

Fractionation of five technical lignins by selective extraction in green solvents and characterisation of isolated fractions



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ABSTRACT

Lignins from softwood, hardwood, grass and wheat straw were fractionated by selective extraction at ambient temperature using green solvents like acetone/water solutions of 10, 30, 50, 70 and 90% (v/v) acetone and ethyl acetate. A comparison between the isolated fractions and unfractionated lignins was made in terms of extraction yield, lignin solubility factor, molecular weight distribution and functional group composition. Low molecular weight (LMW) lignin fractions with narrow dispersity are obtained by extraction with ethyl acetate and acetone–water solution containing 30% acetone, with yields depending on the type and the functional group content of lignins. A significant amount (56%) of the organosolv hardwood lignin with low molecular weight ($M_w = 1868 \text{ g/mol}$) and low dispersity was isolated from ethyl acetate. Insoluble fractions with very high molecular weight (M_w between 10 and 17 kg/mol) are obtained in low yield from acetone–water solutions with 50, 70 and 90% acetone. LMW lignins are in general less condensed and have lower aliphatic hydroxyl content than parent lignins while the HMW fractions have a higher content of condensed hydroxyls. Principal component analysis on the chemical composition of lignins and isolated fractions determined from ^{31}P NMR data showed the high heterogeneity of the technical lignins. Partial least squares models based on FT-IR spectral data were developed to predict the functional group content determined by quantitative ^{31}P NMR analysis of technical lignins and lignin fractions. This approach can be used to develop simple, rapid and accurate analytical tools to monitor and control the selective fractionation of lignin.

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1. Introduction

Large amounts of lignin are produced yearly as by-product of pulping and significant increase of lignin's availability is forecasted in the near future due to the development and implementation of new technologies to produce second generation biofuels and

chemicals from lignocellulosic biomass. Currently, less than 2% of the lignin available is isolated from pulping liquors and used for specialty products, the remainder (i.e. 98%) being burned to generate energy for pulp mills (Gosselink et al., 2004). Although using lignin will save fossil oil, there is great potential to create high value for lignin if it is used as source for biobased chemicals and materials.

Lignin is a unique natural aromatic polymer, consisting of phenylpropanoid units linked by aryl–ether, alkyl–ether and carbon–carbon covalent bonds into a highly condensed cross-linked polymer network, with an average of 1–2 hydroxyl groups per C9-monomer (Hofrichter, 2002). Guaiacyl- (G), syringyl- (S) and p-hydroxyphenyl (H) are the main building blocks of lignin and they are primarily produced during the dehydrogenative radical polymerisation of monolignols (Dashtban et al., 2009; Martinez et al., 2005). The composition of lignin varies significantly between different groups of vascular plants thus generating a wide diversity of lignin polymers, each with its own structure and properties. Most softwood lignins consist predominantly of G units, whereas

Abbreviations: G, guaiacyl; S, syringyl; H, p-hydroxyphenyl; δ , solubility parameter; M_w , weight-average molecular weight; M_n , number-average molecular weight; PD, polydispersity (M_w/M_n); KSL, Kraft softwood lignin; SGWL, Soda grass/wheat straw lignin; OHL, organosolv hardwood lignin; SWL, Soda wheat straw lignin; AWL, alkaline wheat straw lignin.

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hardwood and herbaceous (grass/straw) lignins contain G, S and H in different ratios (Capanema et al., 2005; Obst, 1982).

In the past decades we have witnessed major developments in the processing technology for lignin extraction, resulting in a higher availability of lignin from different plant resources and of constant quality. Next to the traditional lignosulphonates, a large variety of lignins are now available, like thio-lignins from the Kraft pulping and sulphur-free lignins obtained by solvent extraction or alkaline pulping. High variability in lignin structure is introduced during the different pulping processing (Lora, 2008; Lora and Glasser, 2002; Tejado et al., 2007), which might be an advantage since more alternatives for reactivity and functional properties become available. Several industrial applications have been developed and implemented for different types of lignin as materials/additives for wood panel products, biodispersants, polyurethane foams, adhesives, epoxies, polypropylene, among others (Lora and Glasser, 2002; Stewart, 2008) and a multitude of potential applications have been reported exploiting the antioxidative, antimicrobial and other biological functionality of lignin (Vinardell et al., 2008). Significant steps have been made in the understanding of the lignin's structure and reactivity and their relationship with the functional properties required for specific applications. For example, low molecular weight lignin of high purity, containing a large amount of free phenolic hydroxyl groups with adjacent free *ortho*-positions, non-sterically hindered, are best substrates for enzymatic polymerisation with laccases and peroxidases (Fitigau et al., 2013) and as alternative to phenol in the synthesis of phenol–formaldehyde resins (Tejado et al., 2007). G lignins from softwood and GHS lignins from grasses, are a preferred choice for these applications. (Pouteau et al. (2003) has studied the application of lignin with antioxidant properties for propylene stabilisation and showed that the limiting factor was the solubility of lignin in polypropylene. Low molecular weight and low content of total hydroxyl groups (e.g. aliphatic and phenolic) improved the compatibility of lignin with polypropylene and promoted the stabilising effect. These and numerous studies show the importance of heterogeneity of lignins on their performance. Chemical heterogeneity (i.e. composition, molecular weight, level and nature of impurities) influences the chemical reactivity, the thermal behaviour, the accessibility to solvents and the compatibility with polymers and materials. Therefore to enhance high-value applications of lignin it is important to have methodologies to reduce its heterogeneity.

Reducing molecular and structural heterogeneity of lignin by sequential extraction using organic solvents of increasing hydrogen-bonding capacity has been reported. Low molecular weight (LMW) and high molecular weight (HMW) lignin have been obtained by sequential extraction of Alcell lignin (Thring et al., 1996) and Kraft lignin using combination of solvents, like ethyl acetate–methanol (Thring and Griffin, 1994), dichloromethane–propanol–methanol–methanol/dichloromethane (Gosselink et al., 2010; Morck et al., 1986), dichloromethane–acetone–methanol (Methacanon et al., 2010) and hexane–diethyl ether–dichloromethane–methanol–dioxane (Wang et al., 2010). The molecular weight of the isolated fractions increased with increase of the hydrogen bonding capacity and polarity of the solvents. Small oligomeric lignins were extracted with hydrophobic solvents like hexane and diethyl ether in low yield (Wang et al., 2010). Significant differences have been observed in the yield of LMW fractions in solvents with lower hydrogen-binding capacity. For example, the yield of LMW lignin fractions extracted from Kraft softwood lignins with dichloromethane and n-propanol was 2.5–3 times lower than in the case of a grass lignin (Gosselink et al., 2010). The performance of the isolated fractions in different applications, like wood adhesives, was closely related to their molecular weight and molecular properties (Gosselink et al., 2010; Yoshida et al., 1990). Fractionation of lignins from steam-exploded

bamboo and sorghum using alkali/alkaline-ethanol post-treatment was also reported (Sun et al., 2012, 2014a, 2014b, 2014c). The isolated fractions had low molecular weight and showed good antioxidative and radical scavenging properties (Sun et al., 2014b, 2014c).

