

## Facile synthesis of vanillin from fractionated Kraft lignin

Rui Zhang, Riku Maltari, Ming Guo, Jussi Kontro, Aleksi Eronen, Timo Repo\*

Department of Chemistry, University of Helsinki, A. I. Virtasen Aukio 1, P.O. Box 55, 00014 Finland



### ARTICLE INFO

#### Keywords:

Kraft lignin  
Single solvent fractionation  
Vanillin synthesis  
Catalytic oxidation

### ABSTRACT

More than 90 % of global lignin production comes from Kraft pulp mills but due to shortage of economical viable methods to valorise lignin, it is commonly burned for energy. Therefore, finding new routes to utilize Kraft lignin (KL) as a renewable raw material for the chemical industry is of significant economic and environmental importance. Herein we report a novel, two-step procedure for facile synthesis of vanillin from technical KL by combining solvent fractionation and catalytic oxidation reaction. From the studied green solvents, 1-propanol was the most attractive for one-step, single solvent fractionation as it afforded uniform, low molecular weight lignin fractions ( $M_w = 1300 \text{ g}\cdot\text{mol}^{-1}$ ,  $M_n = 580 \text{ g}\cdot\text{mol}^{-1}$ ) with yield of 46 wt%. Using this homogeneous lignin as a raw material,  $\text{CuSO}_4$  catalyzed oxidation reaction proceeds smoothly, and under optimized conditions a high vanillin yield of 10.9 wt% was achieved. The method reported herein is promising as it facilitates straightforward and high yield vanillin synthesis from commercially available technical KL.

### 1. Introduction

Lignin is a renewable resource of organic  $\text{sp}^2$ -hybridized carbon, and it is, therefore, a highly attractive raw material for the manufacturing of sustainable fuels and aromatic chemicals (Rahimi et al., 2014; Upton and Kasko, 2016; Xu et al., 2014). Kraft lignin (KL), a byproduct from the pulp or paper industry, represents the majority of global lignin production and is commercially available in vast quantities (Chakar and Ragauskas, 2004; Gellerstedt, 2015). Despite of its great abundance and potential, KL is severely underutilized as a raw material for the chemical industry and instead simply burned for energy production. Only a small fraction of KL is employed for other applications, such as adhesives, fillers or dispersants, binders or coatings (Bajwa et al., 2019; Behling et al., 2016; Passoni et al., 2016; Ragauskas et al., 2014). Therefore, finding new routes to utilize Kraft lignin (KL) as a renewable raw material for the chemical industry is of significant economic and environmental importance (Azadi et al., 2013; Sun et al., 2018).

The main challenges to convert lignin to added-value chemicals or materials are related to the inherent heterogeneity of lignin itself and its high degree of condensation during the Kraft pulping process (Chakar and Ragauskas, 2004; Crestini et al., 2017; Vázquez et al., 1997). For example, highly reactive benzylic carbocations are prone to attack electron-rich aromatic rings in lignin to form stable carbon-carbon bonds (Shuai et al., 2016). Also the fragmentation of aryl-alkyl ether

linkages alters the original structures and their composition is depending on variations in pulping process (Cui et al., 2014). Currently solvent fractionation is one of the widely studied approaches to reduce the heterogeneity of lignin and offer uniform lignin fractions. Various organic solvents and co-solvent systems are introduced and studies including the impact of solvent fractionation on the antioxidant activity, heating values, and thermal and adsorption properties are reported (Ho Seo et al., 2019; Jääskeläinen et al., 2017; Passoni et al., 2016; Sadeghifar and Argyropoulos, 2015; Sadeghifar et al., 2016; Saito et al., 2014; Wang et al., 2011; Wang et al., 2018a; Yuan et al., 2009).

Successive fractionation of KL by solvents with different polarities has attracted focus. As an example, a continuum of narrow fractions can be isolated from softwood KL by the incremental addition of a nonpolar solvent (hexane) in a polar (acetone) solution (Cui et al., 2014). Also sequential fractionating with acetone – methanol or THF – methanol cosolvent systems and hexane as an antisolvent is possible. As a result, KL fractions determined by the content of hemicellulose, the molecular weight of lignin itself and the type and content of OH groups can be isolated (Wang et al., 2018a). As noteworthy, sequential extraction of KL with series of solvents, like hexane, diethyl ether, methylene chloride, methanol, and dioxane (Wang et al., 2010), or ethyl acetate, ethanol, methanol, and acetone (Tagami et al., 2019) yield various lignin fractions which distinguish themselves with different chemical composition and molecular weight distribution.

However, the successive extractions processes are costly in large

Abbreviations: KL, Kraft Lignin; Con. Ph–OH, condensed phenolic – OH; Nonc. Ph–OH, noncondensed phenolic – OH

\* Corresponding author.

E-mail address: [timo.repo@helsinki.fi](mailto:timo.repo@helsinki.fi) (T. Repo).

<https://doi.org/10.1016/j.indcrop.2020.112095>

Received 23 August 2019; Received in revised form 20 December 2019; Accepted 4 January 2020

Available online 13 January 2020

0926-6690/© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

scale, where separation and recycling of solvents is essential (Passoni et al., 2016). Thus, the use of a single solvent could be beneficial as it also simplifies the treatment procedures. In addition, the applied solvents should be green and not cause health and safety issues as well as detrimental environmental impact (Gillet et al., 2017). Therefore, in terms of greenness and sustainability, bio-based solvents (de Jong et al., 2012) such as ethanol, 1-propanol and 1-butanol were preferred in our study and we focused on one-step single solvent fractionation of KL.

Vanillin is currently one of the only molecular phenolic compounds manufactured on an industrial scale from lignosulfonates. On the other hand, lignosulfonates accounts only less than 10 % of global lignin extracted, while kraft pulp mills contributes over 90 %. Thus, the availability of KL makes it as a very attractive starting material for producing chemicals, including vanillin. However, based on previous reports, the success in vanillin synthesis seems to be largely depending on the source of lignin. The reported vanillin yields from KL are relatively low, presumably because the high degree of condensation, and thus KL is considered as an inappropriate starting material (Araújo et al., 2010; Fache et al., 2015; Silva et al., 2009; Tarabanko et al., 1995a, 1995b; Villar et al., 1997; Wang et al., 2018b). Surprisingly, studies on synthesis of high value-added vanillin from solvent fractionated KL are rare to our knowledge. Having this in mind, we report herein a novel, two-step procedure for high yield vanillin synthesis from technical KL by combining single solvent fractionation and an effective catalytic oxidation system based on  $\text{CuSO}_4$  as a catalyst and  $\text{H}_2\text{O}_2$  as an oxidant.

