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Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy

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Abstract

Fourier-transformed infrared spectroscopy (FT-IR) was evaluated as an analytical technique for the estimation of the chemical composition and functional properties of lignin. A sample set containing various non-wood, hardwood and softwood lignins isolated by different processing technologies was used. The lignin samples were characterised by both conventional chemical analysis and non-destructive methods, such as diffuse reflectance FT-IR. Principal component analysis (PCA) based on the IR-fingerprint spectral region allowed classification of lignins according to origin and processing conditions.

The antioxidative properties of each lignin sample in both aqueous and micellar systems were determined. All lignin samples showed radical scavenging activity, with sisal and abaca lignin being the most effective radical scavengers. The radical scavenging efficiency of the most efficient lignin was about 20% of that of BHT and tocopherol (based on weight), compounds that are commonly used in food and cosmetic industries.

Multivariate analysis was applied to correlate chemical composition and antioxidative properties of lignins with the FT-IR spectral data. Partial least squares (PLS) models were able to predict the major components' concentrations and radical scavenging activity at the 99% confidence level presenting r^2 values higher than 0.80 in most cases.

Keywords: Lignin characterisation; FT-IR; Antioxidant; Multivariate analysis; Structure–function relationship

1. Introduction

Lignin is a phenolic polymer present in large amounts in the cell wall of plants, especially in woody tissues. In contrast to other biopolymers, lignin is a network polymer that results from the dehydrogenative radical polymerisation of monolignols (e.g. *p*-coumaryl-, coniferyl- and sinapyl-alcohols), which are connected via carbon–carbon and ether linkages. The relative amounts of the monolignol units differ

Abbreviations: ABAP, 2,2′-azobis-(2-amidinopropane)-dihydrochloride; ABTS, 2,2′-azinobis-(3-ethylbenzthiazoline)-6-sulfonic acid; BHT, butylated hydroxytoluene; EAI, emulsifying activity index; ESI, emulsifying stability index; PC, principal component; PCA, principal component analysis; PLS, partial least squares regression; RAA, relative analytical accuracy; RMSEP, root mean squared error of prediction; RSA_{ABTS}, radical scavenging activity, measured with the ABTS-peroxidase method; RSA_{ABAP}, radical scavenging activity, measured with the ABAP-linoleic acid method; SDS, sodium dodecylsulfate

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considerably between plants. In softwood lignin, the network is formed primarily by coniferyl moieties (95%), the rest consisting of *p*-coumaryl alcohol-type units and only trace amounts of sinapyl alcohol moieties, while in hardwood and dicotyl crops like hemp and flax, various ratios of coniferyl/sinapyl have been reported (Dence and Lin, 1992). Lignins derived from monocotyl plants (grasses and cereal straw) contain also significant amounts of *p*-coumaryl alcohol residues (Dence and Lin, 1992). Lignins contain several functional chemical groups, such as hydroxyl (phenolic or alcoholic), methoxyl, carbonyl and carboxyl, in various amounts, depending on origin and the applied isolation process (Gosselink et al., 2004; Sun et al., 2001). The distinct network structure as well as the presence of the various chemical substituents confers unique functional properties to lignins.

Lignins from trees, plants and agricultural crops with different chemical composition and properties can be obtained by use of several extraction methods. Commercial chemical pulping processes (sulfite and kraft process) produce lignosulfonates and kraft lignins as residue. Recently commercialized alkaline pulping–precipitation process supply sulphur-free, free-flowing lignins (Abächerli and Doppenberg, 1998). Other delignification technologies use an organic solvent (Cruz et al., 1999) or a high-pressure steam treatment. However, it is practically impossible to isolate pure lignin quantitatively from cell walls in an intact state. The lignin isolated by known methods (physical, chemical or enzymatic treatments) is a mixture of degraded or solubilized lignin from various unidentified morphological regions.

In the past decades, extensive research has been invested not only in the improvement of lignin extraction processes, but also in elucidation of the structure of lignins, characterisation of chemical reactivity and functional properties and development of new applications. It has been shown that lignin is a versatile molecule that possess multiple properties such as antioxidant (i.e. radical scavenger), UV-absorption, anti-fungal and antibiotic activity (Mai et al., 2000; Lu et al., 1998; Barclay et al., 1997). Besides the traditional use as energy source and in leather tanning, lignins are now applied for polymer reinforcement, or as pesticides, dispersants, emulsifiers or sequestrants (i.e. antioxidants). It has been suggested that lignins can be applied for stabilisation of food and feed, due to their antioxidant and antifungal properties. Also, anti-carcinogenic and antibiotic activities of lignins have been reported (Lu et al., 1998). When depolymerised, lignins can be used for the synthesis of valuable chemicals, such as vanillin.

