 1 mL/min. Solutions of pullulan ($M_W$ range = 342 to 805000 Da, PSS, Germany) were used as standards. Chromatograms were processed using a Chromeleon 7.1 software. CFBL, UMPL, and LBoost were dissolved in dimethylsulfoxide (DMSO) containing 0.5% LiBr, at a

concentration of 3 mg/mL, filtered through 0.45 µm PTFE filters, and injected into a SECcurity 1260 system (Polymer Standards Services, Mainz, Germany) equipped with a PSS GRAM guard column and two PSS GRAM columns placed in series (100 and 10000 Å, 300 × 8 mm, 10 µm particle size, Polymer Standards Services, Mainz, Germany) and coupled to UV and RI detectors. The elutions were performed at 60 °C using DMSO containing 0.5% LiBr as the eluent and using a flow rate of 0.5 mL/min. Calibration was performed using solutions of pullulan ($M_W$ range = 342 to 805000 Da, PSS, Germany) in the eluent as standards.

*Thermogravimetric analysis (TGA)*

To investigate the thermogravimetric behavior of the different lignosulfonates and kraft lignins, 5 to 10 mg of each sample was analyzed using a TGA Mettler Toledo. A heating ramp from 25 to 750 ˚C at a rate of 10 °C/min was used under nitrogen atmosphere (50 mL/min). Blank curve subtraction was used for baseline correction, and TG curves were processed using a STAR$^e$ Evaluation Software (Mettler Toledo) to obtain mass loss percentages, onset temperatures (initial decomposition temperatures), $T_{5\%db}$ (temperature at which 5% of dry mass is lost), $T_{max}$ values (temperature at which decomposition occurs at maximal speed), and mass residues. The measurements were performed in duplicate.

## RESULTS AND DISCUSSION

### General Composition of Lignin Samples

Samples of technical lignins, *i.e.*, enzymatically cross-linked lignin from pre-hydrolysis of softwood (EH), lignosulfonates from different sources (LSD and LSB), as well as filtered (CFBL) and unfiltered (UPML and LBoost) lignins from kraft pulping, were analyzed in terms of sugar content using two different methods: a method based on $H_2SO_4$-mediated hydrolysis and a method based on TFA-mediated hydrolysis. The latter was specifically chosen for the quantification of uronic acids (discussed in the next section), which are not preserved in a strong acidic environment such as that used during $H_2SO_4$-mediated hydrolysis. The total sugar content of each sample found after $H_2SO_4$-based hydrolysis and TFA-based hydrolysis is reported in Table 2. It can be observed that EH contained the highest percentage of carbohydrates (17.9% according to the $H_2SO_4$-based hydrolysis method and 21.9% according to the TFA-based hydrolysis method). All the other samples had a low sugar content (≤3.2% according to the $H_2SO_4$-based hydrolysis method and ≤3.7% according to the TFA-based hydrolysis method), with the CFBL having the lowest percentage of all (0.2% according to the $H_2SO_4$-based hydrolysis method and 0.3% according to the TFA-based hydrolysis method). From an analytical point of view, it can be noted that the total sugar contents found for each sample using the two different methods were sufficiently comparable. The experiments performed in this study did not indicate whether the sugars in the lignin samples were present as covalently bound residues or as free (oligo-) saccharides. Nevertheless, considering that the technical lignins underwent filtrations and/or lignin selective precipitation during the production process, it is likely that the sugars were mostly present as covalently bound residues.

As expected, the three types of analyzed kraft lignins were mainly composed of Klason lignin (91.5% for CFBL, 93.5% for UPML, and 91.0% for LBoost) and contained low percentages of ASL (≤5%). On the other hand, sulfonated lignin was the major component of EH (LS = 72.8%), LSD (LS = 80.5%), and LSB (LS = 68.5%). It is notable

that the content of Klason lignin varied remarkably between EH (Klason content = 32.0%) and the two lignosulfonates (Klason content = 3.3 and 1.3% for LSD and LSB, respectively). This phenomenon can likely be explained by the fact that the lignin polymerization occurring during EH production increased the lignin fraction that is not soluble in the acidic water medium, therefore resulting in a higher percentage of Klason lignin. Unlike for technical lignins, where the determinations of Klason and ASL are complementary, when measuring Klason and LS for lignosulfonates samples one needs to keep track of the fact that the Klason lignin fraction is also accounted for during LS determination. Therefore, for EH, LSD, and LSB, Klason lignin cannot be summed with LS to estimate the total lignin content, and LS *per se* can be considered a good estimation of the total lignin content.

**Table 2.** Content of Carbohydrates, Klason Lignin, Acid-soluble Lignin (ASL), Sulfonated Lignin (LS), and Ash (on sample dry weight basis) found for EH, LSD, LSB, CFBL, UPML, and LBoost

| Sample | Carbohydrates (%) | Klason (%) | ASL (%) | LS (%) | Ash (%) |
|---|---|---|---|---|---|
| EH | 17.9[a]<br>21.9[b] | 32.0[c] | n.d. | 72.8 | 19.1 |
| LSD | 3.2[a]<br>3.7[b] | 3.3[c] | n.d. | 80.5 | 28.9 |
| LSB | 1.6[a]<br>2.4[b] | 1.3[c] | n.d. | 68.5 | 21.9 |
| CFBL | 0.2[a]<br>0.3[b] | 91.5 | 5.0 | n.d. | 3.3 |
| UPML | 1.7[a]<br>2.2[b] | 93.5 | 3.8 | n.d. | 2.1 |
| LBoost | 1.7[a]<br>2.2[b] | 91.0 | 4.7 | n.d. | 1.3 |

[a] According to $H_2SO_4$-hydrolysis
[b] According to TFA-hydrolysis
[c] Included also into LS (%)
n.d.= not determined

The much higher sugar content found for EH in comparison to LSD was likely due to the fact that EH is isolated during an earlier stage of the pulping process, when hemicelluloses are still at least partially preserved and anchored to lignin. The general molecular organization that can be assigned to EH polymer is related to that which was earlier hypothesized for lignin-carbohydrate complexes obtained after hot water extraction or from TMP process waters (Oinonen *et al.* 2013, 2015). According to that model, lignin-carbohydrate complexes have been described as a complex network where different hemicellulosic chains are covalently cross-linked *via* lignin bridges, after oxidative, enzyme-catalyzed coupling (Bi *et al.* 2015). Nevertheless, there are at least two important differences between the lignin-carbohydrate complexes described earlier by our group and EH. Firstly, EH is a sulfonated material, and secondly, EH has a lower carbohydrate/lignin ratio. In fact, for lignin-carbohydrate complexes extracted from process water of TMP and further cross-linked *via* laccase, carbohydrate/Klason lignin ratios ranging between 0.9/1.0 to 1.3/1.0 have been reported (Oinonen *et al.* 2013). Furthermore, for complexes extracted from hot water treatments of dried industrial chips of Norway spruce (*P. abies*) and further cross-linked in the same manner, an even higher carbohydrate/Klason lignin ratio has been found, *i.e.*, 2.8/1.0 (Oinonen *et al.* 2013). One of the reasons for the lower

carbohydrate/lignin ratio found for EH in this work (carbohydrate/Klason lignin ratio = 0.7/1.0 and carbohydrate/LS ratio = 0.3/1.0) could be that sulfonation occurring during the production of EH was likely responsible for the extraction and solubilization of a higher amount of lignin compared to other processes, such as TMP and hot water extraction. Overall, the presence of a remarkable amount of carbohydrates is a distinctive property of EH in comparison to other commercially available lignin and lignosulfonate-based products, and it is expected to provide a positive effect on EH final applications as barrier films, for instance in food packaging.