We think that in the future selective fractionation of lignin will play an important role in the production of lignins with distinct group functionalities and molecular weight distribution for industrial application.

In this study, we report on the fractionation of five technical lignins from different sources (softwood, hardwood, grass) and obtained by different pulping processes (kraft, soda, organosolv) by selective extraction in green solvents with increasing hydrogen bonding capacity and on the effect of the chemical and molecular fingerprint of lignins on their solubility. The soluble and insoluble fractions isolated were characterised in terms of chemical composition, FT-IR, ^{31}P NMR, molecular weights and dispersity. The similarities/dissimilarities between the fractions and the starting lignins were identified using principal component analysis on chemical data determined by quantitative ^{31}P NMR spectroscopy. We also report on the potential use of quantitative models based on multivariate statistical analysis to predict functional group composition of lignins based on infrared spectral data as a simple and fast analytical tool in the development and the exploitation of processes for the selective fractionation of lignin.

2. Materials and methods

2.1. Materials

Soda wheat straw lignin (coded SWL) and P1000 soda lignin from mixed Sarkanda grass (75%) and wheat straw (25%) were obtained from Greenvalue SA (Lausanne, Switzerland). Organosolv lignin (Alcell) from mixed maple, birch and poplar (hardwoods, coded as OHL) was obtained from Repap Technologies Inc. (Valley Forge, PA, USA). Indulin AT, a Kraft lignin from pine (softwood, coded KSL), was obtained from MeadWestvaco (USA). AWL is a wheat straw isolated by a mild alkaline process, received from TU Dresden (Germany). The lignin content in the samples, expressed as the sum of acid-insoluble and acid-soluble lignin was OHL: 96.5%, SGWL: 90%, SWL: 91%, AWL: 65.6%, and KSL: 90%, and was determined according to standard analytical procedures (Boeriu et al., 2004). The lignin samples were used without further purification. All the reagents used were of analytical reagent grade.

2.2. Methods

2.2.1. Fractionation of lignin in acetone/water mixtures and ethyl acetate

Lignin (1 g) was suspended in 50 ml acetone/water solutions of different composition or in 50 ml ethyl acetate, at 2% concentration (w/v), and the suspension was stirred over night at 20 °C. The insoluble fractions were separated on a glass filter (G4) and dried to constant weight in a vacuum oven at 105 °C. The soluble lignin in acetone–water mixtures was isolated after removal of acetone under reduced pressure and acid precipitation. The extracted lignin was dried in a vacuum oven at 105 °C till constant weight. Yields of soluble and insoluble lignin fractions were calculated as % weight of the unfractionated dry lignin. Experiments were performed in duplicate and the mass balance was in the range 95–98%. The concentration of dissolved lignin in acetone/water solutions was determined after dilution with 1% (w/w) NaOH solution (dilution factor between 10^2 and 10^3), from the UV spectra based on calibration curves build up for each lignin at the following wavelength: 290 nm KSL and OHL, 287 nm for AWL and SGWL, and 285 nm SWL.

The lignin solubility factor in the different solvents used was calculated as the ratio between the amount of lignin in solution (from UV data) and the total lignin content in the unfractionated lignin sample.

2.2.2. FT-IR spectroscopy

Fourier Transform Infrared (FT-IR) spectra of the solid lignin samples were obtained in attenuated total reflectance (ATR) mode on a Varian Scimitar 1000 FT-IR spectrometer equipped with a DTSG-detector PIKE MIRacle ATR equipped with a diamond w/ZnSe lens single reflection plate. Spectra were collected in the range 4000–650 cm⁻¹ with a resolution of 4 cm⁻¹ and with 128 co-added scans.

2.2.3. Alkaline size exclusion chromatography (SEC)

The molar mass distribution of lignins was analysed by alkaline SEC using a TSK gel Toyopearl HW-55F column, 0.5 M NaOH as eluent, UV detection at 280 nm and calibration with sodium-polystyrene sulfonates, according to the procedure as described elsewhere (Gosselink et al., 2010). Mp (peak molecular weight), Mn (number average molecular weight), and Mw (weight-average molecular weight) and dispersity (PD, Mw/Mn) were calculated.

2.2.4. Quantitative ³¹P NMR

³¹P NMR spectra of lignin samples were recorded on a Bruker Avance II 400 MHz spectrometer, after derivatisation with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, using the procedure described by Gosselink (Gosselink et al., 2010). Signal assignment was performed as described by Granata and Agryropoulos (Granata and Agryropoulos, 1995).

2.2.5. Calculation of solubility parameters for lignin and solvents

(a) The Hildebrand solubility parameter (δ) of lignins was calculated from the energy of vaporisation (E) and the molar volume (V) of the phenylpropane repeating units of the polymer (Hildebrand and Scott, 1950), using Eq. (1).

$$\delta = \left(\frac{E}{V} \right)^{1/2} \quad (1)$$

The energy of vaporisation and the molar volume were calculated as $E = \sum \Delta e_i$ and $V = \sum \Delta v_i$, where Δe_i and Δv_i are the additive atomic and functional group contributions (Ni and Hu, 1995; Quesada-Medina et al., 2010). The calculation of E and V for the S, G and H units is given in Table S1 in Supporting information. The δ -values of each lignin sample (Table 1) were computed taking into account the molar ratio of S, G and H units determined from ³¹P NMR data.

(b) The solubility parameter δ of acetone–water mixtures and ethyl acetate was calculated as described elsewhere (Schuerch, 1952). The computation of solvent δ -values is resumed in Table S2, Supporting information. The δ -values of solvents are given in Table 1.

2.2.6. Chemometrics

Principal component analysis (PCA) and partial least squares regression analysis (PLS modelling) were performed with Unscrambler® 7.6 (Camo, Norway). PCA was applied in this work to gain information about dissimilarity between lignin fractions. PLS was applied to correlate FT-IR spectroscopic data with the chemical composition of lignin fractions determined by ³¹P NMR and to build up a calibration model enabling the prediction of important chemical parameters useful for the characterisation of lignin. PCA model was validated using cross-validation and the PLS model was validated using a new set of data, i.e. a validation set.