To enhance the industrial use of lignins, there is need for a continuous supply of lignin products with constant quality as related to purity, chemical composition, and functional properties.

Both process control and product quality control require fast physical methods for the analysis of lignins. Fourier-transform infrared spectroscopy (FT-IR) has been shown to be a powerful tool for the investigation of plant cell wall polymers, such as polysaccharides and lignins (Faix, 1992; Séné et al., 1995; Boeriu et al., 1998; Xiao et al., 2001).

The aim of this study was to investigate the potential of Fourier-transform infrared spectroscopy as a probe for the chemical and functional characterisation of lignins. Also, we report on the application of FT-IR to discriminate between lignins of different origin and extracted according to different processes.

2. Materials and methods

2.1. Materials

Lignins from wheat straw, sisal, abaca, hemp, jute and flax were obtained from Granit SA (Lausanne, Switzerland). Alcell^{TM} organosolv lignin from mixed hardwoods (maple, birch and poplar) was obtained from Repap Technologies Inc. (Valley Forge, PA, USA). Lignosulfonate from softwood (Borresperse 3A and WAFEX-P) and Kraft lignin from softwood (Curan 100 and Curan 2711P) were obtained from Lignotech (Sweden). Indulin AT, a Kraft lignin from softwood, was obtained from Westvaco (USA). Sulphur free lignins from softwood were obtained from Kiram AB (Sweden). Solutions of lignin (0.1%) were prepared in water by adjusting the pH to alkaline values (pH = 11) with 1 M NaOH.

Commercially available antioxidants butylated hydroxy-toluene (BHT) and α-tocopherol, 2,2′-azino-bis-(3-ethylbenzthiazoline)-6-sulfonic acid (AB-TS), 2,2′-azobis-(2-amidinopropane)-dihydrochloride (ABAP), linoleic acid 99%, and horseradish peroxidase (type II, 250 U/mg) were from Sigma-Aldrich

Chemie (Zwijndrecht, The Netherlands). Sodium dodecylsulfate (SDS), hydrogen peroxide 30%, sodium monohydrogen phosphate and sodium dihydrogen phosphate were from Merck (Darmstadt, Germany). Tween 20 was obtained from Brunschwick Scientific (Suffolk, UK). Sunflower oil (commercial) was purified with silicagel in order to remove its additives.

2.2. Analytical methods

2.2.1. Chemical composition (Gosselink et al., 2004)

Three hundred and seventy-five milligram lignin was hydrolysed with 3.75 ml ice-cooled 12 mol l^{-1} sulphuric acid during 1 h at 30 °C. After dilution with 36.25 ml demineralised water and 5 ml solution of inositol in demineralised water at 5 g l^{-1} (internal standard), hydrolysis was performed during 3 h in boiling water. After cooling in ice-water, the hydrolysate was centrifuged for 15 min at 4000 rpm. Acid insoluble lignin was determined after filtration and hot water washing over a G4 glass filter crucible (TAPPI T249 om-94, 1999; TAPPI T222 om-83, 1999). Acid soluble lignin was spectrophotometrically determined at 205 nm (TAPPI UM250 um-83, 1999). Sugar residues were determined as their corresponding alditol acetates after subsequent reduction and acetylation (TAPPI T249 om-94, 1999) using gas liquid chromatography with a flame ionisation detector on a CP-Sil 88 capillary column (Chrompack, Middelburg, The Netherlands). Uronic acids in the sulphuric acid hydrolysate were spectrophotometrically determined at a wavelength of 520 nm (Blumenkrantz and Asboe-Hanssen, 1973).

2.2.2. Phenolic hydroxyl and carboxylic groups: non-aqueous titration

The phenolic hydroxyl and carboxylic groups were determined by a non-aqueous potentiometric titration with tetra-*n*-butylammonium hydroxide (TnBAH) (Dence, 1992; Gosselink et al., 2004). 0.15 g lignin on dry weight basis and 0.02 g *p*-hydroxybenzoic acid as internal standard were used and the dissolving time of the lignins in DMF was extended to 15 min.