## 2. Material and methods

### 2.1. Materials

Kraft lignin Indulin AT (KL, softwood, Meadwestvaco, US) was obtained commercially and used as received. Solvents and chemicals were obtained from commercial retailers and were used as received.

### 2.2. Fractionation of KL

In a typical experiment, desired amount of KL was added into a round bottom flask with certain amount of a specific alcohol (lignin/alcohol = 1 g/60 mL). The mixture was stirred (600 rpm/min) and refluxed for 4 h. After reaction, the mixture was cooled to room temperature, then soluble fraction and solid fraction were separated by vacuum filtration. Solid fraction was dried in oven at 105 °C for 24 h to determine the dry mass of the residual and conversion of KL. Solvents in soluble fraction were evaporated to obtain the different lignin fractions.

### 2.3. Catalytic oxidation of lignin

About 40 mg of KL and all fractions were added in glass vials and dissolved in 10 mL 2 M NaOH. Subsequently,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  was added followed by the addition of  $\text{H}_2\text{O}_2$  (33 wt% in  $\text{H}_2\text{O}$ ). The vials were sealed with a Teflon cap, placed in an oil bath and stirred at the desired temperature for the respective reaction time. Upon completion, the reaction mixture was cooled to room temperature and filtered, then quenched with an aqueous  $\text{H}_2\text{SO}_4$  solution (c = 2 M, 8 mL). Remaining solid lignin materials were filtered out and dried at 105 °C for further analysis. The aqueous phase was extracted with ethyl acetate (5 × 8 mL) and the organic phase was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and after dilution subsequently measured by an Agilent 5973–6890 N gas chromatography mass spectrometer equipped with a HP-5 MS UI column (30 m × 0.250 mm × 0.25 μm). Acetophenone was employed as an internal standard and He was used as a carrier gas at a flow rate of 1.1 mL/min. The initial oven temperature 60 °C was held for 0.5 min and then increased to 90 °C at a rate of 10 °C/min followed by the temperature ramp of 20 °C/min to 300 °C, and in the end the oven was held at 300 °C for 6 min. The detector temperature was 250 °C.

### 2.4. GPC measurements

GPC (Gel permeation chromatography) measurements were performed on 1260 Infinity HPLC (Agilent) with Acquity APC XT 200 2.5 and 45 1.7 μm columns (Waters) at 40 °C. Calibration was performed using polystyrene standards (Scientific Polymer Products and Fluka Analytical). UV-detector (280 nm) was employed for monitoring, while THF was used as an eluent with a flow rate of 0.8 mL/min. Before measurement, all samples were acetylated by acetic anhydride and pyridine at 50 °C for 24 h based on the reference (Hu et al., 2012). Residual reagents were removed by evaporation with ethanol, toluene and finally chloroform. The samples were dissolved in THF with a concentration of 1 mg/mL and filtered prior to injection.

### 2.5. NMR analysis

NMR (Nuclear magnetic resonance) spectra was acquired on a Bruker Avance III 500-MHz spectrometer with a BBFO broad band probe. For each run, 120 mg of lignin samples were dissolved in 0.7 mL of  $\text{DMSO-d}_6$ . Heteronuclear single quantum coherence (HSQC) were carried out with the following parameters: acquired from 12 to 0 ppm in F2 ( $^1\text{H}$ ) with 1024 data points (acquisition time 85.2 ms), 215 to 0 ppm in F1 ( $^{13}\text{C}$ ) with 256 data points (acquisition time 4.7 ms), a 1.4 s delay. The  $\text{DMSO-d}_6$  solvent peak ( $\delta\text{C}/\delta\text{H}$  at 39.5/2.49) was used for chemical shift calibration. The volume integration of contours in HSQC plots were conducted by using Bruker's TopSpin 4.0.3 (Microsoft version) software. The cross-signals were identified and the regions of interest were integrated based on a saved template to minimise the introduction of errors. The integrals were then normalised to get the relative content.

$^{31}\text{P}$  NMR spectra was acquired with a 90° pulse flip angle, 1.6 s acquisition time, 5 s relaxation time and 85,000 Hz spectral width in collecting 200 scans on Bruker Avance III 500-MHz spectrometer. About 40 mg samples were weighted precisely and added into NMR tube with the addition of 0.6 mL of 1.6:1 pyridine: $\text{CDCl}_3$  solvent, 0.2 mL of internal standard (N-hydroxy-5-norbornene-2,3-dicarboxylic acid imide, 9.27 mg/mL) and 50 μL of relaxation agent  $\text{Cr}(\text{AcAc})_3$  (5.6 mg/mL), followed by 0.15 mL of phosphitylation reagent 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) just right before the measurement. Phosphitylation product ( $\delta$  132.2 ppm) was set as reference peak. The integration of  $^{31}\text{P}$  NMR spectra was carried out by Bruker's TopSpin 4.0.3 and based on a saved template referred from a published method (Pu et al., 2011). The integration regions were as follows: 151.100–151.850 ppm (internal standard), 149.006–145.000 ppm (aliphatic–OH), 145.000–140.396 ppm (condensed phenolic–OH, con. Ph–OH), 140.396–137.203 ppm (noncondensed phenolic–OH, nonc. Ph–OH) and 135.995–133.897 ppm (carboxylic acids, –COOH).

### 2.6. Elemental analysis

Elemental analysis was conducted on an Elementar Analysensysteme (HANAU, Germany) model vario MICRO cube, operated in CHNS mode. Sulfanilamide was used as a standard.

### 2.7. IR

IR (Infrared Spectroscopy) spectra were recorded on a Bruker Alpha-P Fourier transform infrared spectrometer in the range of 4000–400  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$  and 32 scans.