Furthermore, the ash content is remarkably higher in EH and lignosulfonates compared to kraft lignins. This higher ash content in lignosulfonates compared to kraft lignin is in line with previously reported data (Vishtal and Kraslawski 2011), and it can be likely explained by the fact that the combustion products from sulfonic acid groups present in lignosulfonates may at least partially remain as solid material in the ash. Moreover, inorganic counter-ions ($Na^+$ and $Ca^{2+}$), which are heavily present in lignosulfonate samples, reasonably contribute to a large ash content. Interestingly, the ash content found for CFBL is slightly higher than that found for LBoost. This can be explained by the fact that a higher content of inorganic counter-ions is expected in CFBL, due to its higher quantity of ionizable groups, *i.e.* phenolic and carboxyl groups, compared to that of LBoost (5.35 mmol/g in CFBL vs 5.16 mmol/g in LBoost, Table 4). Finally, it has to be noted that for EH and LSD, by summing the carbohydrate content, the LS content, and the ash content, values slightly higher than 100% are obtained. This may be due to the fact that the quantification of the different components is performed by three independent methods. Moreover, it is likely that the quantification of LS in EH samples is susceptible to overestimation during the UV-based measurements due to the expected higher absorptivity of EH compared to that of lignosulfonate samples, which are not treated with laccase. For LSB, there is a certain percentage of material (<10%) that could not be identified as carbohydrates, lignin, or ash. This percentage may comprise protein-derived or lignin-derived degradation products as well as organic acids and lipophilic substances (Oinonen *et al.* 2013).

## Sugar Composition

Acidic hydrolysis of samples in the presence of $H_2SO_4$ was used to quantify the content of expected neutral monosaccharides, *i.e.*, arabinose, rhamnose, galactose, glucose, xylose, and mannose. Since uronic acids, *i.e.*, monosugars bearing a carboxyl acid functionality, are not expected to be preserved and therefore detectable after $H_2SO_4$-based hydrolysis, they were quantified after milder hydrolysis conditions using TFA. A general overview of the neutral sugar composition of the analyzed samples is given in Fig. 1. As briefly discussed above, it is likely that these sugars are present in lignin samples as saccharide-residues covalently bound to the lignin backbone. These covalent linkages may derive from lignin-carbohydrate complexes (LCC) present in wood that have survived the pulping process or may represent new covalent bridges formed during pulping (Lawoko *et al.* 2005). EH and LSD show similar sugar compositions, with a high content of galactose (17.4% and 16.9% for EH and LSD, respectively), glucose (15.4% and 17.8% for EH and LSD, respectively), and mannose (45.9% and 49.7% for EH and LSD, respectively), with galactose/glucose/mannose ratios of 1/0.9/2.6 and 1/1/2.9 for EH and LSD, respectively. This profile mirrors a clear high content of galactoglucomannan, naturally present in large amounts into softwood plants. EH and LSD contain a certain amount of arabinose (4.9% and 1.8% for EH and LSD, respectively) and xylose (14.8% and 12.0% for EH and LSD,

respectively), probably derived from arabinoxylan, and they contain equally low amounts (≤1.8%) of rhamnose. LSB, which is produced from hardwood, shows a remarkably different profile, where xylan (61.5%) is the major sugar component, due to the expected high content of arabinoxylan and glucuronoxylan residues. LSB contains also a certain amount of galactoglucomannan. Kraft lignin samples, *i.e.*, CFBL, UPML, and LBoost, show similar sugar compositions, where galactose appears to be the major component (39.9%, 46.3%, and 46.0% for CFBL, UPML, and LBoost, respectively). This high galactose content in combination with a relatively low content of glucose and mannose indicate a galactose/glucose/mannose ratio remarkably different from that known for natural galactoglucomannan. These findings show that glucose and mannose are lost to a large extent during alkaline pulping, whereas galactose is better preserved. A possible explanation for this could be that the galactose present in the side chains of galactoglucomannan may be the sugar that is mainly involved in the covalent bonding between hemicellulose and lignin due to their higher mobility and consequently higher reactivity. Likely, the galactose-lignin bond is less susceptible to hydrolysis under alkaline conditions than a regular glycosidic bond (Berglund *et al.* 2018), and this may result in the observed higher contents of galactose in kraft lignin samples. CFBL, UPML, and LBoost also contain large percentages of arabinose and xylose, derived from arabinoxylan chains.

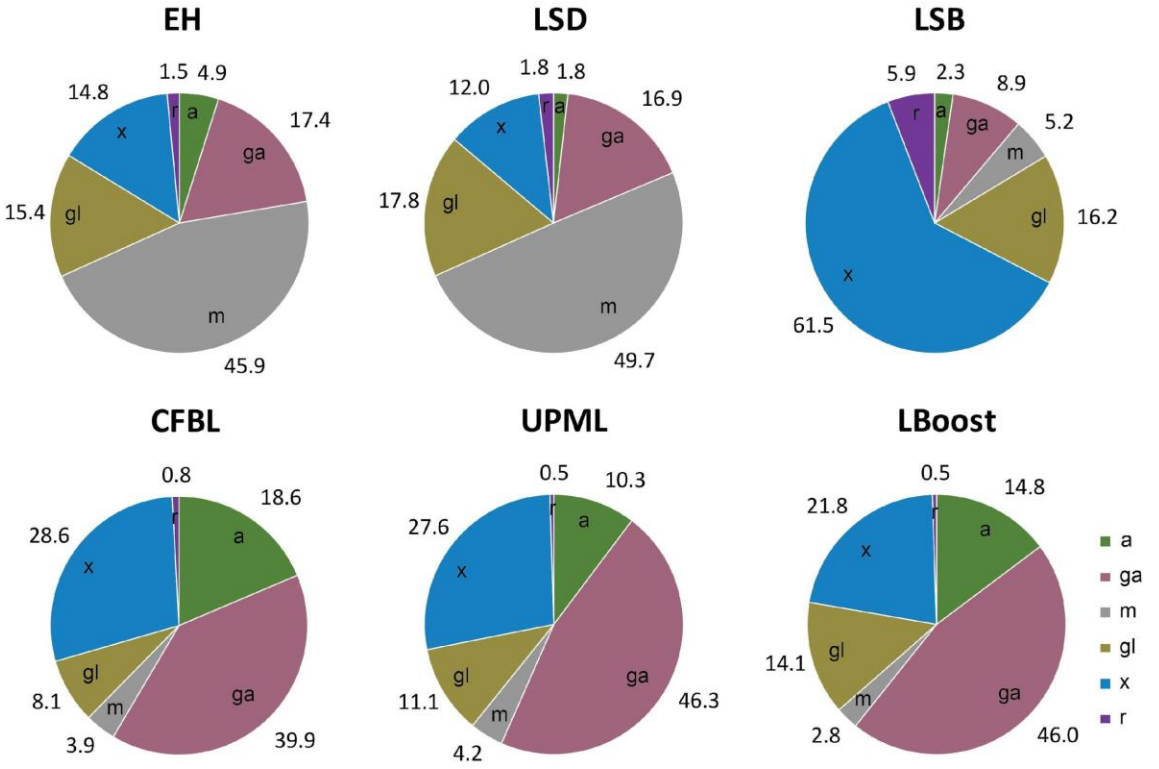



**Fig. 1.** Content of neutral monosugars, arabinose (a), galactose (ga), mannose (m), glucose (gl), xylose (x), and rhamnose (r), obtained after $H_2SO_4$-based hydrolysis for EH, LSD, LSB, CFBL, UPML, and LBoost

All materials contained low amounts of uronic acids (Table 3), *i.e.*, glucuronic and galacturonic acid, and EH showed the highest content of these components (0.6% of the total sample weight) compared with all other analyzed samples. The difference between EH and LSD can be likely explained by the fact that EH is isolated in a much earlier stage

of the pulping process compared to LSD, during which the less harsh conditions may contribute to a better preservation of uronic acids. It is to be noted that LSB contains 0.4% of uronic acids, where the glucuronic acid contribution can be attributed to the expected glucuronoxylan in hardwood. The percentages of neutral sugars found after TFA-mediated hydrolysis are reported in Fig. 2 and were fairly comparable to those found after $H_2SO_4$-mediated hydrolysis.