The ash content of lignin was gravimetrically determined after incineration at 525 °C for 8–12 h till black carbon particles have been disappeared (TAPPI T211 om-93, 1999).

2.2.3. FT-IR spectroscopy

IR spectra of lignins were obtained on a Bio-Rad Fourier transform infrared spectrometer (FTS-60 A) equipped with a MTC detector and diffuse reflectance (DRIFT) accessory. MID-IR spectra of lignin samples (5% (w/w) lignin in KBr) were recorded between 700 and 4000 cm^{-1} at a resolution of 4 cm^{-1} in the diffuse reflectance mode, using KBr as reference. Sixty-four Interferograms were co-added for a high signal to noise ratio. The spectra were baseline corrected for further analysis.

2.3. Free radical scavenging properties of lignins

2.3.1. Radical scavenging activity against ABTS radicals

The efficiency of lignin samples to scavenge the ABTS radical in watery solutions was determined at 20 °C using a method adapted from Cano et al. (1998). The ABTS radical was generated by peroxidase in the presence of hydrogen peroxide, in oxygen-free solutions. In a typical reaction, 1.50 ml of ABTS solution (1.5 mM, in 0.1 M citrate buffer pH 4.5) diluted to a total volume of 3.0 ml with 0.1 M citrate buffer pH 4.5 are mixed with 10 μl of hydrogen peroxide solution (100 mM). The reaction is started by injection of 75 μl of peroxidase solution (1 mg/ml). Complete generation of the ABTS radical (followed spectrophotometrically) occurs in about 10 min. The ABTS radical ($\lambda_{max} = 414$ nm, $\varepsilon_{414} = 31.1\ mM^{-1}\ cm^{-1}$) was stable for 1 day, in oxygen-free solution, under nitrogen. The disappearance of the ABTS radical in time as result of interaction with antioxidant lignins was followed spectrophotometrically at 414 nm with an UV-Vis Perkin-Elmer Lambda 2S spectrophotometer against a reference containing the same antioxidant (e.g. lignin) concentration as the reaction mixture. The standard reaction mixture was 100 μl of the ABTS radical solution diluted buffer 0.1 M citrate buffer, pH 4.5 to a total volume of 3.0 ml (corresponding to an absorbance of about 1.0 at $\lambda = 414$ nm). The reaction was initiated by the injection of the solution of test compounds (5–50 μl). The radical scavenging activity (RSA_{ABTS}, mean value of two measurements) was expressed as the amount of ABTS radical (in μmol) scavenged per minute by 1 mg antioxidant. The antioxidant activity of lignin samples was com-

pared with that of a commercial antioxidant BHT (0.3 mM).

2.3.2. Chain-breaking antioxidant activity

The ability of lignins to inhibit lipid peroxidation in micellar systems was determined based on the measurement of the rate of the ABAP-induced generation of linoleic acid peroxy radical, using the method adapted from Pryor et al. (1993). The rate of oxidation of linoleic acid with or without antioxidant was determined by following the development of absorption at 234 nm due to the conjugated diene hydroperoxide of linoleic acid, using an UV-Vis Perkin-Elmer Lambda 2S spectrophotometer, with thermostated cuvette holder for temperature control. The linoleic acid solution (32 mM, oxygen free, 1% Tween 20, v/v) was prepared with 200 μl of linoleic acid, 200 μl of Tween 20 and 0.2 M phosphate buffer pH 7.4 for a final volume of 20 ml, under continuous nitrogen flow. The reaction mixture with a total volume of 2.5 ml contained of 0.2 M phosphate buffer, pH 7.4, saturated with air, 100 μl of linoleic acid (32 mM) and variable amounts (0–50 μl) of 0.1% lignin solutions or 0.5 mM tocopherol solution. The reaction mixture was heated to 45 °C and then the reaction was initiated by the addition of 10 μl of 0.5 mM ABAP solution. The change in the absorbance at 234 nm was measured in time against a reference containing 2.5 ml 0.2 M phosphate buffer, pH 7.4 + 10 μl of ABAP solution. The rate of formation of linoleic acid peroxide in non-inhibited and inhibited reaction was determined from the linear phase of the kinetic trace (initial rate conditions), using $\varepsilon_{LAP} = 25\,mM^{-1}\,cm^{-1}$ as the molar extinction coefficient of linoleic acid peroxide at 234 nm. The radical scavenging activity of lignins (RSA_{ABAP}, mean value of three measurements) was expressed as the decrease of the rate of formation linoleic acid peroxide in an inhibited reaction (k_{inh}) as compared to the non-inhibited ABAP promoted oxidation (k_p).