**Table 1**  
Yields and molecular weight data of the extracted lignin fractions.

	Yields (wt%)	Mw (g·mol <sup>-1</sup> )	Mn (g·mol <sup>-1</sup> )	PD
KL	–	6095	1466	4.16
Ethanol	48.1	1900	1027	1.85
1- Propanol	46.4	1299	557	2.33
1- Butanol	47.3	1457	762	1.91

### 3. Results and discussion

#### 3.1. Fractionation of KL

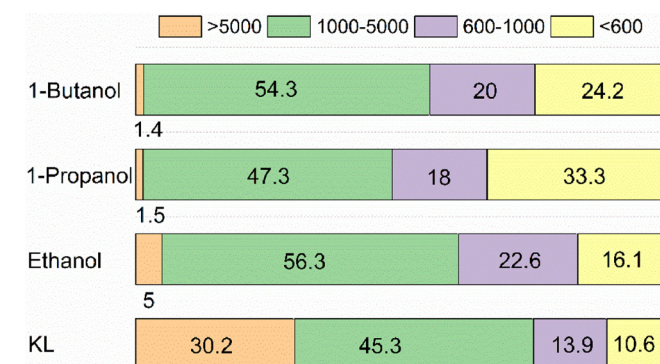
##### 3.1.1. Fractionation yields and molecular weight distributions of fractions

Under refluxing conditions, ethanol, 1-propanol and 1-butanol dissolved KL significantly and gave soluble fractions with yields around 47 wt% (Table 1). Mw and Mn values of extracted lignin are markedly lower in comparison to the initial KL, or in other words, high molecular weight fraction (Mw > 5000 g·mol<sup>-1</sup>) of lignin is effectively removed by the solvent extractions. From the series of solvents, ethanol gave lignin fraction with Mw ~ 1900 g·mol<sup>-1</sup> while 1-propanol offered a lignin fraction with Mw ~ 1300 g·mol<sup>-1</sup>, which is slightly lower than values with 1-butanol (Mw ~ 1460 g·mol<sup>-1</sup>). Improved homogeneity of the extracted lignin fractions in comparison to the initial KL is also reflecting in the polydispersity (PD) values, which are around 2 for the extracted lignin oligomers.

When the high molecular weight fraction is removed, the medium molecular weight (1000–5000 g·mol<sup>-1</sup>) fraction constitutes nearly half of the extracted lignin (Fig. 1). Intriguingly, depending on the applied solvent, distribution of the low molecular weight oligomers is markedly different in the extracts; lignin oligomers with Mw < 600 g·mol<sup>-1</sup> accounts for 16–33 % of the material and are particularly enriched in the 1-propanol extract. Presumably hydrophobic and hydrophilic properties of 1-propanol (M. Hansen, 2012; Thielemans and Wool, 2005) are matching those of the lignin oligomers, which makes it remarkable in the series of studied solvents. The increasing ratio of oligomers with Mw < 600 g·mol<sup>-1</sup> is actually seen in slightly increasing PD value in lignin extracts in the order of ethanol, 1-butanol and 1-propanol. After all, the low molecular weight lignin fractions, Mw ~ 600–1000 g·mol<sup>-1</sup> and < 600 g·mol<sup>-1</sup>, represent together 39–51 % of the extracted lignin and this property makes them attractive starting materials for further transformations.

##### 3.1.2. Structure and functional groups of KL and fractions

KL and all fractions were characterized by 2D HSQC, IR and <sup>31</sup>P NMR and the main signals were assigned based on previous works (Constant et al., 2016; Hu et al., 2016; Wang et al., 2018a; Yao et al., 2018). In comparison to KL, obvious decrease of the carbohydrate signals was observed after the alcohol extractions (Fig. 2), particularly



**Fig. 1.** Molecular weight (Mw) distributions of KL and fractions after extractions with alcohols (From GPC results in THF using polystyrene standards).

those signals belonging to α-L-(1→4) arabinosyl units (ara) and β-D-(1→4) xylosyl units (xyl) that are covalently attached to lignin to form xylan-enriched lignin carbohydrate complexes (Wang et al., 2018a). The poor solubility of carbohydrates in alcohols is consistent with the literature observations (Duval et al., 2016). Accordingly, it is clear that the alcohol extractions described above are a straightforward method to markedly reduce carbohydrates content of lignin.

Four general interunit linkages, arylglycerol-β-aryl ethers (β-O-4), phenyl coumaran (β-5), pinoresinols (β-β), and secoisolariciresinols (E) were investigated (Table 2). All lignin fractions derived from the alcohol extractions contained lower ratio of β-O-4, β-5 and β-β linkages compared with original KL. All of them were decreased in similar relative ratios, while β-O-4 is the most abundant and its reduction was the highest in absolute values among the linkages. As it is very unlikely that β-O-4, β-5 and β-β structures are broken under applied reaction conditions, presumably a part of lignin is removed together with alcohol insoluble carbohydrate fractions and high molecular weight fractions in KL. Meanwhile, secoisolariciresinol substructures (E) are present in increased concentrations in low molecular weight lignin fractions. These results are in accord with previous reports on lignin fractionation (Gioia et al., 2018; Jiang et al., 2016a). For the three alcohols employed in this study, 1-propanol gave lignin fractions with the highest content of E structures, along with the lowest content of β-β and β-5 linkages and the lowest molecular weight.

Signals of KL and all fractions presented in IR spectra are basically the same, expect for those belong to characteristic vibrations of guaiacyl unit (1150 cm<sup>-1</sup> and 1121 cm<sup>-1</sup>, aromatic C–H in-plane deformation). They are clearly presented in soluble fractions, thus indicating concentrated G units.  $\bar{\nu}$  = 1591 cm<sup>-1</sup> represent CC= stretching vibrations in benzene ring of lignin. Signals at 1511 cm<sup>-1</sup> and 1425 cm<sup>-1</sup> are assigned to aromatic ring vibration of the phenylpropane groups plus C–C bounds and C–H in-plane deformation with aromatic ring stretching. A semiquantitative method was employed to determine the relative content of aliphatic–OH and phenolic–OH. The intensity of signal at 1511 cm<sup>-1</sup> was set as reference. Peak at 1365 cm<sup>-1</sup> is originated from the O–H in plane deformation vibration (phenolic OH), while peaks at 1080 and 1030 cm<sup>-1</sup> are assigned to aliphatic secondary and primary hydroxyl group (Passoni et al., 2016). As listed in Table 3, all fractions contained more phenolic–OH and less aliphatic–OH compared with initial KL.

<sup>31</sup>P NMR was employed to determine the accurate content of functional groups. The amount of aliphatic–OH in KL (2.44 mmol/g) decreased after extraction while the content of phenolic–OH and –COOH increased (Table 2). This is in line with the 2D HSQC study, as removal of carbohydrates should reduce the amount of aliphatic–OH. Moreover, the amount of aliphatic–OH groups decreased with the decreasing of molecular weight, as the reduction of sidechain structures. Similar phenomena have been previously reported (Brodin et al., 2009; Cui et al., 2014; Mörck et al., 1988). The increase in phenolic–OH indicating the augment of end groups, which correlates with the decrease of molecular weight after the fractionation. The results also accorded with the previous report where solvent extractable fractions were composed of lower molecular weight polyphenolic fragments (Crestini et al., 2017). The augment of phenolic–OH groups in the lignin extracts also confirmed the increased concentrations of aromatic-containing structures. Importantly, the lignin fraction from 1-propanol has lower amount of condensed phenolic–OH groups than original KL, which would favor the further use of the extract as a starting material for various chemical transformations.