**Table 3.** Content of Uronic Acids, *i.e.*, Glucuronic and Galacturonic Acid (% of sample dry weight) found after TFA-hydrolysis

| Sample | Uronic Acids Content (%, TFA-hydrolysis) |
|---|---|
| EH | 0.6 |
| LSD | 0 |
| LSB | 0.4 |
| CFBL | traces |
| UPML | traces |
| LBoost | traces |

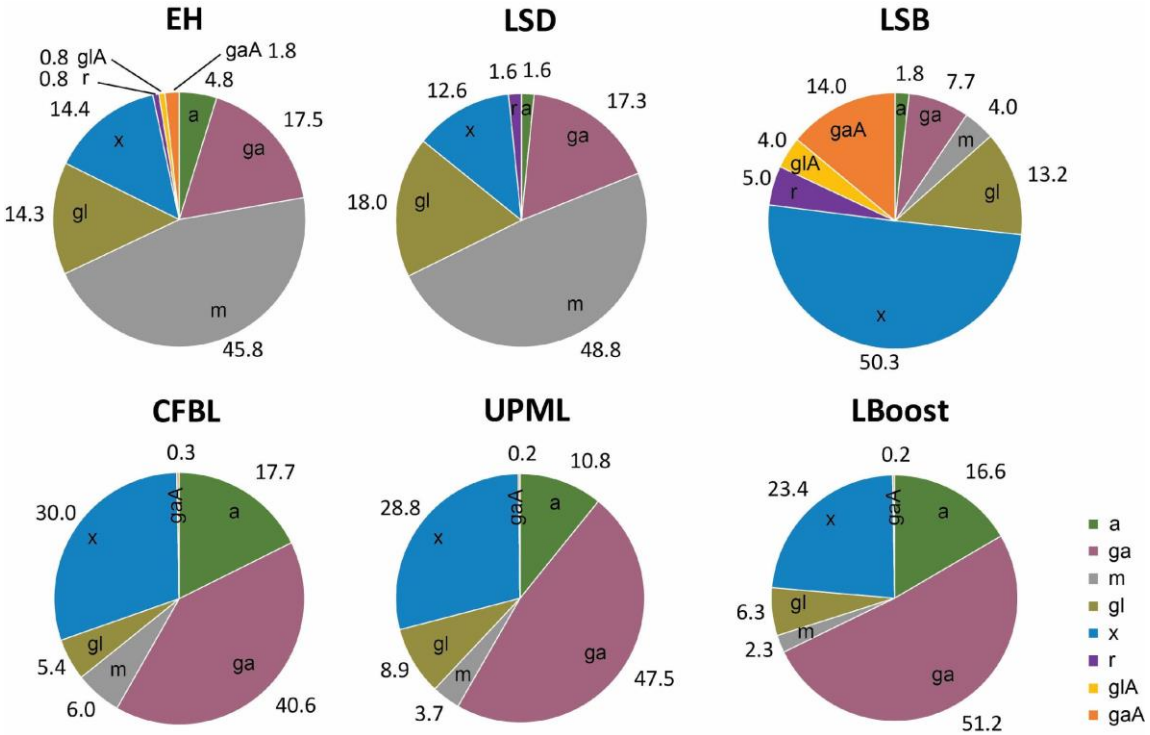


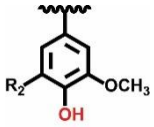
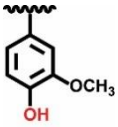
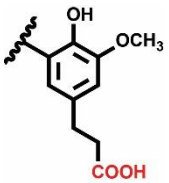

**Fig. 2.** Content of neutral monosugars, arabinose (a), galactose (ga), mannose (m), glucose (gl), xylose (x), and rhamnose (r), and uronic acids, glucuronic acid (glA) and galacturonic acid (gaA), obtained after TFA-based hydrolysis for EH, LSD, LSB, CFBL, UPML, and LBoost. Rhamnose (purple) was not detectable in CFBL, UPML, and LBoost; glucuronic acid (yellow) was not detectable in LSD, CFBL, UPML, and LBoost; galacturonic acid (orange) was not detectable in LSD.

## Functional Groups within the Technical Lignins

The technical lignins were analyzed for the identification and quantification of functional groups using $^{31}$P-NMR. The analysis showed that samples varied remarkably in the content of aliphatic and phenolic hydroxyl groups as well as of carboxyl groups (Table 4). EH, LSD, and LSB underwent a preliminary ion exchange step before dissolution in

the DMF/Pyr mixture. As it can be observed from Table 4, the standard deviations for these samples were relatively low. Moreover, the batch-to-batch variation of TBA content in EH-TBA, LSD-TBA, and LSB-TBA was minimal (data not shown), and the content of TBA was accurately measured gravimetrically on the sample scale (250 mg) that was used. These observations indicated that the TBA ion exchange was an appropriate treatment to make ionic lignin derivatives soluble in the organic mixture (DMF/Pyr) that is used for a classic $^{31}$P-NMR-based analysis.

**Table 4.** Content of Aliphatic OH, Phenolic OH (C5-substituted and non-condensed phenols), and Carboxyl Functionalities (mmol/g of sample dry weight) for EH, LSD, LSB, CFBL, UPML, and LBoost according to $^{31}$P-NMR-based Method

| Sample | Aliphatic OH (mmol/g)  | C5-substituted ph-OH (mmol/g)  | Non-condensed guaiacyl OH (mmol/g)  | p-hydroxyphenyl OH (mmol/g)  | Carboxyl OH (mmol/g)  |
|---|---|---|---|---|---|
| EH | 5.44 ± 0.00 | 0.11 ± 0.00 | 0.00 ± 0.00 | 0.03 ± 0.00 | 0.40 ± 0.06 |
| LSD | 3.27 ± 0.06 | 0.17 ± 0.05 | 0.63 ± 0.02 | 0.03 ± 0.01 | 0.74 ± 0.14 |
| LSB | 4.06 ± 0.12 | 1.16 ± 0.08* | 0.43 ± 0.01 | 0.03 ± 0.01 | 1.10 ± 0.12 |
| CFBL | 1.48 ± 0.02 | 1.85 ± 0.20 | 2.71 ± 0.32 | 0.17 ± 0.03 | 0.62 ± 0.18 |
| UPML | 2.01 ± 0.26 | 2.35 ± 0.28 | 2.20 ± 0.28 | 0.24 ± 0.03 | 0.49 ± 0.02 |
| LBoost | 1.98 ± 0.32 | 2.28 ± 0.22 | 2.23 ± 0.15 | 0.17 ± 0.04 | 0.48 ± 0.15 |

* of which 0.97 ± 0.05 mmol/g of syringyl OH
$R_1$ = aliphatic chain from sugar or lignin backbone
$R_2$ = $OCH_3$ (syringyl OH), or β-5, or 4-O-5 or 5-5 (condensed guaiacyl OH)

In line with the expectations, EH was characterized by the highest content of aliphatic OH (5.44 mmol/g) compared with all the other tested materials. This is imputable to the high carbohydrate content that was found for EH. On the other side, CFBL showed the lowest content of aliphatic OH (1.48 mmol/g), in correspondence with its lowest carbohydrate content. The contents of syringyl OH and condensed guaiacyl OH are reported in Table 4 as C5-substituted phenolic content. This is because in $^{31}$P-NMR spectra of softwood-derived materials, it is not trivial to differentiate the peak representative of syringyl OH from that representative of condensed phenolic structures. In fact, only hardwood-derived LSB showed a clear syringyl OH peak, which indicated a syringyl OH content of 0.97 mmol/g. Remarkably, the content of non-condensed guaiacyl OH of EH was nil. This is in line with previously reported data on hemicellulose-lignin hydrids extracted from hot water treatment of industrial sawdust of Norway spruce and further cross-linked *via* laccase-mediated oxidative reaction (Oinonen *et al.* 2015). As described in that study, the nil value of non-condensed guaiacyl OH found for EH could be ascribed to the fact that these phenolic groups, present before laccase treatment, were involved in the cross-linking reaction, and they consequently decreased or disappeared after laccase treatment. This is confirmed by the observation that the EH precursor (polymer before

laccase treatment) showed a certain content of non-condensed guaiacyl phenols (0.73 mmol/g, data not shown in Table 4).