2.4. Emulsifying properties of lignin

A mixture of 0.1% lignin solution, alkaline (14 ml) and sunflower oil (38 ml) was added to a glass container and mixed at 15 000 rpm with an ultraturax for 1 min. A reference emulsion was prepared following the same procedure with demineralized water instead of lignin solutions but containing the same NaOH concentration. 0.2 ml of the fresh emulsion where diluted to 50 ml with 1% SDS solution (1:250 dilution) and the absorbance at 500 nm was measured against 1% SDS solution as reference to obtain $A_{0,500\,nm}$. 10 ml of fresh emulsion were drawn up in a syringe and let stand vertically for a time period $\zeta \geq 30$ min. 0.2 ml from the separated lower phase where diluted to 50 ml with 1% SDS solution (1:250 dilution) and the absorbance at 500 nm was measured against 1% SDS solution as reference to obtain $A_{\zeta,500\,nm}$.

The emulsifying activity index (EAI, mean value of two measurements) was expressed as the value of the absorbance of the emulsion at 500 nm, at time zero ($A_{0,500\,nm}$). The emulsifying stability index (ESI, mean value of two measurements) was expressed as the ratio between the value of the absorbance at 500 nm measured at $\zeta = 24$ h ($A_{24,500\,nm}$) and at $\zeta = 0$ ($A_{0,500\,nm}$), respectively.

2.5. Data analysis

Principal component analysis (PCA) and partial least squares regression (PLS) were performed using the statistical program Unscrambler 6.1 (Camo A/S, Norway). PCA focuses on data reduction, by transforming the originally measured numerical information into new variables, the principal components (PCs), which are linear combinations of the latter (Esbensen et al., 1996). PCA was used to gain information about the dissimilarity between lignin samples and to identify the variables that contribute most to this differentiation. PLS regression applies to the simultaneous analysis of two sets of variables on the same objects to find the latent variables in X that will best predict the latent variables in Y (Martens and Naes, 1987). The method is applied in this work to correlate the FT-IR spectroscopic data (X-matrix) with related chemical data and functional properties (Y-matrix), and to build a calibration model enabling the prediction of a wanted property (y) from a measured spectrum (x). The predictive ability of the calibration models is described by the root mean squares error of prediction (RMSEP; Esbensen et al., 1996). Both the PCA and PLS models were validated using the "full cross-validation" technique to ensure predictive validity, guarding against over-fitting.