##### 3.1.3. Elemental analysis

The content of C, H, N and S of lignin was measured and oxygen content and H/C<sub>eff</sub> ratio was calculated accordingly. High H/C<sub>eff</sub> ratio implies high intrinsic hydrogen content in the biomass-derived feedstocks, which is prerequisite for high yield of aromatic hydrocarbons and olefins (Jiang et al., 2016b). After the fractionation, all the lignin

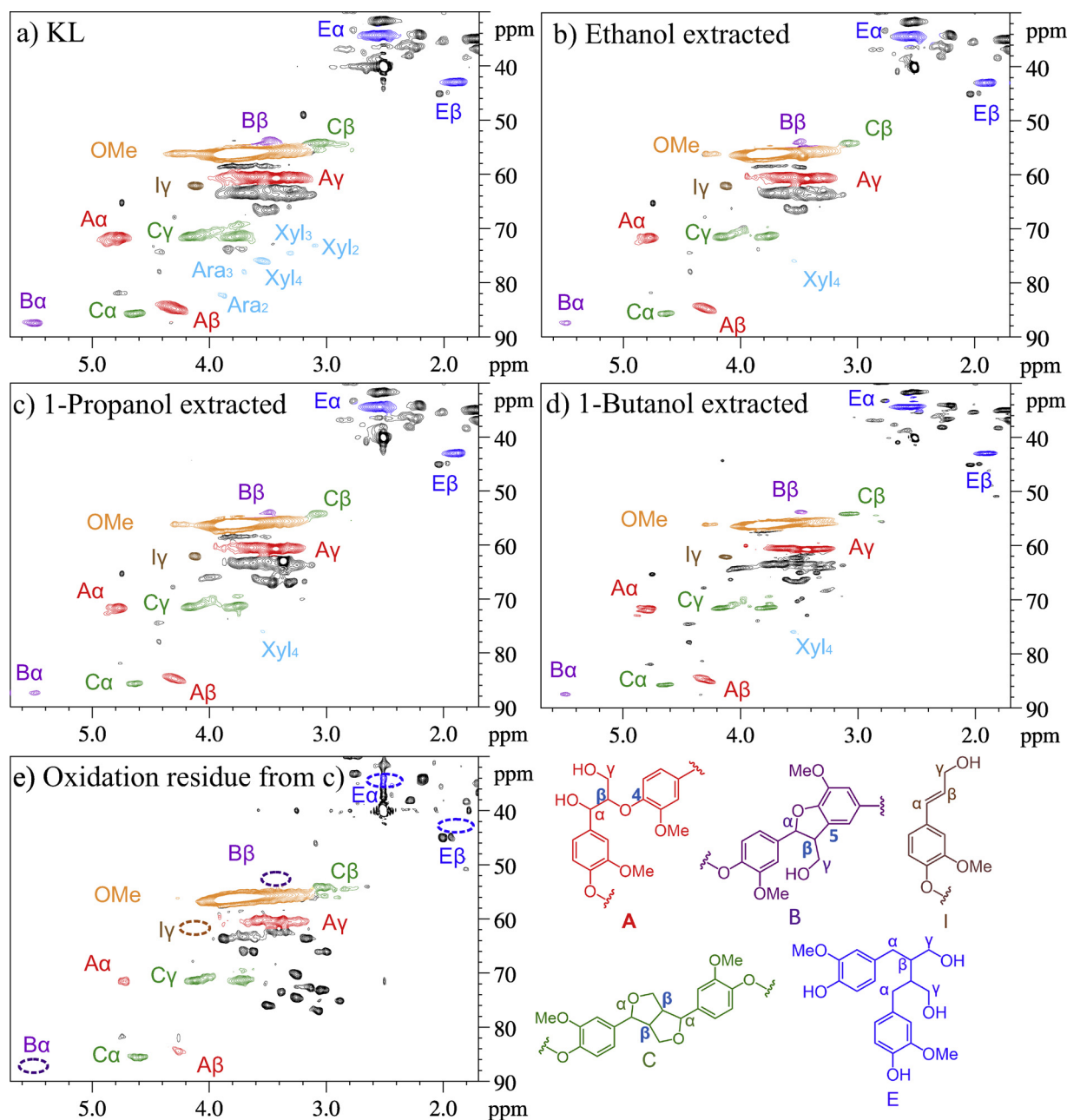


Fig. 2. 2D HSQC spectra of a) KL, b) Ethanol extracted KL, c) 1-Propanol extracted KL, d) 1-Butanol extracted KL and e) Oxidation residue from 1-Propanol extracted KL. Arylglycerol- $\beta$ -aryl ethers ( $\beta$ -O-4) (A), phenyl coumaran ( $\beta$ -5) (B), pinoresinols ( $\beta$ - $\beta$ ) (C), secoisolariciresinols (E) and conferyl alcohol structures (I).

Table 2

Content of interunit linkages, functional groups and vanillin yield of fractions.

	A $_{\alpha}$ <sup>a</sup>	B $_{\alpha}$ <sup>a</sup>	C $_{\alpha}$ <sup>a</sup>	E $_{\beta}$ <sup>a</sup>	Aliphatic-OH <sup>b</sup>	Con. Ph-OH <sup>b</sup>	Nonc. Ph-OH <sup>b</sup>	Total Ph-OH <sup>b</sup>	-COOH <sup>b</sup>	Vanillin Yield (wt%) <sup>c</sup>
KL	47.5	11.9	19.2	21.4	2.44	1.52	2.16	3.68	0.44	6.7
Ethanol	41.8	11.0	17.5	29.7	2.05	1.61	2.77	4.38	0.52	7.8
1-Propanol	39.9	10.2	16.6	33.3	1.71	1.51	2.80	4.31	0.48	9.4
1-Butanol	39.2	10.7	17.8	32.3	1.98	1.59	2.90	4.49	0.51	8.7

<sup>a</sup> Relative content from integrate results (%).

<sup>b</sup> Quantitative results from <sup>31</sup>P NMR (mmol/g).

<sup>c</sup> Oxidation reaction conditions: 120 °C, 2 M NaOH, 10 wt% CuSO<sub>4</sub>·5H<sub>2</sub>O, 270  $\mu$ L 33 wt% H<sub>2</sub>O<sub>2</sub>, 60 min.

extracts were higher with carbon and hydrogen and lower with nitrogen, sulfur and oxygen content than KL (Table 4). Whereas the reduction in oxygen content is related to removal of carbohydrates, the decrease of sulfur content is mainly attributed to the inorganic part in

KL (Jiang et al., 2016a). In general, fractionation provided lignin extracts with increased H/C<sub>eff</sub> ratio and from those 1-propanol extracted fractions showed the highest H/C<sub>eff</sub> ratio and lowest amount of oxygen.