Importantly, CFBL showed the highest content of non-condensed guaiacyl phenols (2.71 mmol/g) and a remarkably high total content of phenols (4.73 mmol/g). Intuitively, the low cut-off membrane used for isolating CFBL played a role in selecting a polymer fraction with a well-defined low molecular weight, which resulted from a more extensive cleavage of α-aryl and β-aryl ether bonds. In support of this, a study can be mentioned in which kraft lignin was ultrafiltered using different molecular weight cut-off membranes. In that study, it was observed that by reducing the molecular weight cut-off of the membranes, the lignin phenolic content was increased (Keyoumu *et al.* 2004). The high content of the phenols is one of the aspects that makes CFBL a material with high potential, because these reactive groups may be efficiently exploited for post-production cross-linking and for different types of chemical modification. All samples show some degree of carboxylation, and LSB is the material with the highest content of carboxyl groups among those analyzed in this study. This can be explained, at least partially, by the content of uronic acids that was found for LSB.

## Molar Mass Distribution

Due to different solubility properties of the samples, two different SEC methods were used. EH, LSD, and LSB were tested using alkaline aqueous conditions, whereas CFBL, UPML, and LBoost were tested using an organic solution as eluent, *i.e.*, DMSO/LiBr (0.5%). Chromatograms of all tested samples (RI and UV signal) are reported in Fig. 3, and the weight average molecular weight ($M_w$), number average molecular weight ($M_n$), and polydispersity index (PDI) calculated according to the RI signal are listed in Table 5. EH showed much higher $M_w$ and $M_n$ values (31.4 kDa and 6.4 kDa, respectively) compared to LSD and LSB.

From the chromatograms reported in Fig. 3, it can be observed that there was a noticeable shift of the main peak to the left side (higher $M_w$) for EH compared to LSD and LSB. The RI chromatogram of EH shares with that of LSD and LSB the retention time of the three secondary peaks, which are eluted at 23.2, 23.9, and 25.2 minutes and which are representative of smaller molecular weight populations. However, these peaks are much less pronounced in EH compared to LSD and LSB, indicating the presence in EH of a large excess of high molecular weight chains over shorter ones.

The relatively high molecular weight of EH is a consequence of the laccase-mediated oxidative treatment, which is responsible for cross-linking lignin moieties resulting in chain growth, as seen for previously described carbohydrate-lignin complexes (Oinonen *et al.* 2013, 2015).

Having a higher molecular weight is often an advantageous property for polymers that target high value applications in material science, for instance as barrier films for food packaging, since a higher molecular weight can have a positive effect on the mechanical and thermal properties of the final product. In fact, in our previous studies it was demonstrated that an increase in molecular weight of carbohydrate-lignin complexes obtained after laccase treatment provided higher thermal stability and resulted in the formation of polymeric films with a much higher E-modulus compared to un-crosslinked materials (Oinonen *et al.* 2013, 2016).

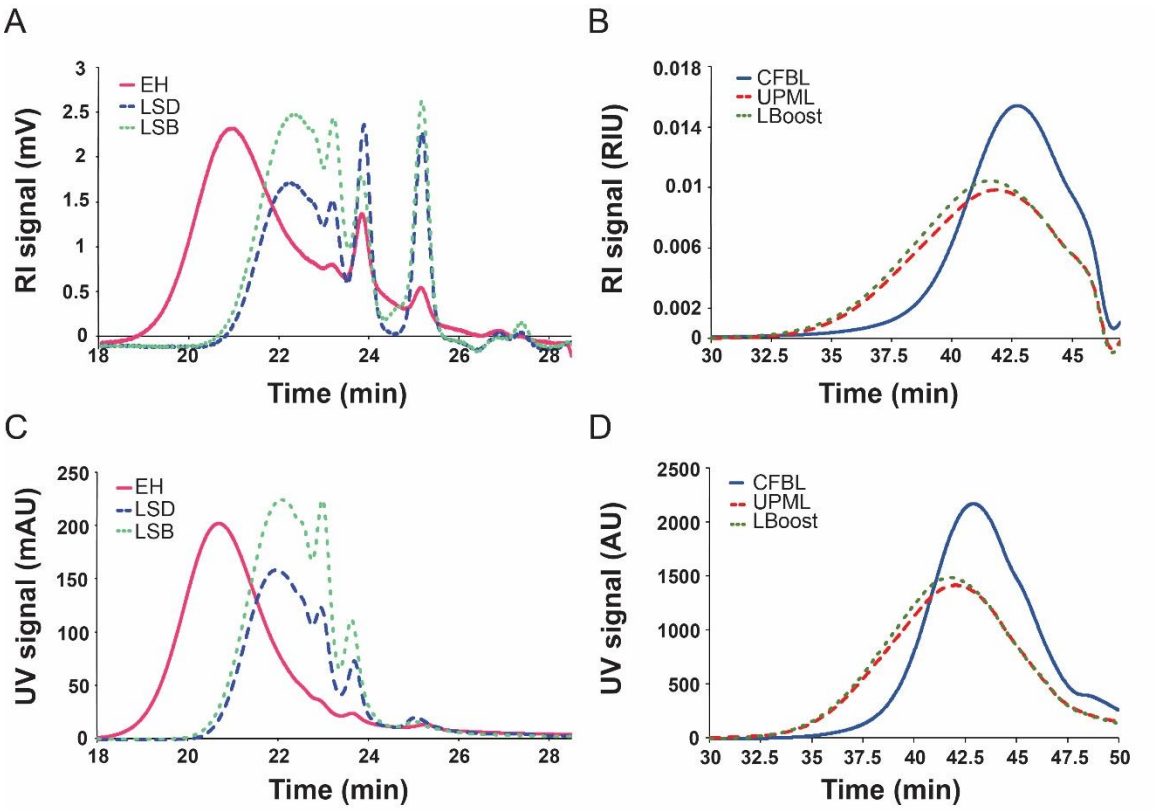



**Fig. 3.** SEC chromatograms (RI signal, A and B, and UV signal, C and D) for EH, LSD, LSB, CFBL, UPML, and LBoost

From the comparison of the RI and UV chromatograms of EH (Fig. 3, panel A and C), it is apparent that all peaks present in the RI chromatogram have a corresponding peak in the UV chromatogram at comparable retention times. Since lignosulfonate typically gives RI and UV signal, and free carbohydrates (as opposite to carbohydrates bounded to lignin) usually give RI response with very poor or nil UV response, this suggests that the high content of carbohydrates in EH (21.9%) is likely bounded to lignosulfonate in the form of LCC.