3. Results and discussion

3.1. Selective extraction of lignin in solvents with increasing polarity

3.1.1. Lignin samples

Five technical lignins from hardwood, softwood, grasses and wheat straw obtained by different isolation technology (e.g. organosolv, kraft and soda) were extracted with solvents with increasing polarity. The lignins studied have molecular masses in the medium to high range (entry 1 in Table 2) between 4200 g/mol (OHL) and 6700 g/mol (AWL). Average molecular weights (Mw) and dispersity (PD) increase from OHL < SGWL < KSL < SWL < AVL. Functional group content determined by ³¹P NMR revealed differences between the lignins (Table S3, Supportive information). The hardwood lignin sample OHL has the composition of a GSH – type lignin, with a high content of S (38.7%), G (25.8%) and condensed phenolic OH (28%), and little amount of H (7.4%) and bound carboxyl (entry 1). KSL (G-type) is composed mainly of G (54.3%) and low amounts of H (6.4%), with high condensed phenolic-OH (39.3%) and aliphatic OH and low content of carboxyl (entry 33). Syringyl content of KSL was below the detection limit of the method, and was set to 0. The samples SGWL (entry 10), SWL (entry 22) and AVL (entry 30) are also GSH-type, but differ from sample OHL by lower syringyl content (29–30.5%), and a higher content of H and carboxyl.

The chemical and structure characteristics of the lignin samples are well identified in the FT-IR spectra (Fig. 1). The spectra contain all lignin characteristic peaks that are well described in literature (Bermello et al., 2002; Casas et al., 2012; Popescu et al., 2007; Rumyantseva et al., 1994). Analysis of the spectra identifies the spectral features related to the differences induced by the type of lignin and isolation process. Important differences are:

In the range below 900 cm⁻¹, characteristic for the multiple substitution of the aromatic ring, we identify for KSL two peaks at 815 cm⁻¹ and 850 cm⁻¹, assigned to G rings and only one overlapping peak at 835 cm⁻¹ in the spectrum of the GSH type lignins which is more intense for OHL (with an S/G ratio of 1.5) than for grass lignins, with an S/G of about 0.8. Differences in the total amount of free aromatic OH are visible at 1365 cm⁻¹, while

Table 1
Calculated values of the Hildebrand solubility parameter (δ -value) of lignins and solvents.

Solubility parameter of lignins					Solubility parameter of solvents	
Lignin	Molar ratio ^a S:G:H	$\sum \Delta e_i$ (cal/mol)	$\sum \Delta v_i$ (cm ³ /mol)	Solubility parameter (δ) (cal/cm ³) ^{1/2}	Acetone/Water (v/v)	Solubility parameter (δ) (cal/cm ³) ^{1/2}
OHL	5.35:3.5:1	245,849.5	1195.1	14.34	90/10	12.01
SGWL	1.2:1.5:1	84,545.5	435.8	13.93	70/30	15.75
SWL	1.8:2.2:1	116,824.8	603.2	13.92	50/50	18.80
AWL	2.1:2.6:1	132,011.7	681.8	13.88	30/70	21.11
KSL	0:8.5:1	193,897.8	1052.2	13.58	10/90	22.75
					Ethyl acetate	7.62

^a The ratio S:G:H of the different lignins has been calculated based on the hydroxyl group content of the phosphorylated lignins as determined by ³¹P NMR analysis (Table 3).

Table 2
Weight-average molecular weight (Mw) and dispersity (PD = Mw/Mn) of lignins and lignin fractions.

Nr.	Fractions		OHL		SGWL		SWL		AWL		KSL	
			Mw/ratio Mw(f)/Mw(u)	PD	Mw/ratio Mw(f)/Mw(u)	PD	Mw/ratio Mw(f)/Mw(u)	PD	Mw/ratio Mw(f)/Mw(u)	PD	Mw/ratio Mw(f)/Mw(u)	PD
1	Unfractionated		4217	7	4622	7.7	5671	6.8	6638	7.4	4879	8.4
2a	Acetone/Water	Soluble	ND	ND	3035/0.6	5.9	ND	ND	ND	ND	3553/0.7	7.9
2b	10:90	Insoluble	4539/1.1	6.9	4984/1.1	7.2	6762/1.2	7.5	6879/1.0	7.1	5872/1.2	7.3
3a	Acetone/Water	Soluble	1171/0.3	3.6	1857/0.4	2.5	1825/0.3	3.5	1761/0.3	4.6	2244/0.5	2.8
3b	30:70	Insoluble	5175/1.2	6.4	6555/1.4	6.6	8101/1.4	6.8	8030/1.2	6.5	9012/1.5	8.6
4a	Acetone/Water	Soluble	2010/0.5	4.6	3325/0.7	3.3	3777/0.7	6.4	3123/0.5	4.8	4279/0.9	3.5
4b	50:50	Insoluble	7624/1.8	6.5	14,768/4.2	7.4	17,173/3.0	9.0	12792/1.9	6.6	5260/1.1	8.6
5a	Acetone/Water	Soluble	3738/0.9	6.5	3936/0.9	7.1	4434/0.8	3.4	5451/0.8	7.0	4435/0.91	3.8
5b	70:30	Insoluble	ND	ND	16,979/3.7	9.7	10,795/1.9	7.5	15,632/2.4	6.3	6338/1.3	5.9
6a	Acetone/Water	Soluble	3974/0.9	6.7	3937/0.9	6.7	4843/0.9	6.3	5811/0.9	7.1	3601/0.7	3.7
6b	90:10	Insoluble	ND	ND	15,971/3.5	8.1	13554/2.4	6.9	14166/2.1	5.6	14342/2.94	7.3
7a	Ethyl Acetate	Soluble	1868/0.4	4.4	1318/0.3	2.6	1324/0.2	3.6	1714/0.3	4.8	1369/0.3	2.0
7b		Insoluble	6910/1.6	5.8	5951/1.3	5.5	6485/1.1	5.5	7472/1.1	5.7	4944/1.0	7.9

ND, not determined;

Mw(f)/Mw(u) = the ratio between the Mw of an isolated fraction and the Mw of the unfractionated parent lignin sample.

the decrease of the aliphatic-OH is illustrated by the absorption at 1030 cm^{-1} . A significant difference is also visible in the area 1658 cm^{-1} and 1710 cm^{-1} , specific for the conjugated and non-conjugated C=O, respectively. A clear absorption at 1658 cm^{-1} is observed in the spectra of samples KSL and SWL, while the AWL has a clear peak at 1710 cm^{-1} , associated to non-condensed C=O. Strong absorption band is found at 1080 cm^{-1} (AWL spectrum) that is assigned to aliphatic ether groups.