**Table 3**  
Relative content of phenolic–OH and aliphatic–OH groups.

	$\bar{\nu} = 1365 \text{ cm}^{-1}$ phenolic–OH	$\bar{\nu} = 1080 \text{ cm}^{-1}$ aliphatic secondary OH	$\bar{\nu} = 1030 \text{ cm}^{-1}$ aliphatic primary OH
KL	9.5	15.1	86.5
Ethanol	10.8	13.4	70.1
1-Propanol	9.9	10.5	66.9
1-Butanol	10.9	11.8	67.3

**Table 4**  
Elemental analysis of KL and fractions.

	C	H	N	S	O	H/C <sub>eff</sub> <sup>a</sup>
KL	62.6	5.7	0.7	1.6	29.35	0.40
Ethanol	65.1	6.1	0.4	1.1	27.28	0.49
1-Propanol	68.1	6.3	0.4	1.1	24.08	0.58
1-Butanol	66.4	5.9	0.4	1.2	26.08	0.48

<sup>a</sup> H/C<sub>eff</sub> = (mol of H – 2 × mol of O)/mol of C.

### 3.2. Vanillin synthesis

#### 3.2.1. Catalytic oxidation

Catalytic oxidation reactions were investigated for vanillin synthesis using the lignin extracts as starting material. We focused on copper (II)-based catalysts to enhance the oxidation reaction, as they are fairly well-studied catalytic systems, known to be efficient in alkaline water solutions (Korpi et al., 2007; Ninomiya et al., 2018) and compatible with lignocellulosic biomass (Hakola et al., 2010; Kallioinen et al., 2013). Based on initial experiments, Cu(II)/H<sub>2</sub>O<sub>2</sub> system afforded a simple reaction setup and gave higher vanillin yield than reactions with pressurized O<sub>2</sub> (5 bar). The use of H<sub>2</sub>O<sub>2</sub> overcame the low concentration and low mass transfer rate of O<sub>2</sub> to the alkaline water solution. Accordingly, we selected Cu(II)/H<sub>2</sub>O<sub>2</sub> catalytic system for further studies. Catalytic oxidations were conducted by using original KL and three KL fractions with different molecular weight, interunit linkages and hydroxy groups content (Table 2). To our surprise, the obtained vanillin yields are remarkably high in comparison to other reports with KL (Silva et al., 2009; Villar et al., 2001; Voitl and Rohr, 2010). This implied the success of our catalytic oxidation system.

In general, vanillin yields are largely influenced by the source of lignin (Araújo et al., 2010; Tarabanko et al., 1995a, 1995b; Wang et al., 2018b). As showed in Table 2, fractionated KL gave vanillin yield higher than untreated KL. Notably, the fraction from 1-propanol treatment, enriched with the lignin oligomers, afforded vanillin with the highest yield of 9.4 wt%, which is 40 % higher in comparison to the yield from the original KL (6.7 wt%). To improve vanillin yields, we conducted further experiments with the 1-propanol extracted lignin and investigated the effect of reaction temperature and time. It was worth mentioning that the yield of vanillin was up to 7.4 wt% already at 70 °C, and by increasing the temperature the vanillin yield increased; at 130 °C the recorded yield was 10.7 wt% and at 140 °C was 10.9 wt% after 60 min reaction (Table 5). The later value is up to 73 % from the reference maximum, which is achieved by nitrobenzene oxidation of Indulin AT KL at 170 °C (Hu et al., 2016). Accordingly, the herein developed two-step procedure combining single solvent fractionation and catalytic oxidation offers a straightforward, high yield synthesis of vanillin.

To gain an insight to the oxidation reaction, effect of each individual reaction component at 130 °C in 60 min reaction time were investigated. At first, the reaction does not take place without NaOH (Table 6, entry 1). Alkaline reaction conditions are most likely needed for efficient substrate deprotonation (Korpi et al., 2007; Tarabanko et al., 1995a, 1995b; Tarabanko et al., 2004) and to enhance vanillin synthesis. As shown earlier the phenoxyl anion reacts further to a

**Table 5**  
Effect of reaction temperature and time on vanillin yield.

Entry	Temperature (°C)	Time (min)	Vanillin Yield (wt%)
1	70	60	7.4
2	100	60	8.5
3	110	60	9.4
4	120	60	9.4
5	130	60	10.7
6	140	60	10.9
7	130	30	7.4
8	130	45	8.7
9 <sup>a</sup>	130	90	9.4
10 <sup>a</sup>	130	120	9.4

Reaction conditions: lignin fractions from 1-propanol extracted, 2 M NaOH, 10 wt% CuSO<sub>4</sub>·5H<sub>2</sub>O, 270 μL 33 wt% H<sub>2</sub>O<sub>2</sub>.

<sup>a</sup> Increased reaction time caused vanillin overoxidation to acids, thus decreased the yield.

**Table 6**  
Effect of individual reaction component on vanillin yield.

Entry	Cu(II) (wt%)	H <sub>2</sub> O <sub>2</sub> (μL)	Vanillin Yield (wt%)
1 <sup>a</sup>	10	270	–
2 <sup>b</sup>	0	0	1.8
3	0	270	3.4
4	5	270	10.2
5	10	270	10.7
6	10	0	6.1
7	10	135	8.9
8	10	405	8.6
9	10	504	7.6
10 <sup>c</sup>	10	270	8.4

Reaction conditions: lignin fractions from 1-propanol extracted, 130 °C, 2 M NaOH, 270 μL 33 wt% H<sub>2</sub>O<sub>2</sub>, 60 min.

<sup>a</sup> Without NaOH.

<sup>b</sup> Without CuSO<sub>4</sub>·5H<sub>2</sub>O and H<sub>2</sub>O<sub>2</sub>.

<sup>c</sup> Using recycled CuO.

phenoxyl radical through an electron detachment and this is the rate-determining step in the overall reaction (Wu and Heitz, 1995). The resulting phenoxyl radical is further converted to quinonemethide, for which the nucleophilic addition of hydroxide ion at γ-carbon position occurs. The formed coniferaldehyde undergoes a retroaldol cleavage to give vanillin.