**Table 5.** Weight Average Molecular Weight ($M_w$, Da), Number Average Molecular Weight ($M_n$, Da), and Polydispersity Index (PDI) for EH, LSD, LSB, CFBL, UPML, and LBoost

| Sample | $M_w$ (Da) | $M_n$ (Da) | PDI |
|---|---|---|---|
| EH | 31415[a] | 6403[a] | 4.9[a] |
| LSD | 10482[a] | 1999[a] | 5.2[a] |
| LSB | 11981[a] | 5247[a] | 2.3[a] |
| CFBL | 2044[b] | 666[b] | 3.1[b] |
| UPML | 5718[b] | 922[b] | 6.2[b] |
| LBoost | 6038[b] | 962[b] | 6.3[b] |

[a] Determined using alkaline SEC with 10 mM NaOH aqueous solution as eluent
[b] Determined using organic SEC with DMSO/LiBr (0.5%) as eluent

CFBL showed noticeably lower $M_w$ and $M_n$ values, as well as a lower PDI compared to UPML and LBoost. This effect can be explained by the fact that CFBL is isolated *via* ultra-filtration by using a low molecular weight cut-off to increase the yield of the low molecular weight polymeric fraction for high-value applications (Keyoumu *et al.* 2004; Helander *et al.* 2013; Giummarella *et al.* 2016). In fact, for the conversion of technical lignin into biofuel and biochemicals, the depolymerization of lignin is a crucial step, and in this scenario, having lignin with a lower molecular weight can be advantageous (Azadi *et al.* 2013; Xu *et al.* 2014). Importantly, in a previous study carried out by Giummarella *et al*. (2016), the solubility and viscosity properties of CFBL were investigated and compared to LBoost. In that study it was found that although the two types of lignin were soluble in the same set of solvents, CFBL could be dissolved in much higher concentrations and resulted in solutions with lower viscosities (Giummarella *et al.* 2016). These findings are in line with the lower molecular weight found for CFBL in the present work in comparison to LBoost. The SEC properties found for LBoost in this study were also in accordance with the previously reported data (Duval *et al.* 2016).

## Thermogravimetric Behavior

The thermogravimetric (TG) curves and first derivatives of TG curves (DTG) of EH, LSD, LSB, CFBL, UPML, and LBoost are shown in Fig. 4.

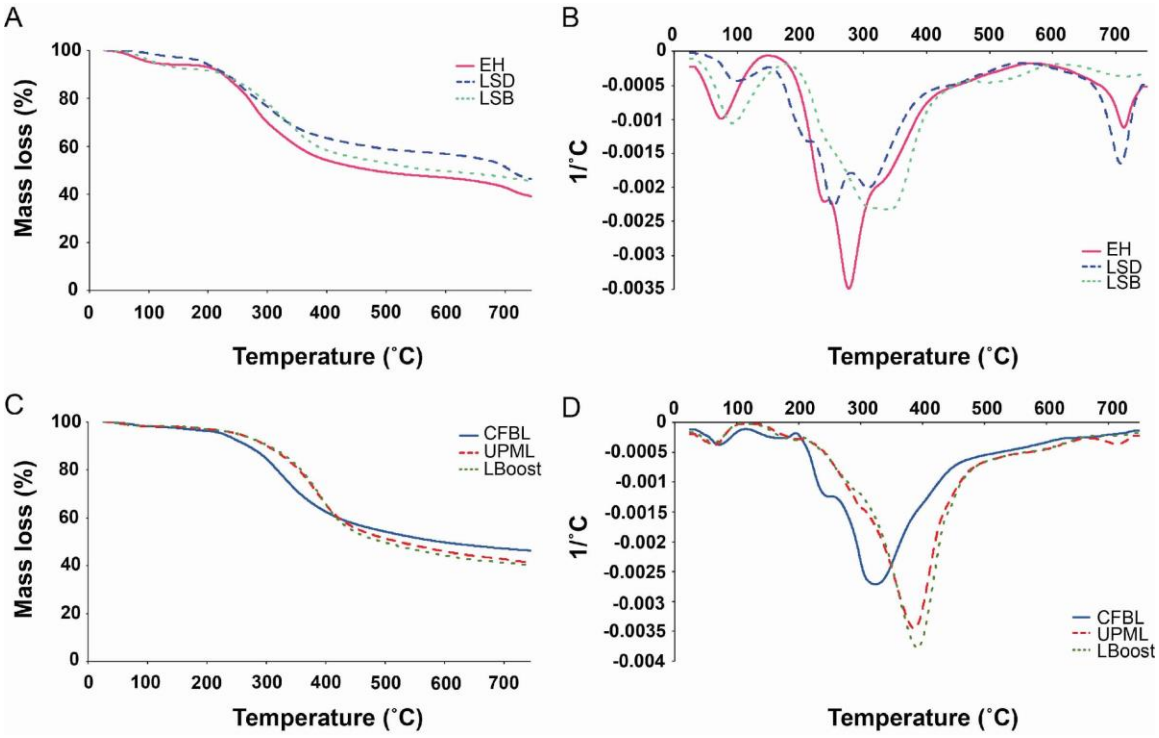



**Fig. 4.** Thermogravimetric (TG) curves (A, C) and first derivatives of TG curves (DTG) (B, D) for EH, LSD, LSB, CFBL, UPML, and LBoost

Thermogravimetric parameters, *i.e.*, mass loss (%), onset temperature (initial decomposition temperature), $T_{5\%db}$ (temperature at which 5% of dry mass is lost), and $T_{max}$ (temperature at which decomposition proceeds at maximal speed) are reported in Table 6. For EH, LSD, and LSB, the area between 25 and 150 °C was attributed to the loss of moisture. In line with expectations, during this step the samples sustained a minimal mass

loss (5.8%, 2.9%, and 7.9% for EH, LSD, and LSB, respectively). The heating step that caused the largest mass loss was that between approximately 150 and 600 °C (denoted as the 1st decomposition in Table 6). The value of $T_{5\%db}$ varied between samples (230.0, 215.4, and 250.1 °C for EH, LSD, and LSB, respectively), and the pattern of the DTG curves in this tract was particularly complex, as previously found for lignosulfonate-based samples (Fig. 4B) (Sahoo *et al.* 2011). For LSD and LSB, two clear minima were identified in this area, centered at 250.4 and 306.2 °C for LSD, and at 340.0 and 496.9 °C for LSB. The main minimum found for EH was centered at 275.0 °C, and it was flanked by two shoulders, one between 150 and 250 °C (identified minimum at 236.0 °C) and another one between 300 and 400 °C; a final tail was also observed at higher temperatures (between 400 and 570 °C). The $T_{max}$ values for EH, LSD, and LSB followed the same trend as the values of $T_{5\%db}$ (*i.e.*, LSB>EH>LSD), indicating that EH has a typical stability between those of LSD and LSB. In fact, the onset temperatures (or $T_{5\%db}$) and $T_{max}$ values of the main decomposition area are generally used to compare material stability (Gordobil *et al.* 2016).

**Table 6.** Thermogravimetric Parameters of EH, LSD, LSB, CFBL, UPML, and LBoost

| | | EH | LSD | LSB |
|---|---|---|---|---|
| Moisture Area | Mass loss (%) | 5.8 ± 0.2 | 2.9 ± 0.1 | 7.9 ± 0.0 |
| 1st Decomposition | Temp range (˚C) | 150-570 | 150-550 | 180-610 |
| | $T_{5\%db}$ (˚C) | 230.0 ± 0.2 | 215.4 ± 0.1 | 250.1 ± 0.4 |
| | $T_{max}$ (˚C) | 275.0 ± 0.0 | 250.4 ± 0.1 | 340.0 ± 0.4 |
| | Mass loss (%) | 46.9 ± 0.5 | 39.4 ± 0.1 | 42.7 ± 0.1 |
| 2nd Decomposition | Temp range (˚C) | 570-750 | 550-750 | 610-750 |
| | Onset (˚C) | 672.3 ± 3.4 | 662.7 ± 1.2 | 628.4 ± 1.9 |
| | $T_{max}$ (˚C) | 708.5 ± 0.3 | 702.6 ± 0.6 | 711.1 ± 1.9 |
| | Mass loss (%) | 8.6 ± 0.1 | 11.5 ± 0.0 | 4.0 ± 0.0 |
| Residue | Mass (%) | 38.8 ± 0.3 | 46.3 ± 0.1 | 45.5 ± 0.1 |
| | | CFBL | UPML | LBoost |
| Moisture Area | Mass loss (%) | 2.0 ± 0.0 | 1.8 ± 0.0 | 1.7 ± 0.1 |
| 1st Decomposition | Temp range (˚C) | 120-750 | 120-670 | 120-750 |
| | $T_{5\%db}$ (˚C) | 244.8 ± 0.4 | 272.8 ± 2.9 | 271.4 ± 1.8 |
| | $T_{max}$ (˚C) | 320.8 ± 3.2 | 385.7 ± 0.4 | 390.1 ± 1.3 |
| | Mass loss (%) | 51.8 ± 0.1 | 54.8 ± 0.2 | 58.5 ± 0.6 |
| 2nd Decomposition | Temp range (˚C) | - | 670-750 | - |
| | Onset (˚C) | - | 676.8 ± 0.5 | - |
| | $T_{max}$ (˚C) | - | 708.7 ± 1.3 | - |
| | Mass loss (%) | - | 2.5 ± 0.2 | - |
| Residue | Mass (%) | 46.3 ± 0.1 | 41.0 ± 0.4 | 39.9 ± 0.6 |