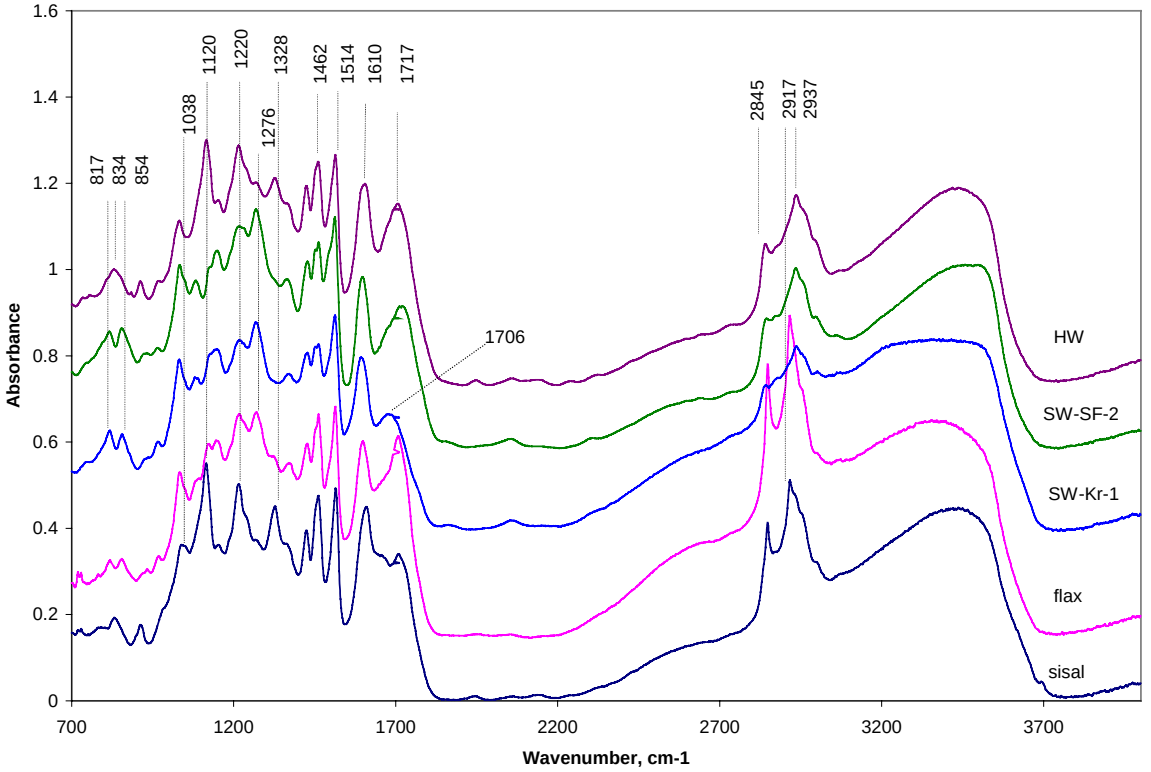


Fig. 1. FT-IR spectra of lignin samples.

3. Results and discussion

3.1. Spectroscopic characterisation of lignins

Fig. 1 shows the infrared spectra of some typical non-wood (sisal and flax) sulphur-free lignins, a hardwood lignin (sulphur-free, solvent extraction) and two softwood lignins, a lignosulphonate and a sulphur-free lignin. FT-IR spectra reflect the chemical structure as well as the purity of lignins. Common features as well as particular vibrations, specific to each lignin, are found in the spectra. Below we present an analysis of the recorded spectra of the lignin samples used in this study, based on the assignments given by Faix (1992).

All lignins show a broad band at 3410–3460 cm^{-1}, attributed to the hydroxyl groups in phenolic and aliphatic structures, and the bands centred around 2938 and 2842 cm^{-1}, predominantly arising from CH stretching in aromatic methoxyl groups and in methyl and methylene groups of side chains. Lignins from hemp, jute and flax show strong bands in this region, with peaks at 2917 and 2847 cm^{-1}, arising from CH stretching in aliphatic methylene group that can originate from fatty acids present in the lignin preparations. These bands are less intense for sisal lignin.

In the carbonyl/carboxyl region, weak to medium bands are found at 1705–1720 cm^{-1}, originating from unconjugated carbonyl/carboxyl stretching, with a shoulder at around 1680 cm^{-1}, that can be associated with the conjugated carbonyl–carboxyl stretching. The band at 1705 cm^{-1} increases in oxidised lignins (flax-ox), in solvent-extracted hardwood lignin and in sulphur-free softwood lignin, but is absent in softwood lignosulfonates and Kraft lignin. Weak absorptions around 1650 cm^{-1}, resulting in the asymmetry and broadening of the more intense bands at 1705 and 1600 cm^{-1}, respectively, may originate from both protein impurity and water associated with lignin. Softwood lignosulfonate SW-LS-1 shows a weak

band at 1771 cm^{-1} (spectrum not showed) that may originate from aromatic acetoxy groups.

Aromatic skeleton vibrations at 1600, 1515 and 1426 cm^{-1} and the C–H deformation combined with aromatic ring vibration at 1462 cm^{-1} are common for all lignins, although the intensity of the bands may differ. The spectral region below 1400 cm^{-1} is more difficult to analyse, since most bands are complex, with contribution from various vibration modes. However, this region contains vibrations that are specific to the different monolignol units and allows the structural characterisation of lignins. The spectra of all lignin samples show the vibrations characteristic for the guaiacyl unit (1269 cm^{-1}, G ring and C=O stretch; 1140 cm^{-1}, CH in-plane deformation; 854 and 817 cm^{-1}, C–H out-of-plane vibrations in position 2, 5 and 6 of guaiacyl units) but the intensity of the bands vary significantly between samples.

Spectra of hardwood and all non-wood lignins show a band at 1326 cm^{-1}, which is characteristic for syringyl (S) ring plus guaiacyl (G) ring condensed and the vibration at 843 cm^{-1}, that arise from the C–H out-of-plane in position 2 and 6 of S units.