The oxidation reaction gave only low yields of vanillin (3.4 wt%) if CuSO<sub>4</sub>·5H<sub>2</sub>O was removed (Table 6, entry 3), while addition of 5 wt% CuSO<sub>4</sub>·5H<sub>2</sub>O increased the yield significantly (10.2 wt%). Further increase of the CuSO<sub>4</sub>·5H<sub>2</sub>O loading did not improve the yield markedly, e.g. 10 wt% CuSO<sub>4</sub> loading gave 10.7 wt% vanillin (Table 6, entry 5). It is likely that the role of Cu<sup>2+</sup> herein is to be an electron acceptor (Wu and Heitz, 1995), which takes part in the rate limiting step and facilitates conversion of phenolate anion to phenoxyl radical, thus catalyzing the production of vanillin. While assisting vanillin synthesis, Cu<sup>2+</sup> as an electron acceptor reduces to Cu<sup>+</sup>. On the other hand, the formed Cu<sup>+</sup> are readily oxidized back to Cu<sup>2+</sup> in the presence of H<sub>2</sub>O<sub>2</sub> (supported by the fact that in the absence of H<sub>2</sub>O<sub>2</sub>, the vanillin yield obtained was only 6.1 wt% in entry 6), thus completing the catalytic redox cycle (Chen et al., 2014; Figiel et al., 2007; Korpi et al., 2007). Under alkaline conditions Cu<sup>2+</sup> species are likely to form Cu(OH)<sub>2</sub> which reacts further and forms CuO. We also confirmed the existence of CuO as a black precipitation after the reaction by XRD. Whereas CuO is linked to the deactivation of catalytic species in aerobic oxidation, here with H<sub>2</sub>O<sub>2</sub> it can efficiently catalyze vanillin synthesis (Table 6, entry 10). This is an intriguing observation as solid CuO is a robust material and its removal by filtration makes the catalyst recycling straightforward.

### 3.2.2. Effect of structure and molecular weight of extracted fraction on vanillin yield

To get an insight to the effect of lignin structure on vanillin synthesis, a lignin residue after the oxidation was characterized by 2D HSQC (Fig. 2). Clearly,  $\beta$ -5 and secoisolariciresinol substructures E were entirely degraded along with  $\text{C}_\gamma\text{-H}_\gamma$  in coniferyl alcohol end groups ( $\text{I}_\gamma$ ). The intensity of  $\beta$ -O-4 linkages reduced sharply, while  $\beta$ - $\beta$  in pinoresinol almost remain unchanged. Considering the structure of above mentioned, signals that disappeared or decreased all contain aliphatic hydroxyl groups. Dabral et al. (Dabral et al., 2015) found aliphatic -OH had a strong effect on the decomposition of lignin model compounds under basic conditions, while other studies demonstrated that  $\beta$ - $\beta$  pinoresinol structures were resistant to oxidation (Villar et al., 1997). Herein, the high vanillin yield is mainly related to the low molecular weight of fractions and particularly the cleavage of  $\beta$ -O-4,  $\beta$ -5, E and coniferyl substructures. In this respect, 1-propanol was the most interesting solvent for fractionation as it gave lignin fractions with the lowest molecular weight, the highest content of E structures and the lowest content of  $\beta$ - $\beta$  linkages, thus afforded the highest yield of vanillin from the studied extracts.

Purification of vanillin from the alkaline reaction mixture is often complicated due to remaining unreacted lignin and impurities like carbohydrates and other side products. In this respect, high yield pathway with uniform fractionated lignin should be beneficial in vanillin isolation. The recent review by Fache et al. (2015) is important as it summarized publications and patents related to vanillin separation technics (Fache et al., 2015). In addition to that, a highly effective methods include vanillin recovering using consecutive membrane, ion-exchange and crystallization processes is reported by Silva et al. (Silva et al., 2009).

## 4. Conclusions

A straightforward fractionation of KL with green, bio-based alcohols (ethanol, 1-propanol and 1-butanol) offered lignin fractions with improved homogeneity, low molecular weight and high content of carbon and phenolic-OH groups. As shown herein, the followed catalytic oxidation reaction with all isolated, low fractions gave vanillin yield higher than unfractionated KL. Particularly, the 1-propanol extract, enriched with 51 wt% of lignin oligomers ( $M_w < 1000 \text{ g}\cdot\text{mol}^{-1}$ ) was noteworthy as it afforded vanillin with the highest yield of 9.4 wt%. The high yield of vanillin was mainly related to the low molecular weight of fractions, also partially to the content of different linkages. The cleavage of  $\beta$ -O-4,  $\beta$ -5 and secoisolariciresinol E structures was response for vanillin production. After reaction optimization, remarkable yield of 10.9 wt% was obtained. The overall success of using KL as a renewable and sustainable resource for high yield vanillin synthesis was achieved by an integrated procedure, which efficiently bridges fractionation of KL and chemical conversion of the obtained fractions.

### Declaration of competing interest

The authors declare no competing financial interest.

### CRedit authorship contribution statement

**Rui Zhang:** Investigation, Methodology, Data curation, Formal analysis, Writing - original draft. **Riku Maltari:** Conceptualization, Software, Writing - review & editing. **Ming Guo:** Conceptualization. **Jussi Kontro:** Software, Methodology. **Aleksi Eronen:** Software, Methodology. **Timo Repo:** Funding acquisition, Project administration, Supervision, Validation, Writing - review & editing.

### Acknowledgements

This work was funded by University of Helsinki, Finland. R. Z. is

grateful for financial support from CSC (China Scholarship Council), File No. 201706240138), China and EDUFI (EDUFI Fellowship, TM-18-10860), Finland; R. M. received support from EU Horizon 2020, project No 720918 (FALCON); M. G. received support from CSC; J. K. received support from the Academy of Finland, project No 298882 (AROMAFUNG); A. E. received support from Magnus Ehrnrooth foundation.

### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.indcrop.2020.112095>.