The complexity of the DTG curve pattern was due to the fact that several phenomena occur in that temperature range. For EH, which contains a relatively large amount of carbohydrates, it can be assumed that pyrolysis of xylan and galactoglucomannan occurs in the first part of the decomposition process (200 to 350 °C). This was confirmed by comparing the DTG curve of EH with those of xylan and galactoglucomannan (Fig. 5). Reasonably, it has been previously reported that during hemicellulose pyrolysis, cleavage of glycosidic bonds and fragmentation of xylan side chains are believed to occur first, followed by the decomposition of the generated monomers (Moriana *et al.* 2014). Decomposition of the lignosulfonate backbone likely

starts already during the sugar decomposition, but it proceeds until much higher temperature, due to the high thermal stability of lignosulfonate inter-unit linkages and aromatic rings (Tejado *et al.* 2007). The EH, LSD, and LSB DTG curves showed a last decomposition step (denoted as 2nd decomposition in Table 6) with minima centered between 703 and 711 °C, as previously observed in literature for lignosulfonate (Sahoo *et al.* 2011). During this last step, the further decomposition of previously generated combustion products likely occurs, with a typical release of $CO_2$ and CO due to the cleavage of C-O-C and C=O bonds (Sahoo *et al.* 2011). The residual mass after the heating ramp to 750 °C for EH, LSD, and LSB ranged between 38.8 and 46.3%, and this is in line with previously reported char values for lignosulfonate samples (Sahoo *et al.* 2011). The char composition of lignosulfonate samples likely consists of highly condensed structures, which are formed at high temperatures, and ash rich in minerals derived from lignosulfonate counter-ions.

The DTG curves of CFBL, UPML, and LBoost showed a first mass loss area between 25 and 120 °C, during which moisture was lost (mass loss ≤ 2.0%). All lignin samples presented a broad main mass loss region (mass loss ranging between 51.8% and 58.5%) above 120 °C, which in case of CFBL and LBoost leveled off above 600 °C. Only for UPML was a clear new minimum also observed at 708.7 °C, and a further mass loss of 2.5% occurred between 670 and 750 °C. During lignin thermal decomposition, it is believed that cleavage of the inter-unit linkages and fragmentation of the side-chains occurred below 500 °C, whereas above this temperature, pyrolysis of some aromatic rings can occur (Tejado *et al.* 2007; Gordobil *et al.* 2016). The values of $T_{5\%db}$ and the $T_{max}$ of the main decomposition area (denoted as 1st decomposition in Table 6) were noticeably lower for CFBL compared to those of UPML and LBoost, pointing out a lower thermal stability of CFBL, which can be considered a favorable feature for biofuel application. This important difference between CFBL and the other two technical lignins analyzed in this study may be due to the much lower molecular weight of CFBL compared to UPML and LBoost. In fact, the molecular weight together with structural aspects (*e.g.*, degree of condensation, linkages, and functional groups) is one of the most important factors affecting thermal stability (Gordobil *et al.* 2016). After the whole heating ramp, a residual mass ranging between 39.9 and 46.3% was left in all kraft lignin samples due to the presence of un-volatile, highly condensed structures (Tejado *et al.* 2007).

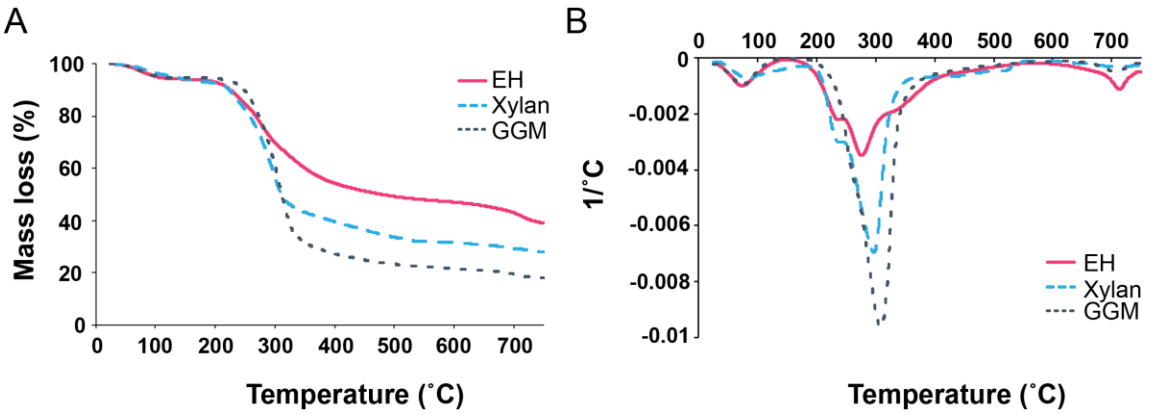



**Fig. 5.** Thermogravimetric (TG) curves (A) and first derivatives of TG curves (DTG) (B) for EH, xylan, and galactoglucomannan (GGM)

## CONCLUSIONS

1. Ecohelix (EH) is a complex and hybrid polymeric network composed of covalently attached carbohydrates and sulfonated lignin. The sugar composition of EH mirrors that of hemicelluloses typically present in softwood.
2. The high content of carbohydrates found for EH is in line with its amount of aliphatic hydroxyl groups, which was much higher than that found for other commercially available lignosulfonates.
3. EH has a much higher molecular weight compared to that of the commercially available lignosulfonates analyzed in this study.
4. The carbohydrate content in combination with a relatively high molecular weight and a good thermal stability renders EH a suitable candidate for high-value applications, such as for the development of polymeric films.
5. Ultrafiltered lignin (CFBL) presented a lower amount of carbohydrates, lower molecular weight, and lower thermal stability than those of other technical kraft lignins analyzed in this study. As such, CFBL is an upgraded technical lignin with high potential for the production of biofuel and biochemicals.
6. The remarkably high content of phenolic functionalities of CFBL opens a large range of possibilities for chemical functionalization and further development.

## ACKNOWLEDGMENTS

The research leading to these results was financially supported by Nordforsk, within the Nordic Green Growth Research and Innovation Program (project name: “High-value products from lignin”). Ecohelix AB, Domsjö Fabriker AB, CleanFlow AB, and Professor Mikael Lindström are thanked for providing the material described in this study. The authors are grateful to Martin Lawoko and Nicola Giummarella for helpful discussions during this study.

## REFERENCES CITED

Albersheim, P., Nevins, D. J., English, P. D., and Karr, A. (1967). "A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography," *Carbohydrate Research* 5(3), 340-345. DOI: 10.1016/S0008-6215(00)80510-8.

Alekhina, M., Ershova, O., Ebert, A., Heikkinen, S., and Sixta, H. (2015). "Softwood kraft lignin for value-added applications: Fractionation and structural characterization," *Industrial Crops and Products* 66(Supplement C), 220-228. DOI: 10.1016/j.indcrop.2014.12.021.

Areskogh, D., and Henriksson, G. (2011). "Immobilisation of laccase for polymerisation of commercial lignosulphonates," *Process Biochemistry* 46(5), 1071-1075. DOI: 10.1016/j.procbio.2011.01.024.