Common for the spectra of all lignin samples are a weak band at 1370–1375 cm^{-1} originating from phenolic OH and aliphatic C–H in methyl groups and a strong vibration at 1215–1220 cm^{-1} that can be associated with C–C plus C–O plus C=O stretching. The aromatic C–H deformation at 1035 cm^{-1} appears as a complex vibration associated with the C–O, C–C stretching and C–OH bending in polysaccharides. Carbohydrate originating vibrations are associated also with other vibrations in the spectral region 1000–1300 cm^{-1}. The influence of polysaccharide impurities on the spectral profile is most visible for sample SW-LS-2 (not shown).

3.2. Chemical characterisation of lignins

Lignin samples were characterised with respect to lignin and sugar content and the amount of hydroxyl and carboxyl groups (Table 1). The lignin content of samples varied between 64 and 99%. It is obvious that not the extraction process but the purification steps applied determine the purity of the samples, since very pure lignins were obtained by either solvent extraction (HW, 96.5%), alkaline extraction (SW-SF-2, 99.2%) and Kraft process (SW-Kr-3, 96.8%). The total sugar content of lignin samples ranged between 0.65 and 7.7%, with one sample containing 24.5% total sugar. In plant cell wall, lignin is often associated with cell wall carbohydrate polymers such as with

Table 1
Chemical characteristics of lignin samples

Sample number	Raw material	Lignin type (commercial name)	Sample code	Lignin content (%)	COOH (mmol/g)	Phenolic OH (mmol/g)	Total sugars (%)
1	Hemp	Soda	He	Nd	2.1	1.58	2.4
2	Sisal	Soda	Si	Nd	1.18	2.29	7.7
3	Abaca	Soda	Ab	Nd	1.14	2.73	5.5
4	Straw	Soda	St	Nd	2.1	2.43	Nd
5	Jute	Soda	Ju	Nd	1.78	2.38	Nd
6	Flax	Soda	Fl-1	87.8	1.9	1.1	1.7
7	Softwood	Lignosulfonate (Borresperse 3A)	SW-LS-1	Nd	3.5	1.1	1.3
8	Softwood	Kraft (Indulin AT)	SW-Kr-1	90.0	2.5	1.8	2.06
9	Softwood	Lignosulfonate (Wafex P)	SW-LS-2	Nd	1.2	1.1	24.5
10	Flax	Soda (Bioplast)	Fl-2	87.8	1.9	1.1	1.7
11	Flax	Soda, oxidised	Fl-ox	92.1	1.8	0.9	1.6
12	Softwood	Kraft (Curan 100)	SW-Kr-2	88.6	1.7	0.8	2.26
13[a]	Softwood	Soda, precipitation high pH	SW-SF-1	64.7	Nd	Nd	1.77
14	Softwood	Kraft	SW-Kr-3	96.8	2.0	2.5	0.71
15	Mixed hardwoods	Organosolv (Alcell)	HW	96.5	0.78	2.4	0.32
16	Softwood	Soda, precipitation low pH	SW-SF-2	99.2	1.4	1.0	0.65
17	Softwood	Kraft (Curan 2711P)	SW-Kr-4	80.3	2.7	1.6	2.58

Nd, not determined.

[a] Lignin 13 contains about 40% ash.

xylans and pectins via ferulic acid bridges. Although the lignin/polysaccharide interaction is genetically encoded, the results clearly indicate that the amount of carbohydrate polymers (expressed as total sugar) in lignin products is determined by the efficiency of the pulping process and the extraction and purification steps used. The amounts of carboxyl and phenolic OH groups are significantly different between the samples. High concentrations of phenolic OH groups (between 2.3 and 2.7 mmol/g) were found in lignin samples isolated from sisal, abaca, wheat straw, jute, softwood (SW-Kr-3) and hardwood.

3.3. Antioxidant properties

The antioxidant efficiency of lignin samples was determined using two different model systems.

The first approach was to measure the ability of lignin samples to react with free radicals in aqueous solution. The $ABTS^{+\bullet}$ cation radicals were generated by an enzymatic system consisting of peroxidase and hydrogen peroxide. The antioxidant activities of lignins were determined by measuring the rate of disappearance of the ABTS radical in the presence of lignin. All lignin samples tested reacted with the ABTS radical (Fig. 2), but their efficiency was about 20–30% (on weight) of that measured for the commercial antioxidant BHT (data not shown). Lignin samples from abaca, sisal and jute and the softwood kraft lignins SW-Kr-1 and SW-Kr-3 show the highest scavenging activity against the ABTS radical.