3.1.2. Yield of LMW and HMW lignin fractions

Ethyl acetate and acetone/water solutions containing 10, 30, 50, 70 and 90% (v/v) acetone were used to fractionate the five technical lignins selected in this study. The solvents selected covered a broad range of solubility parameters, with δ -values from 7.62 to 22.7 (cal/cm^3)^{1/2} (Table 1). The application of acetone/water mixtures and ethyl acetate for lignin extraction has been described before (Quesada-Medina et al., 2010; Schuerch, 1952; Thring and Griffin, 1994; Thring et al., 1996), but not for the set of technical lignins and the conditions described in this study. All lignins showed high

solubility in acetone–water solutions containing above 50% acetone (v/v) and yields of soluble fractions higher than 80% were obtained (Fig. 2). However, significant differences are observed for ethyl acetate and acetone–water solutions of 10 and 30% acetone. The yield increased from 10–12% in acetone–water 10:90 to 62% for KSL and 32–40% for OHL and grass/straw lignins in 30:70 acetone–water. Except KSL, the hardwood and grass lignins showed relatively high solubility in ethyl acetate that allowed yields of soluble lignin of 20% for AWL and SWL, 29% for SGWL and 56% for OHL.

The differences observed in the behaviour of the lignins in the solvents tested can be partly explained by the solubility parameter (δ -value) theory of Hildebrand and Scott (Hildebrand and Scott, 1950) that states that polymers are soluble in solvents with solubility parameters close to their own. Fig. 3 shows the solubility factor of the tested lignins as a function of the difference between the solubility parameters of the lignin and the solvent ($\delta_{\text{lignin}} - \delta_{\text{solvent}}$), further referred to as Delta- δ .

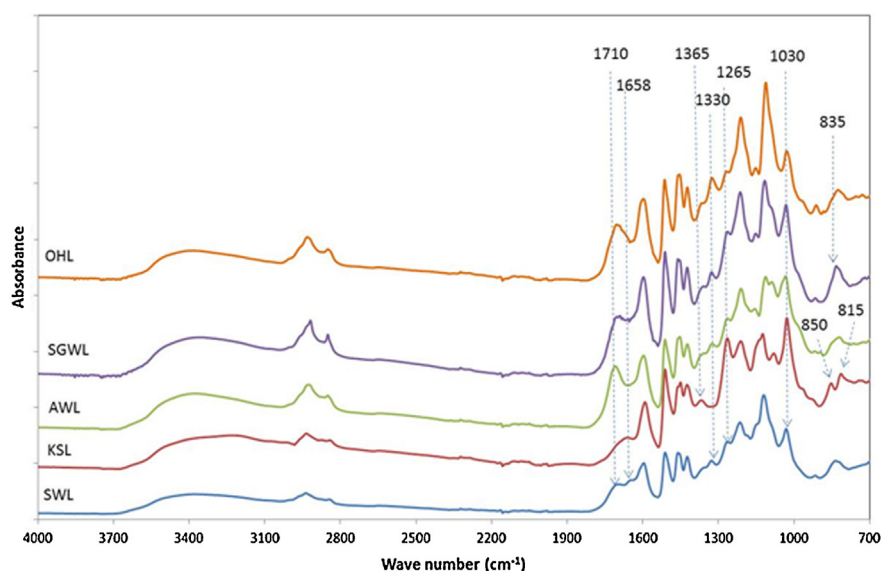


Fig. 1. FT-IR spectra of tested lignins.

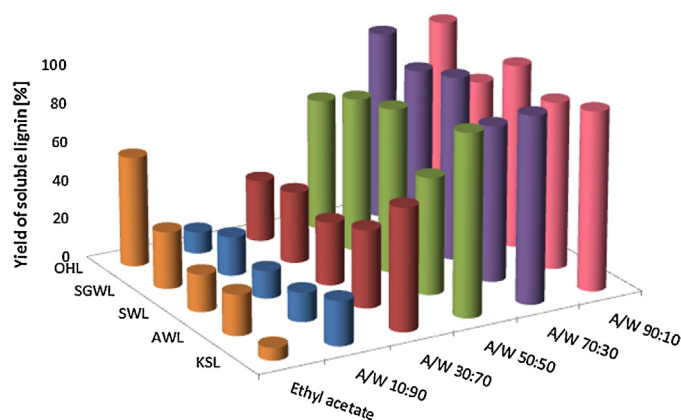


Fig. 2. Yield of lignin fractions soluble in acetone/water mixtures and ethyl acetate.

Although all lignins follow the Hildebrand theory, there are significant differences between lignins that can be related to the type, (i.e. GSW vs G) and source of lignin and the isolation process. The Kraft softwood lignin KSL and the grass lignins SGWL, SWL and AWL show an almost Gaussian solubility pattern and reach maximum solubility in the $\Delta\delta$ range from -5 to $+2$ (cal/cm^3)^{1/2}, corresponding to acetone solutions with 50–90% (v/v). This behaviour can be related to the high content of the polar carboxyl and sulfide groups in grass and KSL lignins, respectively, that enhances the hydrogen bonding and polar interaction.

The organosolv hardwood lignin OHL, more hydrophobic and with low content of carboxyl groups, attains maximum solubility in a narrow $\Delta\delta$ range from -2 to $+2$ (cal/cm^3)^{1/2} and shows high solubility values in ethyl acetate ($\Delta\delta = 6.7$ (cal/cm^3)^{1/2}). Grass lignins SGWL, SWL and AWL show also relatively high solubility factor in ethyl acetate (i.e. 0.3, 0.2 and 0.4, respectively, while KSL has a very low solubility factor of 0.1 in ethyl acetate).

These results confirm earlier studies showing that the solubility of lignin in organic solvents is a very complex phenomenon that is not yet fully understood. Application of the solubility parameter gives a good indication about lignin–solvent interactions and

facilitates the choice of solvents. However, the use of Hansen parameter (Hansen, 1967), that extends the uni-dimensional Hildebrand parameter with dispersion, polar and hydrogen binding might give a better understanding of lignin–solvent interactions, as suggested by Stefanis and Panayiotou (2008) and Vebber et al. (2014).

3.2. Molecular, spectroscopic and chemical characterisation of isolated lignin fractions

The soluble and insoluble fractions isolated by selective extraction with acetone–water mixtures and ethyl acetate, respectively, were characterised by alkaline-SEC ³¹P NMR, to determine the molecular weight distribution and the functional group content.

3.2.1. Molecular weight distribution and dispersity of lignin fractions

Extraction of technical lignin materials with both ethyl acetate and acetone/water solutions results in the separation of lignin fractions with more defined molecular weight distribution and lower dispersity (Table 2).