Areskogh, D., Li, J., Gellerstedt, G., and Henriksson, G. (2010a). "Investigation of the molecular weight increase of commercial lignosulfonates by laccase catalysis," *Biomacromolecules* 11(4), 904-910. DOI: 10.1021/bm901258v.

Areskogh, D., Li, J., Gellerstedt, G., and Henriksson, G. (2010b). "Structural modification of commercial lignosulphonates through laccase catalysis and ozonolysis," *Industrial Crops and Products* 32(3), 458-466. DOI: 10.1016/j.indcrop.2010.06.016.

Areskogh, D., Li, J., Nousiainen, P., Gellerstedt, G., Sipilä, J., and Henriksson, G. (2010c). "Oxidative polymerisation of models for phenolic lignin end-groups by laccase," *Holzforschung* 64(1), 21-34. DOI: 10.1515/hf.2010.001

Areskogh, D., Nousiainen, P., Li, J., Gellerstedt, G., Sipilä, J., and Henriksson, G. (2010d). "Sulfonation of phenolic end groups in lignin directs laccase-initiated reactions towards cross-linking," *Industrial Biotechnology* 6(1), 50-59. DOI: 10.1089/ind.2010.6.050.

Argyropoulos, D. S. (1994). "Quantitative phosphorus-31 NMR analysis of lignins, a new tool for the lignin chemist," *Journal of Wood Chemistry and Technology* 14(1), 45-63.

ASTM D1102-84 (2013). "Standard test method for ash in wood," ASTM International, West Conshohocken, PA.

Azadi, P., Inderwildi, O. R., Farnood, R., and King, D. A. (2013). "Liquid fuels, hydrogen and chemicals from lignin: A critical review," *Renewable and Sustainable Energy Reviews* 21(Supplement C), 506-523. DOI: 10.1016/j.rser.2012.12.022.

Berglund, J., Azhar, S., Lawoko, M., Lindström, M., Vilaplana, F., Wohlert, J., and Henriksson, G. (2018). "The structure of galactoglucomannan impacts the degradation under alkaline conditions," *Cellulose* DOI: 10.1007/s10570-018-1737-z.

Bi, R., Oinonen, P., Wang, Y., and Henriksson, G. (2015). "A method for studying effects on lignin-polysaccharide networks during biological degradation and technical processes of wood," *BioResources* 11(1), 1307-1318. DOI: 10.15376/biores.11.1.1307-1318.

Boeriu, C. G., Bravo, D., Gosselink, R. J. A., and van Dam, J. E. G. (2004). "Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy," *Industrial Crops and Products* 20(2), 205-218. DOI: 10.1016/j.indcrop.2004.04.022.

Chakar, F. S., and Ragauskas, A. J. (2004). "Review of current and future softwood kraft lignin process chemistry," *Industrial Crops and Products* 20(2), 131-141. DOI: 10.1016/j.indcrop.2004.04.016.

De Ruiter, G. A., Schols, H. A., Voragen, A. G. J., and Rombouts, F. M. (1992). "Carbohydrate analysis of water-soluble uronic acid-containing polysaccharides with high-performance anion-exchange chromatography using methanolysis combined with TFA hydrolysis is superior to four other methods," *Analytical Biochemistry* 207(1), 176-185. DOI: 10.1016/0003-2697(92)90520-H.

Dence, C. W. (1992). "The determination of lignin," in: *Methods in Lignin Chemistry*, S. Y. Lin and C. W. Dence (eds.), Springer, Berlin, Heidelberg, pp. 33-61.

Duval, A., and Lawoko, M. (2014). "A review on lignin-based polymeric, micro- and nano-structured materials," *Reactive and Functional Polymers* 85(Supplement C), 78-96. DOI: 10.1016/j.reactfunctpolym.2014.09.017.

Duval, A., Vilaplana, F., Crestini, C., and Lawoko, M. (2016). "Solvent screening for the fractionation of industrial kraft lignin," *Holzforschung* 70(1), 11-20. DOI: 10.1515/hf-2014-0346.

Felby, C., Hassingboe, J., and Lund, M. (2002). "Pilot-scale production of fiberboards made by laccase oxidized wood fibers: Board properties and evidence for cross-

linking of lignin," *Enzyme and Microbial Technology* 31(6), 736-741. DOI: 10.1016/S0141-0229(02)00111-4.

FitzPatrick, M., Champagne, P., Cunningham, M. F., and Whitney, R. A. (2010). "A biorefinery processing perspective: Treatment of lignocellulosic materials for the production of value-added products," *Bioresource Technology* 101(23), 8915-22. DOI: 10.1016/j.biortech.2010.06.125.

Gall, D. L., Ralph, J., Donohue, T. J., and Noguera, D. R. (2017). "Biochemical transformation of lignin for deriving valued commodities from lignocellulose," *Current Opinion in Biotechnology* 45(Supplement C), 120-126. DOI: 10.1016/j.copbio.2017.02.015.

Garcia-Ubasart, J., Colom, J. F., Vila, C., Hernández, N. G., Blanca Roncero, M., and Vidal, T. (2012). "A new procedure for the hydrophobization of cellulose fibre using laccase and a hydrophobic phenolic compound," *Bioresource Technology* 112(Supplement C), 341-344. DOI: 10.1016/j.biortech.2012.02.075.

Garcia-Ubasart, J., Esteban, A., Vila, C., Roncero, M. B., Colom, J. F., and Vidal, T. (2011). "Enzymatic treatments of pulp using laccase and hydrophobic compounds," *Bioresource Technology* 102(3), 2799-2803. DOI: 10.1016/j.biortech.2010.10.020.

Ghatak, H. R. (2011). "Biorefineries from the perspective of sustainability: Feedstocks, products, and processes," *Renewable and Sustainable Energy Reviews* 15(8), 4042-4052. DOI: 10.1016/j.rser.2011.07.034.

Gillet, S., Aguedo, M., Petitjean, L., Morais, A. R. C., da Costa Lopes, A. M., Lukasik, R. M., and Anastas, P. T. (2017). "Lignin transformations for high value applications: Towards targeted modifications using green chemistry," *Green Chemistry* 19(18), 4200-4233. DOI: 10.1039/C7GC01479A.

Giummarella, N., Lindgren, C., Lindström, M. E., and Henriksson, G. (2016). "Lignin prepared by ultrafiltration of black liquor: Investigation of solubility, viscosity, and ash content," *BioResources* 11(2), 3494-3510. DOI: 10.15376/biores.11.2.3494-3510.

Gordobil, O., Moriana, R., Zhang, L., Labidi, J., and Sevastyanova, O. (2016). "Assessment of technical lignins for uses in biofuels and biomaterials: Structure-related properties, proximate analysis and chemical modification," *Industrial Crops and Products* 83, 155-165. DOI: 10.1016/j.indcrop.2015.12.048.

Helander, M., Theliander, H., Lawoko, M., Henriksson, G., Zhang, L., and Lindström, M. E. (2013). "Fractionation of technical lignin: Molecular mass and pH effects," *BioResources* 8(2), 2270-2282.

Isikgor, F. H., and Becer, C. R. (2015). "Lignocellulosic biomass: A sustainable platform for the production of bio-based chemicals and polymers," *Polymer Chemistry* 6(25), 4497-4559. DOI: 10.1039/C5PY00263J.

Keyoumu, A., Sjödahl, R., Henriksson, G., Ek, M., Gellerstedt, G., and Lindström, M. E. (2004). "Continuous nano- and ultra-filtration of kraft pulping black liquor with ceramic filters: A method for lowering the load on the recovery boiler while generating valuable side-products," *Industrial Crops and Products* 20(2), 143-150. DOI: 10.1016/j.indcrop.2004.04.017.