Further, the effect of lignin on the oxidation of unsaturated compounds by molecular oxygen has been evaluated. The oxidation of linoleic acid in micellar solution initiated with azo-compounds (ABAP) has been studied in the absence and presence of lignins and tocopherol (Vitamin E), which is a commonly used antioxidant. Antioxidant activities of lignin compounds were determined by measurements of the rate of generation of linoleic acid peroxide during inhibited peroxidation of linoleic acid initiated by ABAP with known rate of initiation. All lignins show some ability to act as chain-breaking antioxidants during lipid peroxidation, but only lignins from abaca, sisal, hardwood and jute

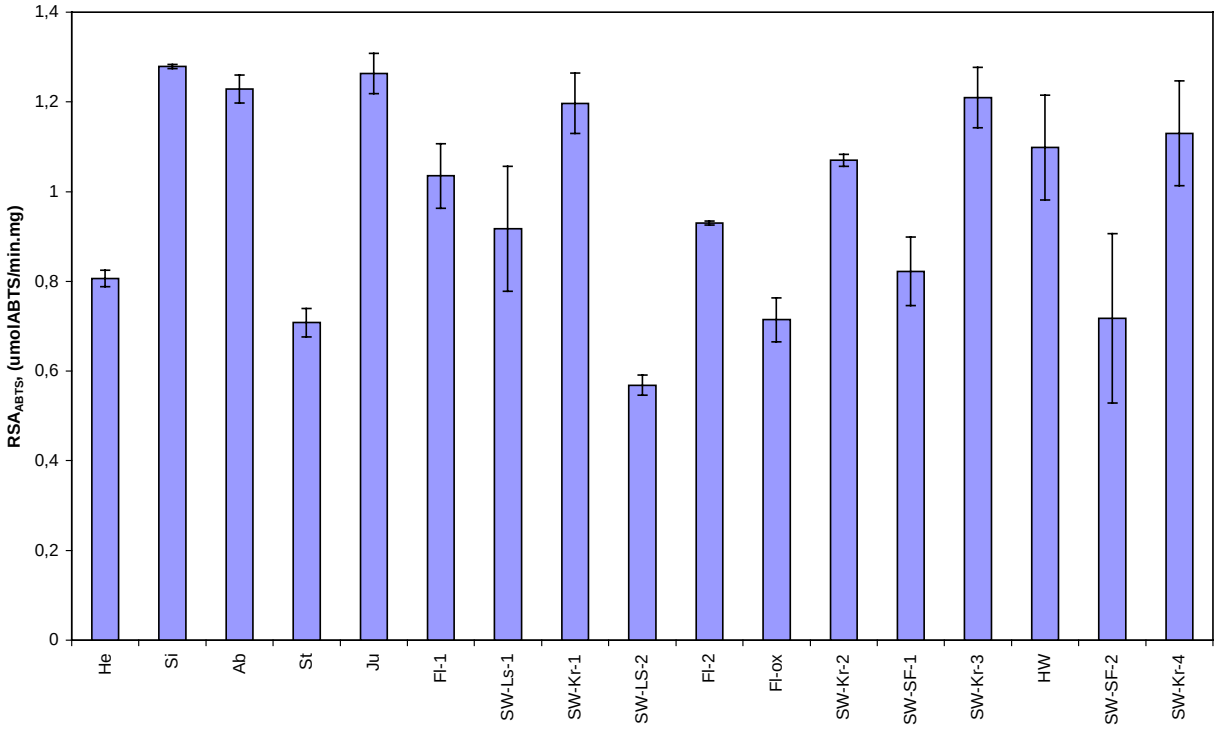


Fig. 2. Relative ABTS-radical scavenging activity of lignin samples.