### References

- Araújo, J.D.P., Grande, C.A., Rodrigues, A.E., 2010. Vanillin production from lignin oxidation in a batch reactor. *Chem. Eng. Res. Des.* 88, 1024–1032.
- Azadi, P., Inderwildi, O.R., Farnood, R., King, D.A., 2013. Liquid fuels, hydrogen and chemicals from lignin: a critical review. *Renew. Sust. Energ. Rev.* 21, 506–523.
- Bajwa, D.S., Pourhashem, G., Ullah, A.H., Bajwa, S.G., 2019. A concise review of current lignin production, applications, products and their environmental impact. *Ind. Crops Prod.* 139, 111526.
- Behling, R., Valange, S., Chatel, G., 2016. Heterogeneous catalytic oxidation for lignin valorization into valuable chemicals: what results? What limitations? What trends? *Green Chem.* 18, 1839–1854.
- Brodin, I., Sjöholm, E., Gellerstedt, G., 2009. Kraft lignin as feedstock for chemical products: the effects of membrane filtration. *Holzforsch* 63, 290–297.
- Chakar, F.S., Ragauskas, A.J., 2004. Review of current and future softwood kraft lignin process chemistry. *Ind. Crops Prod.* 20, 131–141.
- Chen, F., Zhao, X., Liu, H., Qu, J., 2014. Reaction of  $\text{Cu}(\text{CN})_3^{2-}$  with  $\text{H}_2\text{O}_2$  in water under alkaline conditions: cyanide oxidation,  $\text{Cu}^+/\text{Cu}^{2+}$  catalysis and  $\text{H}_2\text{O}_2$  decomposition. *Appl. Catal. B Environ.* 158–159, 85–90.
- Constant, S., Wienk, H.L.J., Frissen, A.E., Peinder, P.D., Boelens, R., van Es, D.S., Grisel, R.J.H., Weckhuysen, B.M., Huijgen, W.J.J., Gosselink, R.J.A., Bruijninx, P.C.A., 2016. New insights into the structure and composition of technical lignins: a comparative characterisation study. *Green Chem.* 18, 2651–2665.
- Crestini, C., Lange, H., Sette, M., Argyropoulos, D.S., 2017. On the structure of softwood kraft lignin. *Green Chem.* 19, 4104–4121.
- Cui, C., Sun, R., Argyropoulos, D.S., 2014. Fractional precipitation of softwood Kraft lignin: isolation of narrow fractions common to a variety of lignins. *ACS Sustain. Chem. Eng.* 2, 959–968.
- Dabral, S., Mottweiler, J., Rinesch, T., Bolm, C., 2015. Base-catalysed cleavage of lignin  $\beta$ -O-4 model compounds in dimethyl carbonate. *Green Chem.* 17, 4908–4912.
- de Jong, E., Higson, A., Walsh, P., Wellisch, M., 2012. Product developments in the bio-based chemicals arena. *Biofuels Bioprod. Bioref.* 6, 606–624.
- Duval, A., Vilaplana, F., Crestini, C., Lawoko, M., 2016. Solvent screening for the fractionation of industrial kraft lignin. *Holzforsch* 70, 11–20.
- Fache, M., Boutevin, B., Caillol, S., 2015. Vanillin production from lignin and its use as a renewable chemical. *ACS Sustain. Chem. Eng.* 4, 35–46.
- Figiel, P.J., Leskelä, M., Repo, T., 2007. TEMPO-Copper(II) Diimine-catalysed oxidation of benzylic alcohols in aqueous media. *Adv. Synth. Catal.* 349, 1173–1179.
- Gellerstedt, G., 2015. Softwood kraft lignin: raw material for the future. *Ind. Crops Prod.* 77, 845–854.
- Gillet, S., Aguedo, M., Petitjean, L., Morais, A.R.C., da Costa Lopes, A.M., Łukasik, R.M., Anastas, P.T., 2017. Lignin transformations for high value applications: towards targeted modifications using green chemistry. *Green Chem.* 19, 4200–4233.
- Gioia, C., Lo Re, G., Lawoko, M., Berglund, L., 2018. Tunable thermosetting epoxies based on fractionated and well-characterized lignins. *J. Am. Chem. Soc.* 140, 4054–4061.
- Hakola, M., Kallioinen, A., Kemell, M., Lahtinen, P., Lankinen, E., Leskelä, M., Repo, T., Riekkola, T., Siika-aho, M., Uusitalo, J., Vuorela, S., von Weymarn, N., 2010. Liberation of cellulose from the lignin cage: a catalytic pretreatment method for the production of cellulosic ethanol. *ChemSusChem* 3, 1142–1145.
- Ho Seo, J., Jeong, H., Lee, H.W., Choi, C.S., Bae, J.H., Lee, S.M., Kim, Y.S., 2019. Characterization of solvent-fractionated lignins from woody biomass treated via supercritical water oxidation. *Bioresour. Technol.* 275, 368–374.
- Hu, F., Jung, S., Ragauskas, A., 2012. Pseudo-lignin formation and its impact on enzymatic hydrolysis. *Bioresour. Technol.* 117, 7–12.
- Hu, Z., Du, X., Liu, J., Chang, H.-m., Jameel, H., 2016. Structural characterization of pine Kraft lignin: BioChoice Lignin vs Indulin AT. *J. Wood Chem. Technol.* 36, 432–446.
- Jääskeläinen, A.S., Liittä, T., Mikkelsen, A., Tamminen, T., 2017. Aqueous organic solvent fractionation as means to improve lignin homogeneity and purity. *Ind. Crops Prod.* 103, 51–58.
- Jiang, X., Savithri, D., Du, X., Pawar, S., Jameel, H., Chang, H.-M., Zhou, X., 2016a. Fractionation and characterization of Kraft lignin by sequential precipitation with various organic solvents. *ACS Sustain. Chem. Eng.* 5, 835–842.
- Jiang, Z., Zhang, H., He, T., Lv, X., Yi, J., Li, J., Hu, C., 2016b. Understanding the cleavage of inter- and intramolecular linkages in corn cob residue for utilization of lignin to produce monophenols. *Green Chem.* 18, 4109–4115.
- Kallioinen, A., Hakola, M., Riekkola, T., Repo, T., Leskelä, M., von Weymarn, N., Siika-aho, M., 2013. A novel alkaline oxidation pretreatment for spruce, birch and sugar cane bagasse. *Bioresour. Technol.* 140, 414–420.