Fractions extracted from SGWL, SWL and KSL in acetone/water 50/50 as well as fractions extracted from all lignins in acetone/water mixtures 70/30 and 90/10 (v/v) have average molecular weight close to the starting lignins, but lower dispersity (Table 2, entries 4a, 5a, 6a). These soluble fractions are medium-size oligomers containing 20–30 monomers, and they represent the major fraction, being obtained in high yields (see Section 3.1.2). The minor fractions that are insoluble in acetone–water mixtures of 50, 70 and 90% acetone have very high molecular weight (i.e. up to 17,000 g/mol, Table 2, entries 4b, 5b and 6b), corresponding to a 2 to 4.2 fold increase of the Mw, as compared with the parent unfractionated lignins. These high molecular weight (HMW) insoluble fractions are higher oligomers, with a degree of polymerisation “n” ranging between 30 and 100. The fractions soluble in acetone/water 50/50 (v/v) isolated from lignins AWL and OHL have lower Mw and PD (i.e. AWL: 3120 g/mol and 4.8, OHL: 2010 g/mol and 4.6, respectively; entry 4a, Table 2) than the fractions soluble in

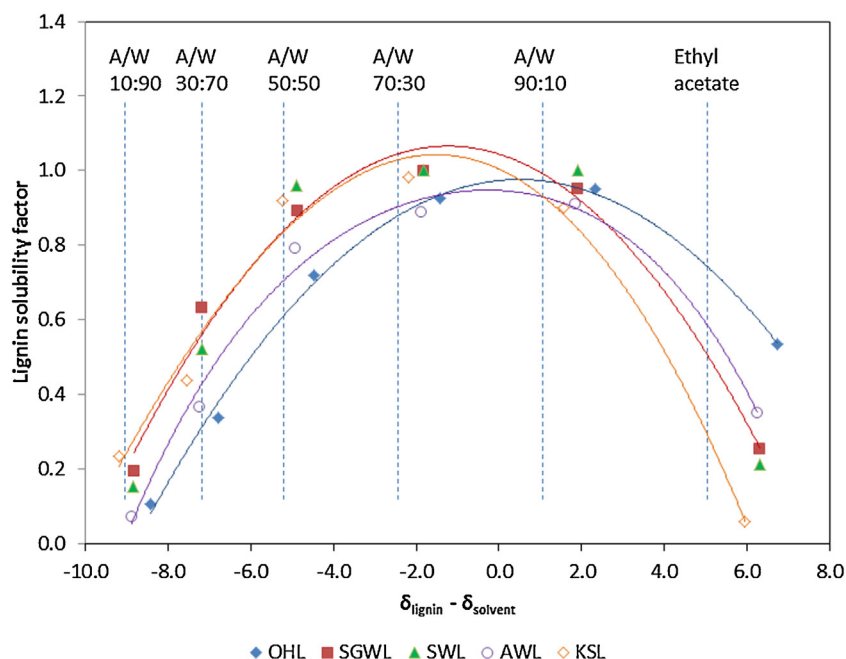


Fig. 3. Lignin solubility factor in acetone/water mixtures and ethyl acetate, as a function of the difference between the average solubility parameter of lignins and the solubility parameter of each solvent tested.

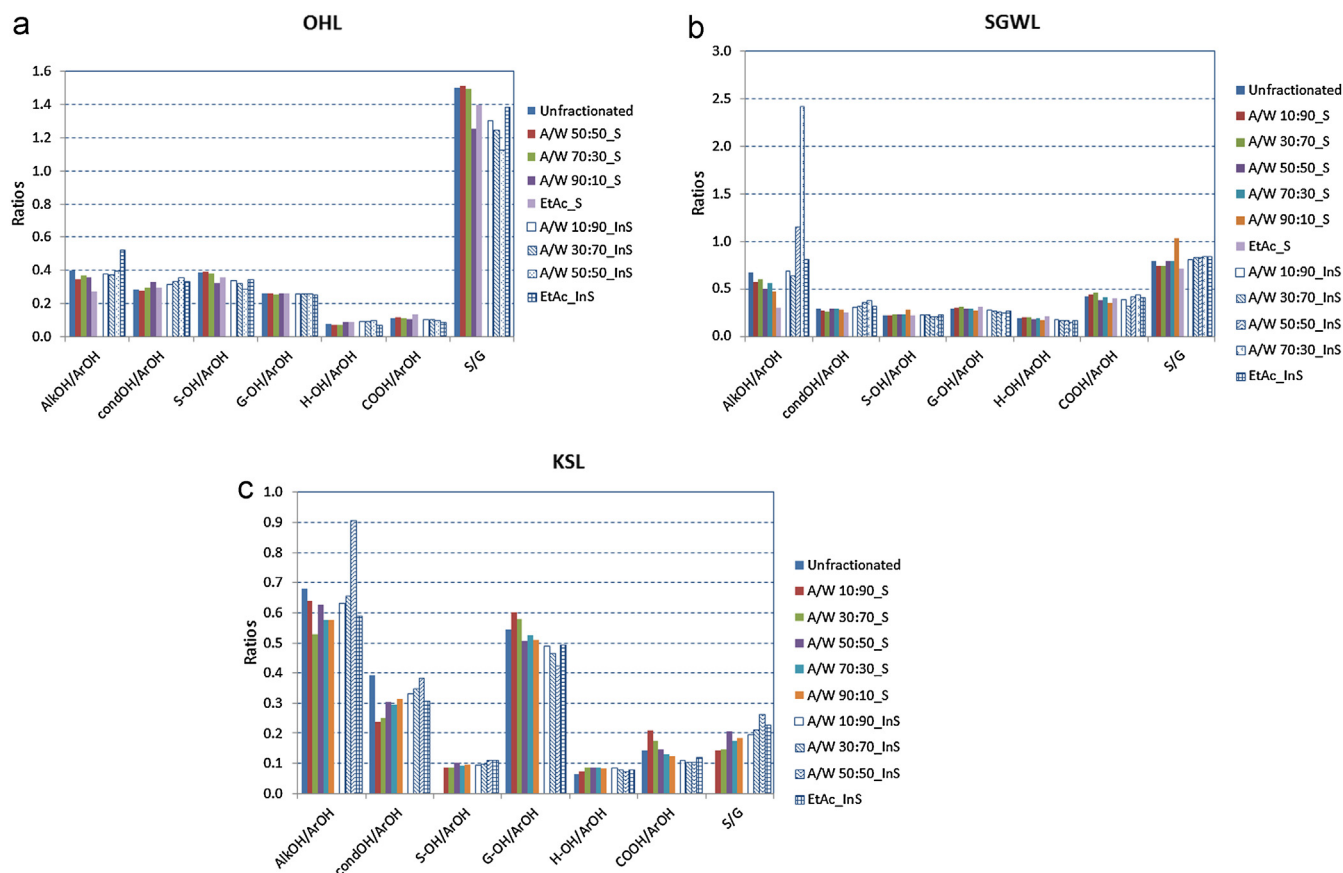


Fig. 4. Comparative analysis of some relevant chemical characteristics of soluble and insoluble isolated fractions of OHL (a), SGWL (b) and KSL (c) lignins.

acetone/water 70/30 and 90/10 (v/v), corresponding to oligomers with $10 < n < 20$. The related insoluble fractions have high Mw and lower PD in the same range as the insoluble fractions isolated from acetone/water mixtures 70/30 and 90/10 (v/v).