Lawoko, M., Henriksson, G., and Gellerstedt, G. (2005). "Structural differences between the lignin−carbohydrate complexes present in wood and in chemical pulps," *Biomacromolecules* 6(6), 3467-3473. DOI: 10.1021/bm058014q.

Li, M., Pu, Y., and Ragauskas, A. J. (2016). "Current understanding of the correlation of lignin structure with biomass recalcitrance," *Frontiers in Chemistry* V4, 45. DOI: 10.3389/fchem.2016.00045

Lin, S. Y. (1992). "Ultraviolet spectrophotometry," in: *Methods in Lignin Chemistry*, S. Y. Lin and C. W. Dence (eds.), Springer, Berlin, Heidelberg, pp. 217-232.

Mansouri, N.-E. E., and Salvadó, J. (2006). "Structural characterization of technical lignins for the production of adhesives: Application to lignosulfonate, kraft, soda-anthraquinone, organosolv and ethanol process lignins," *Industrial Crops and Products* 24(1), 8-16. DOI: 10.1016/j.indcrop.2005.10.002.

McKendry, P. (2002a). "Energy production from biomass (Part 1): Overview of biomass," *Bioresource Technology* 83(1), 37-46. DOI: 10.1016/S0960-8524(01)00118-3.

McKendry, P. (2002b). "Energy production from biomass (Part 2): Conversion technologies," *Bioresource Technology* 83(1), 47-54. DOI: 10.1016/S0960-8524(01)00119-5.

McKendry, P. (2002c). "Energy production from biomass (Part 3): Gasification technologies," *Bioresource Technology* 83(1), 55-63. DOI: 10.1016/S0960-8524(01)00120-1.

Monteil-Rivera, F., Phuong, M., Ye, M., Halasz, A., and Hawari, J. (2013). "Isolation and characterization of herbaceous lignins for applications in biomaterials," *Industrial Crops and Products* 41(Supplement C), 356-364. DOI: 10.1016/j.indcrop.2012.04.049.

Moriana, R., Zhang, Y., Mischnick, P., Li, J., and Ek, M. (2014). "Thermal degradation behavior and kinetic analysis of spruce glucomannan and its methylated derivatives," *Carbohydrate Polymers* 106, 60-70. DOI: 10.1016/j.carbpol.2014.01.086.

Nadif, A., Hunkeler, D., and Käuper, P. (2002). "Sulfur-free lignins from alkaline pulping tested in mortar for use as mortar additives," *Bioresource Technology* 84(1), 49-55. DOI: 10.1016/S0960-8524(02)00020-2.

Nakamura, K., and Go, N. (2005). "Function and molecular evolution of multicopper blue proteins," *Cellular and Molecular Life Sciences* 62(18), 2050-2066. DOI: 10.1007/s00018-004-5076-x.

Oh, Y. H., Eom, I. Y., Joo, J. C., Yu, J. H., Song, B. K., Lee, S. H., Hong, S. H., and Park, S. J. (2015). "Recent advances in development of biomass pretreatment technologies used in biorefinery for the production of bio-based fuels, chemicals and polymers," *Korean Journal of Chemical Engineering* 32(10), 1945-1959. DOI: 10.1007/s11814-015-0191-y.

Oinonen, P., Areskogh, D., and Henriksson, G. (2013). "Enzyme catalyzed cross-linking of spruce galactoglucomannan improves its applicability in barrier films," *Carbohydrate Polymers* 95(2), 690-696. DOI: 10.1016/j.carbpol.2013.03.016.

Oinonen, P., Krawczyk, H., Ek, M., Henriksson, G., and Moriana, R. (2016). "Bioinspired composites from cross-linked galactoglucomannan and microfibrillated cellulose: Thermal, mechanical, and oxygen barrier properties," *Carbohydrate Polymers* 136, 146-153. DOI: 10.1016/j.carbpol.2015.09.038.

Oinonen, P., Zhang, L., Lawoko, M., and Henriksson, G. (2015). "On the formation of lignin polysaccharide networks in Norway spruce," *Phytochemistry* 111(Supplement C), 177-184. DOI: 10.1016/j.phytochem.2014.10.027.

Ragauskas, A. J., Beckham, G. T., Biddy, M. J., Chandra, R., Chen, F., Davis, M. F., Davison, B. H., Dixon, R. A., Gilna, P., Keller, M., Langan, P., Naskar, A. K., Saddler, J. N., Tschaplinski, T. J., Tuskan, G. A., and Wyman, C. E. (2014). "Lignin valorization: Improving lignin processing in the biorefinery," *Science* 344(6185), 1246843. DOI: 10.1126/science.1246843.

Sahoo, S., Seydibeyoğlu, M. Ö., Mohanty, A. K., and Misra, M. (2011). "Characterization of industrial lignins for their utilization in future value added applications," *Biomass and Bioenergy* 35(10), 4230-4237. DOI: 10.1016/j.biombioe.2011.07.009.

SCAN-CM 71:09 (2009). "Pulps – Carbohydrate content," Scandinavian Pulp, Paper and Board Testing Committee, Stockholm, Sweden.

Sheldon, R. A. (2014). "Green and sustainable manufacture of chemicals from biomass: State of the art," *Green Chemistry* 16(3), 950-963. DOI: 10.1039/C3GC41935E.

Tamaki, Y., and Mazza, G. (2010). "Measurement of structural carbohydrates, lignins, and micro-components of straw and shives: Effects of extractives, particle size and crop species," *Industrial Crops and Products* 31(3), 534-541. DOI: 10.1016/j.indcrop.2010.02.004.

Tejado, A., Peña, C., Labidi, J., Echeverria, J. M., and Mondragon, I. (2007). "Physico-chemical characterization of lignins from different sources for use in phenol–formaldehyde resin synthesis," *Bioresource Technology* 98(8), 1655-1663. DOI: 10.1016/j.biortech.2006.05.042.

Ten, E., and Vermerris, W. (2015). "Recent developments in polymers derived from industrial lignin," *Journal of Applied Polymer Science* 132, Article ID 240969. DOI: 10.1002/app.42069

Tomani, P. (2010). "The lignoboost process," *Cellulose Chemistry & Technology* 44(1), 53.

Tomani, P., Axegård, P., Berglin, N., Lovell, A., and Nordgren, D. (2011). "Integration of lignin removal into a kraft pulp mill and use of lignin as a biofuel," *Cellulose Chemistry and Technology* 45(7), 533.

Upton, B. M., and Kasko, A. M. (2016). "Strategies for the conversion of lignin to high-value polymeric materials: Review and perspective," *Chemical Reviews* 116(4), 2275-2306. DOI: 10.1021/acs.chemrev.5b00345.

Van Heiningen, A. (2006). "Converting a kraft pulp mill into an integrated forest biorefinery," *Pulp and Paper Canada* 107(6), 38-43.

Vishtal, A. G., and Kraslawski, A. (2011). "Challenges in industrial applications of technical lignins," *BioResources* 6(3), 3547-3568.

Widsten, P., and Kandelbauer, A. (2008). "Laccase applications in the forest products industry: A review," *Enzyme and Microbial Technology* 42(4), 293-307. DOI: 10.1016/j.enzmictec.2007.12.003.

Xu, C., Arancon, R. A. D., Labidi, J., and Luque, R. (2014). "Lignin depolymerisation strategies: Towards valuable chemicals and fuels," *Chemical Society Reviews* 43(22), 7485-7500. DOI: 10.1039/C4CS00235K.

Yan, P., Xu, Z., Zhang, C., Liu, X., Xu, W., and Zhang, Z. C. (2015). "Fractionation of lignin from eucalyptus bark using amine-sulfonate functionalized ionic liquids," *Green Chemistry* 17(11), 4913-4920. DOI: 10.1039/C5GC01035G.

Article submitted: June 20, 2018; Peer review completed: August 10, 2018; Revised version received and accepted: August 17, 2018; Published: August 22, 2018.
DOI: 10.15376/biores.13.4.7606-7627