- Korpi, H., Figiel, P.J., Lankinen, E., Ryan, P., Leskelä, M., Repo, T., 2007. On in situ prepared Cu-Phenanthroline complexes in aqueous alkaline solutions and their use in the catalytic oxidation of veratryl alcohol. *Eur. J. Inorg. Chem.* 2007, 2465–2471.
- Hansen, C.M., 2012. Hansen Solubility Parameters: A User's Handbook, second edition. .
- Mörck, R., Reimann, A., Kringstad Knut, P., 1988. Fractionation of Kraft lignin by successive extraction with organic solvents. III. Fractionation of Kraft lignin from Birch. *Holzforschung Int. J. Biol. Chem. Phys. Technol. Wood* 42, 111.
- Ninomiya, K., Ochiai, K., Eguchi, M., Kuroda, K., Tsuge, Y., Ogino, C., Taima, T., Takahashi, K., 2018. Oxidative depolymerization potential of biorefinery lignin obtained by ionic liquid pretreatment and subsequent enzymatic saccharification of eucalyptus. *Ind. Crops Prod.* 111, 457–461.
- Passoni, V., Scarica, C., Levi, M., Turri, S., Griffini, G., 2016. Fractionation of industrial softwood Kraft lignin: solvent selection as a tool for tailored material properties. *ACS Sustain. Chem. Eng.* 4, 2232–2242.
- Pu, Y., Cao, S., Ragauskas, A.J., 2011. Application of quantitative  $^{31}\text{P}$  NMR in biomass lignin and biofuel precursors characterization. *Energy Environ. Sci.* 4, 3154–3166.
- Ragauskas, A.J., Beckham, G.T., Bidy, 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., Wyman, C.E., 2014. Lignin valorization: improving lignin processing in the biorefinery. *Science* 344, 1246843.
- Rahimi, A., Ulbrich, A., Coon, J.J., Stahl, S.S., 2014. Formic-acid-induced depolymerization of oxidized lignin to aromatics. *Nature* 515, 249–252.
- Sadeghifar, H., Argyropoulos, D.S., 2015. Correlations of the antioxidant properties of softwood kraft lignin fractions with the thermal stability of its blends with polyethylene. *ACS Sustain. Chem. Eng.* 3, 349–356.
- Sadeghifar, H., Wells, T., Le, R.K., Sadeghifar, F., Yuan, J.S., Jonas Ragauskas, A., 2016. Fractionation of organosolv lignin using acetone:water and properties of the obtained fractions. *ACS Sustain. Chem. Eng.* 5, 580–587.
- Saito, T., Perkins, J.H., Vautard, F., Meyer, H.M., Messman, J.M., Tolnai, B., Naskar, A.K., 2014. Methanol fractionation of softwood kraft lignin: impact on the lignin properties. *ChemSusChem* 7, 221–228.
- Shuai, L., Amiri, M.T., Questell-Santiago, Y.M., Héroguel, F., Li, Y., Kim, H., Meilan, R., Chapple, C., Ralph, J., Luterbacher, J.S., 2016. Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization. *Science* 354, 329–333.
- Silva, E.A.Bd., Zabkova, M., Araújo, J.D., Cateto, C.A., Barreiro, M.F., Belgacem, M.N., Rodrigues, A.E., 2009. An integrated process to produce vanillin and lignin-based polyurethanes from Kraft lignin. *Chem. Eng. Res. Des.* 87, 1276–1292.
- Sun, Z., Fridrich, B., de Santi, A., Elangovan, S., Barta, K., 2018. Bright side of lignin depolymerization: toward new platform chemicals. *Chem. Rev.* 118, 614–678.
- Tagami, A., Gioia, C., Lauberts, M., Budnyak, T., Moriana, R., Lindström, M.E., Sevastyanova, O., 2019. Solvent fractionation of softwood and hardwood kraft lignins for more efficient uses: compositional, structural, thermal, antioxidant and adsorption properties. *Ind. Crops Prod.* 129, 123–134.
- Tarabanko, V.E., Koropatchinskaya, N.V., Kudryashev, A.V., Kuznetsov, B.N., 1995a. Influence of lignin origin on the efficiency of the catalytic oxidation of lignin into vanillin and syringaldehyde. *Russ. Chem. B+* 44, 367–371.
- Tarabanko, V.E., Fomova, N.A., Kuznetsov, B.N., Ivanchenko, N.M., Kudryashev, A.V., 1995b. On the mechanism of vanillin formation in the catalytic oxidation of lignin with oxygen. *React. Kinet. Catal. Lett.* 55, 161–170.
- Tarabanko, V.E., Petukhov, D.V., Selyutin, G.E., 2004. New mechanism for the catalytic oxidation of lignin to vanillin. *Kinet. Catal.* 45, 569–577.
- Thielemans, W., Wool, R.P., 2005. Lignin esters for use in unsaturated thermosets: lignin modification and solubility modeling. *Biomacromolecules* 6, 1895–1905.
- Upton, B.M., Kasko, A.M., 2016. Strategies for the conversion of lignin to high-value polymeric materials: review and perspective. *Chem. Rev.* 116, 2275–2306.
- Vázquez, G., Antorrena, G., González, J., Freire, S., 1997. The Influence of pulping conditions on the structure of acetosolv eucalyptus lignins. *J. Wood. Chem. Technol.* 17, 147–162.
- Villar, J.C., Caperos, A., García-Ochoa, F., 2001. Oxidation of hardwood kraft-lignin to phenolic derivatives with oxygen as oxidant. *Wood Sci. Technol.* 35, 245–255.
- Villar, J.C., Caperos, A., García-Ochoa, F., 1997. Oxidation of hardwood kraft-Lignin to phenolic derivatives. Nitrobenzene and copper oxide as oxidants. *J. Wood. Chem. Technol.* 17, 259–285.
- Voitl, T., Rohr, P.Rv., 2010. Demonstration of a process for the conversion of Kraft lignin into vanillin and methyl vanillate by acidic oxidation in aqueous methanol. *Ind. Eng. Chem. Res.* 49, 520–525.
- Wang, K., Xu, F., Sun, R., 2010. Molecular characteristics of Kraft-AQ pulping lignin fractionated by sequential organic solvent extraction. *Int. J. Mol. Sci.* 11, 2988–3001.
- Wang, Q., Chen, K., Li, J., Yang, G., Liu, S., Xu, J., 2011. The solubility of lignin from bagasse in a 1,4-butanediol/water system. *BioResources* 6, 3034–3043.
- Wang, Y.Y., Li, M., Wyman, C.E., Cai, C.M., Ragauskas, A.J., 2018a. Fast fractionation of technical lignins by organic cosolvents. *ACS Sustain. Chem. Eng.* 6, 6064–6072.
- Wang, Y., Sun, S., Li, F., Cao, X., Sun, R., 2018b. Production of vanillin from lignin: the relationship between  $\beta$ -O-4 linkages and vanillin yield. *Ind. Crops Prod.* 116, 116–121.
- Wu, G., Heitz, M., 1995. Catalytic Mechanism of  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  in alkaline  $\text{O}_2$  oxidation of lignin. *J. Wood. Chem. Technol.* 15, 189–202.
- Xu, C., Arancon, R.A., Labidi, J., Luque, R., 2014. Lignin depolymerisation strategies: towards valuable chemicals and fuels. *Chem. Soc. Rev.* 43, 7485–7500.
- Yao, S.G., Mobley, J.K., Ralph, J., Crocker, M., Parkin, S., Selegue, J.P., Meier, M.S., 2018. Mechanochemical treatment facilitates two-step oxidative depolymerization of Kraft lignin. *ACS Sustain. Chem. Eng.* 6, 5990–5998.
- Yuan, T.Q., He, J., Xu, F., Sun, R.C., 2009. Fractionation and physico-chemical analysis of degraded lignins from the black liquor of Eucalyptus pellita KP-AQ pulping. *Polym. Degrad. Stabil.* 94, 1142–1150.