Distinct fractions with low molecular weight and low dispersity are obtained from all lignin extracts with ethyl acetate ($1300 < Mw < 1900$, $2.0 < PD < 4.8$, entry 7a in Table 2) and acetone/water 30/70 (v/v) ($1100 < Mw < 2200$, $2.5 < PD < 4.6$, entry 3a in Table 2). These soluble LMW lignin fractions are low aromatic oligomers, ranging from hexamers to dodecamers. The fractions insoluble in ethyl acetate and 10% acetone/water mixtures have slightly higher molecular weight than the parent lignins (i.e. ratio $Mw(f)/Mw(u)$ between 1 and 1.3 except for acetate insoluble OHL fraction, with $Mw(f)/Mw(u) = 1.6$) and lower dispersity (Table 2, entry 7b).

Surprisingly, soluble lignin fractions extracted with acetone/water 10/90 (v/v) show medium-high Mw and relatively high dispersity (3035 g/mol and 5.9 for SGWL fraction; 3553 g/mol and 7.9 for KSL fraction; entry 2a, Table 2). Since only lignin and/or phenolic compounds are detected in SEC analysis with detection at 280 nm, we assumed that these soluble lignin fractions consist of lignin–carbohydrate complex. This assumption was confirmed by FT-IR and ^{31}P NMR analysis, as it will be discussed later. The largest fraction separated with acetone/water 10/90 (v/v) consists of insoluble lignin with Mw and dispersity in the same range as the native, unfractionated lignin samples (entry 2b, Table 2).

3.2.2. Functional group composition of soluble and insoluble lignin fractions

42 of the 56 lignin samples obtained by selective extraction of the five technical lignins with ethyl acetate and acetone/water

mixtures were analysed by quantitative ^{31}P NMR analysis and the concentration of the following functional groups, expressed in mmol/g, were determined: aliphatic hydroxyl (AlkOH), condensed hydroxyl (CondOH), syringyl-OH (S), guaiacyl-OH (G), p-hydroxyphenyl-OH (H), carboxyl (COOH) and total aromatic-OH (ArOH). The results of the analysis are given *in extenso* in Table S3 (Supportive information). A significant increase in the S-content of KSL was observed after fractionation with acetone/water mixtures and ethyl acetate. Although the S content of unfractionated KSL lignin is almost zero (i.e. below the limit of detection of the method) and the general acceptance that the syringyl content of lignin in typical softwoods is less than 0.1% of the total phenylpropane units (Obst and Landucci, 1986), in this work we observe concentration of S in the range of 10% of the total ArOH-content of the samples in all soluble and insoluble fractions extracted from KSL lignin. We associate this with the higher purity of the extracted samples.

Differences are observed between the functional group composition of the unfractionated lignins and the soluble and insoluble fractions obtained after solvent extraction. To facilitate the discussion, the relative distribution of functional groups in lignin was calculated as the ratio of the (functional group content) and ArOH, i.e. $AlkOH/ArOH$, $S/ArOH$, $G/ArOH$, $H/ArOH$, $COOH/ArOH$. The relative functional group composition and the S/G ratio for three lignins and their fractions, i.e. OHL, SGWL (as representative of grass lignins) and KSL are given in Fig. 4a–c, respectively.

Data in Fig. 4 allows to identify the following trends:

Soluble lignin fractions:

- All soluble lignin fractions have lower values of the $AlkOH/ArOH$ ratio than the parent lignins; the ratio $AlkOH/ArOH$ decreases

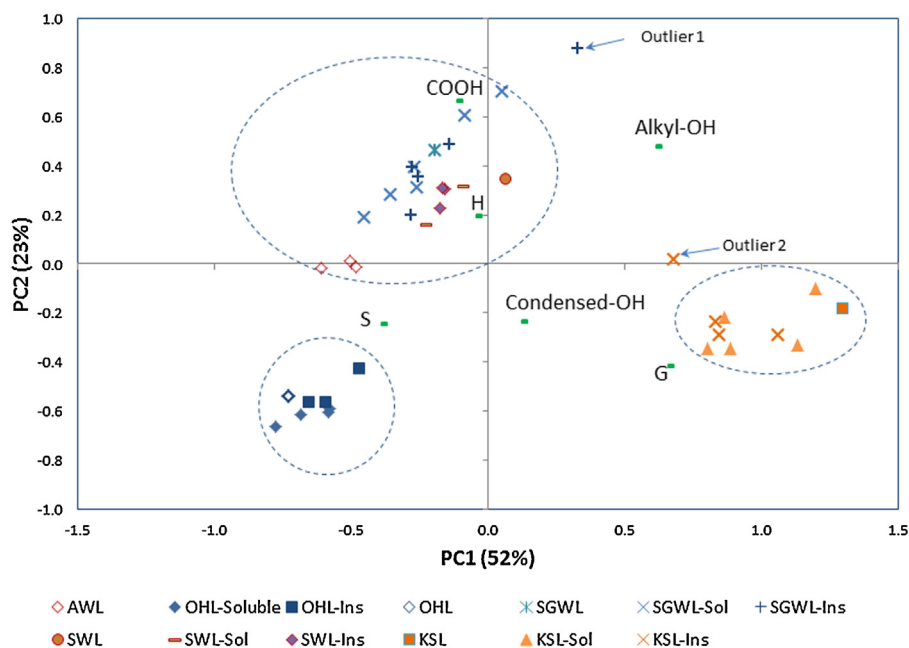


Fig. 5. Plot of scores (samples) and loadings (^{31}P NMR chemical data) on principal component 1 (PC 1) and 2 (PC 2).

with the decrease of solvents' polarity and hydrogen bonding capacity.

- Soluble lignin fractions have lower values of the CondOH/ArOH ratio than parent lignins, except for OHL fractions soluble in ethyl acetate and acetone/water 70:30 and 90:10; the ratio increases with decrease of solvents' polarity. This latest effect is more pronounced for KSL soluble fractions.
- Lignin fractions soluble in acetone/water 10:90 and 30:70 have a higher COOH/ArOH ratio as compared to parent lignins, and the ratio decreases with the decrease of solvent polarity. This effect is more pronounced for KSL fractions (Fig. 4c). KSL fractions soluble in acetone/water 10:90 and 30:70 have higher COOH/ArOH ratio than the unfractionated KSL lignin; the ratio decreases continuously with the decrease of solvent polarity, being the lowest for ethyl acetate soluble fraction.
- Soluble fractions isolated from AOHL and SGWL have in general lower S/G ratio than the unfractionated lignins; S/G increases with the decrease of solvent polarity. This latest effect is more obvious for KSL fractions.

Insoluble lignin fractions:

- All insoluble lignin fractions have higher AlkOH/ArOH, CondOH/ArOH and S/G ratios than unfractionated lignin, which

increase with the decrease of solvent's polarity. The COOH/ArOH ratio is usually lower.

We can conclude that, in general, the low molecular weight (LWW) soluble lignin fractions extracted by ethyl acetate and acetone/water mixtures with 10 and 30% acetone have a lower content of condensed-OH and aliphatic-OH groups and have consequently a less condensed structure than the parent lignins. On the contrary, the high molecular weight (HMW) soluble fractions and all insoluble fractions have higher content of condensed-OH and aliphatic-OH and have a more condensed structure.

3.3. Modelling lignin heterogeneity

Principal component analysis (PCA) was used to find the similarities and dissimilarities between the soluble fractions isolated at different conditions and the unfractionated lignin using the functional group composition determined from quantitative ^{31}P NMR data. A model with three principal components (PC) was developed, with the first 2 PC explaining 75% of the total variance (Fig. 5). Each principal component is associated to a set of "loadings" which are directly related to the contributing chemical data (i.e. functional group content, X-variables). Each sample is "scored" according to their degree of correlation with the loading of each

Table 3

Results of partial least squares (PLS) regression between chemical composition of lignins determined by ^{31}P NMR and absorbance ratios of relevant FT-IR bands.

Statistical data	Alkyl-OH	Condensed-OH	S	G	H	COOH	Aryl-OH	S/G
Calibration model								
Calibration set: 28 samples, namely: 1, 2, 5, 7, 8, 10, 13–15, 16, 18, 20, 22–25, 26, 28, 29, 30, 32, 33, 35, 36, 39–42								
Range	0.91–2.83	0.45–1.19	0.25–1.2	0.29–2.23	0.18–0.84	0.2–1.62	1.17–3.99	0.15–1.5
Calibration								
R_{calib}	0.91	0.88	0.91	0.96	0.87	0.93	0.92	0.95
RMSEP _{calib}	0.197	0.081	0.124	0.135	0.071	0.138	0.238	0.138
Validation								
Validation set: 14 samples, namely 3, 4, 6, 9, 11, 12, 14, 17, 21, 27, 34, 37, 38, 41								
Range	1.12–2.26	0.76–1.16	0.30–1.30	0.64–2.13	0.19–0.65	0.23–1.43	2.32–3.54	0.14–1.51
R_{valid}	0.9	0.94	0.92	0.96	0.92	0.94	0.93	0.94
RMSEP _{valid}	0.149	0.047	0.136	0.143	0.051	0.128	0.178	0.167

R_{calib} , R_{valid} and R_{pred} are the correlation coefficients for the calibration, cross-validation and prediction, respectively; RMSEP_{calib}, RMSEP_{valid} and RMSEP_{pred} are the root mean square error of prediction for calibration, validation and prediction, respectively; PLS 2 models: X-variables: FT-IR spectral data; range: 765–1832 cm^{-1} and 2752–3684 cm^{-1} . Y-variables: Alkyl-OH, Condensed-OH, S, G, H, –COOH, Aryl-OH and S/G.

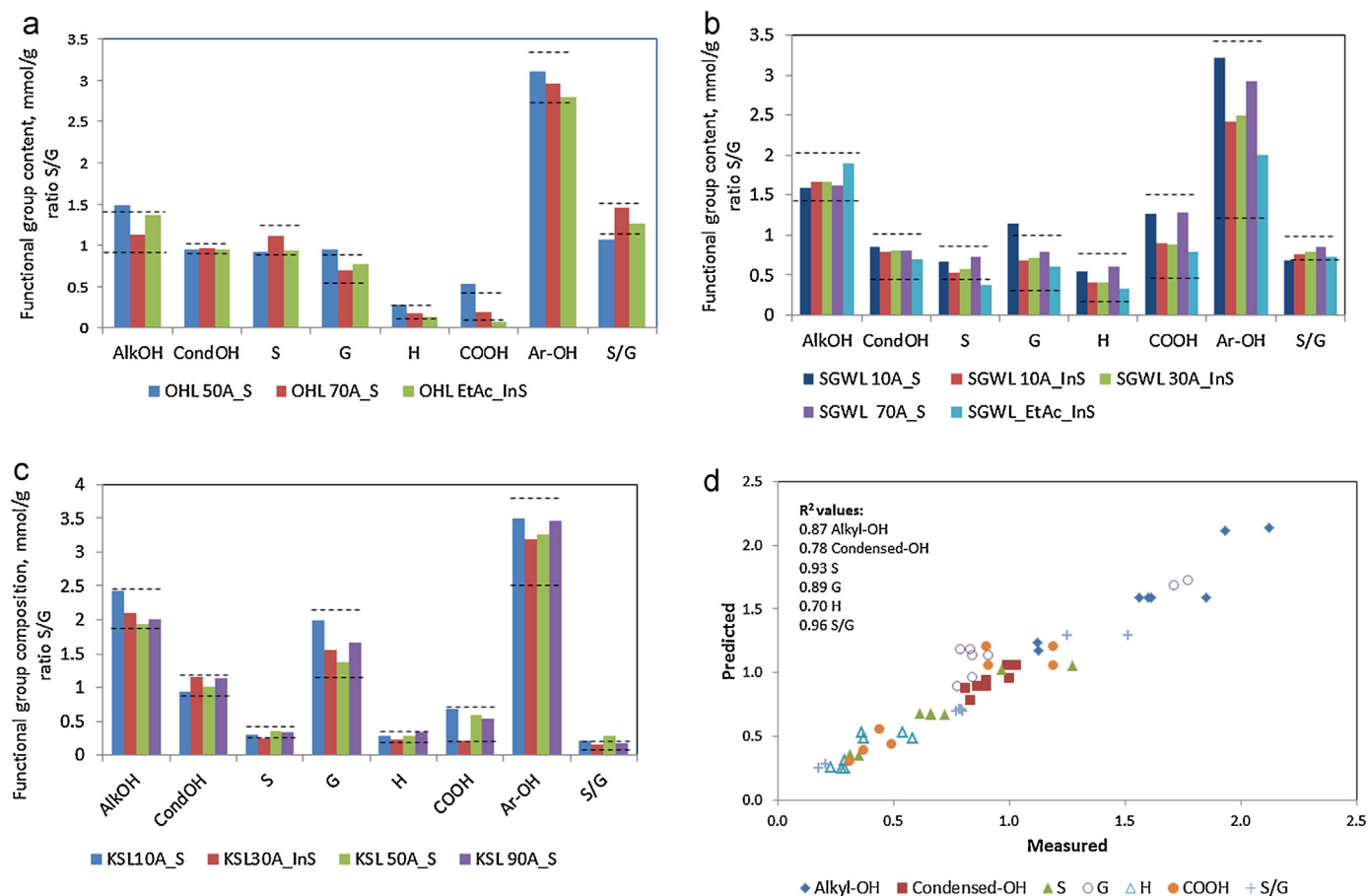


Fig. 6. (a) PLS-Predicted values for chemical composition of a set of 12 lignin soluble and insoluble fractions; (b) Regression lines for alkyl-OH, condensed-OH, p-hydroxyphenyl-OH (H), Syringyl-OH (S), guaiacyl-OH (G), -COOH, aryl-OH and S/G.

principal component. Therefore data that share similar score values will cluster together on the score plot, showing that they possess similar spectral features, similar chemical composition and hence similar structure (Fig. 5).

PC1 is associated with high positive loading for G unit and negative loadings for S unit, while PC2 is dominated on the positive site by carboxyl content and H units. Aliphatic-OH has equal positive loadings on both PC1 and PC2.

The lignin fractions are grouped in three distinct clusters corresponding to the type of lignin. One cluster is represented by all KSL samples, with high G content and low S and carboxyl. The second cluster consists of all OHL samples, with high S content. All grass lignins are clustered together, with high COOH, H and S content and low G. In each cluster, however, the soluble and insoluble samples are broadly distributed along the PCs and this shows the high heterogeneity of the technical lignins currently available. Two fractions are identified as outliers, namely the SGWL insoluble fraction in acetone/water 70:30 (outlier 1) and KSL insoluble fraction in acetone/water 50:50 (outlier 2), which have a very high content of aliphatic hydroxyl. These results bring clear arguments in favour of selective fractionation to produce less heterogeneous lignins for industrial application.

3.4. Quantitative prediction of functional group content of lignin

^{31}P NMR is a very accurate method to determine the functional group content of lignin, however it is laborious and expensive and requires trained technicians and hi-tech equipment. On the

contrary, infrared analysis is a fast, simple and commonly available technique for analysis of organic compounds which has been proven to be useful for quantitative analysis of various compounds in combination with multivariate analysis methods. FT-IR spectra of all isolated soluble and insoluble lignin samples were recorded (data not shown). The spectra showed all the characteristic features of lignin and allowed to identify marked differences between soluble and insoluble fractions as well as between the soluble fractions isolated from the different solvents with increasing polarity used in this study. An inspection of the spectra indicated a good correlation between some critical parameters for lignin composition like S/G ratio determined from the intensity of specific absorptions in the FT-IR spectra and the values determined from ^{31}P NMR analysis (not shown). This was the basis for exploring the potential of relating FT-IR spectra of lignins with their functional group composition determined by ^{31}P NMR. PLS calibration models based on FT-IR spectral data of lignins have been developed for the quantification of lignin content and chemical composition (i.e. phenolic and carboxyl group content) determined by wet analysis (Boeriu et al., 2004), but not for the functional group content determined by ^{31}P NMR. In this study we report for the first time the potential to quantitatively correlate FT-IR spectral data and ^{31}P NMR-determined chemical composition of lignins using chemometrics. The target was to develop and validate a PLS model to predict the following chemical features of lignins: aliphatic and aromatic hydroxyl content (i.e. Alkyl-OH, condensed-OH, S, G and H), carboxyl content (COOH), total aromatic hydroxyl (Aryl-OH) and ratio S/G based on significant FT-IR spectral region. A PLS calibration

model was built using a calibration set consisting of 28 lignin fractions selected to span the whole range of variation for each chemical variable, and the model was validated using a validation set of 14 samples. The composition of the calibration and validation sets and the range of variation of chemical variables (*y*-variables) are given in Table 3. After optimisation, that included removal of non-significant spectral regions, the model was built using truncated spectra covering the frequency range between 765–1832 cm^{-1} and 2752–3684 cm^{-1} . The statistical results of the PLS calibration model are given in Table 3.

The model used 6 principal components to explain 85% of the *y*-variance. The predictive error of the models, expressed as RMSEP, is analytical acceptable for the prediction of the target chemical data. To illustrate the predictive ability of the model, a new set of samples was constructed, consisting of 19 samples, of which 6 samples were duplicates of samples 4, 6, 15, 17, 38 and 40 in Table S3 (Supporting information), which were isolated and characterised in a separate experiment. The other 13 samples included in this prediction set as “unknown” were fractions isolated in the same experiment with fractions included in Table 3, that have been characterised by SEC (Table 2) and FT-IR, but not measured by ^{31}P NMR. For all samples except three (i.e. fractions of SGWL, SWL and KSL insoluble in ethyl acetate, 70:30 acetone–water and 90:10 acetone–water, respectively) the values of the chemical parameters were predicted by the PLS model with low standard deviations. The predicted values of the chemical parameters of the soluble and insoluble fractions isolated from OHL, SGWL and KSL are given in Fig. 6a–c, respectively. The predicted values are in the range of variation of the chemical parameters for the set of lignin fractions measured, as graphically indicated in the figures. For samples 4, 6, 15, 17, 38 and 40, the predicted values of the chemical parameters were very close to the values experimentally calculated from ^{31}P NMR spectra. This is clearly indicated by the regression lines and regression coefficients of the predicted vs. measured values, given in Fig. 6d.

These results indicate that the PLS models based on significant FT-IR spectral regions can be used for the quantitative characterisation of the chemical composition of lignin and lignin fractions separated by selective solvent extraction.

4. Conclusions

Selective extraction with green organic solvents is suited for fractionation of a range of industrial lignins according to their molecular mass since produces defined fractions with narrow molecular weight distribution and defined functional group content. Low molecular weight (LMW) lignin fractions with narrow dispersity are obtained by extraction with ethyl acetate and acetone–water solution containing 30 and 50% acetone, with yields depending on the type and the functional group content of lignins. Lignin–carbohydrate complexes can be isolated from technical lignins by extraction with acetone/water 10:90. Insoluble fractions with very high molecular weight (*M_w* between 10 and 17 kg/mol) are obtained in low yield from acetone–water solutions with 50, 70 and 90% acetone. LMW lignins are in general less condensed while the HMW fractions have a higher content of condensed hydroxyls. Principal component analysis on the chemical composition determined from ^{31}P NMR data showed the high heterogeneity of the technical lignins and give information about discrete changes in the functional group content by extraction. The PLS models developed allow an accurate estimation of the content of syringyl, aliphatic-OH and S/G ratio, and this creates the premises to use this approach for the simple, fast and accurate prediction of structural and chemical information on lignin using FT-IR and chemometrics.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.indcrop.2014.09.019>.

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