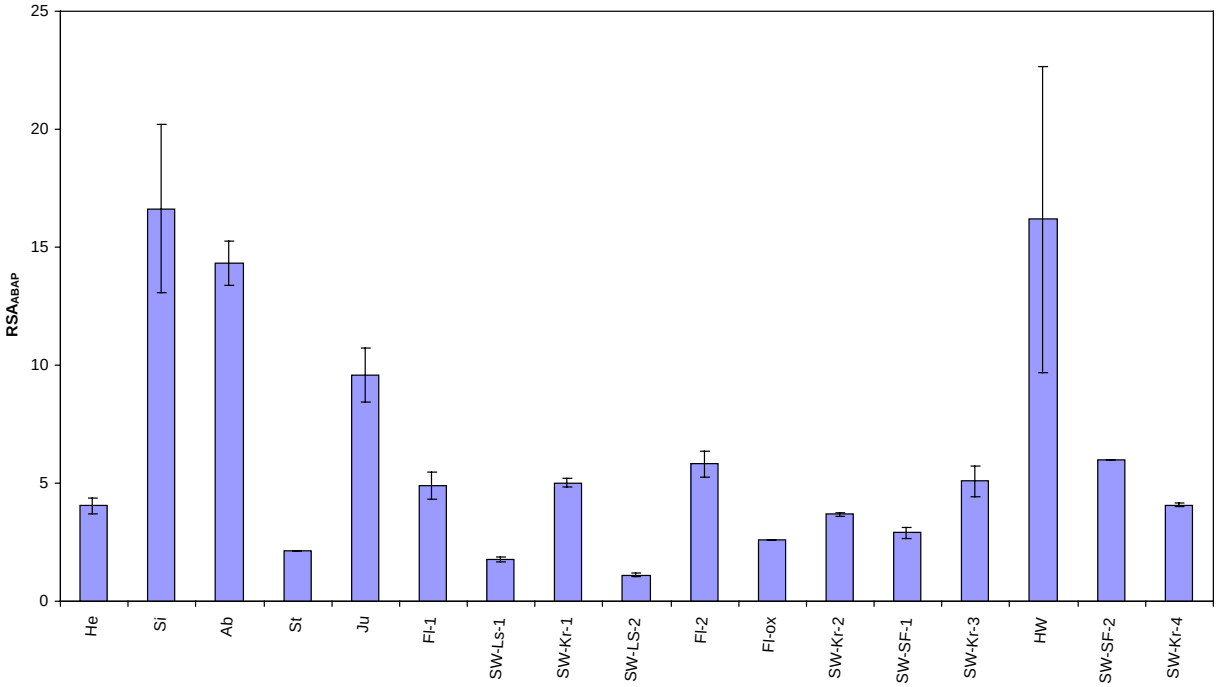


Fig. 3. Relative chain-breaking antioxidant effect of lignin in lipid peroxidation.

and the sulphur-free softwood lignin SW-SF-2 have significant antioxidant effects (Fig. 3). The most effective chain-breaking antioxidant lignin showed about 30% (on weight basis) of the activity of tocopherol in the same system (data not shown).

The results suggest that the radical scavenging activity of lignins, although induced by hydroxyl phenolic groups, is not linearly related to the concentration of the phenolic groups but is influenced also by other structure-related factors such as the steric availability of aromatic hydroxyl groups and the stabilisation of phenolic radicals. Adsorption phenomena at the oil–water interface may be of importance for reactions in micellar systems (e.g. inhibition of oxidation of polyunsaturated fatty acids).

3.4. Emulsion stabilising properties

Lignin samples were tested with respect to their ability to stabilise oil-in-water emulsions. Only lignin samples from jute, wheat straw, flax, hardwood and sisal showed some emulsifying capacity (Fig. 4). However, more lignins were able to stabilise oil-in-water emulsion (Fig. 4). According to the ESI values, lignins can be ordered as follows: Si > St > Ab > Ju > He > Fl. Hardwood lignin does not show an emulsion stabilising effect.

3.5. Classification of lignins based on FT-IR spectral properties

To discriminate between the different lignin samples, PCA was applied to the second derivative of the FT-IR spectra. The use of second derivative instead of original spectra was preferred because the second derivative is able to separate overlapping absorption bands and can correct for large baseline variations. Preliminary analysis showed that the most significant information is contained in the spectral regions ranging from 700 to 1800 cm^{-1} and 2800 to 3100 cm^{-1}. PCA analysis applied to the second derivative of the spectral data in the regions mentioned above resulted in a model that required four PCs to explain about 85% of the variance in the data set. The first two PC's explained 40 and 21% of the variance, respectively (data not shown). The most influential frequencies for

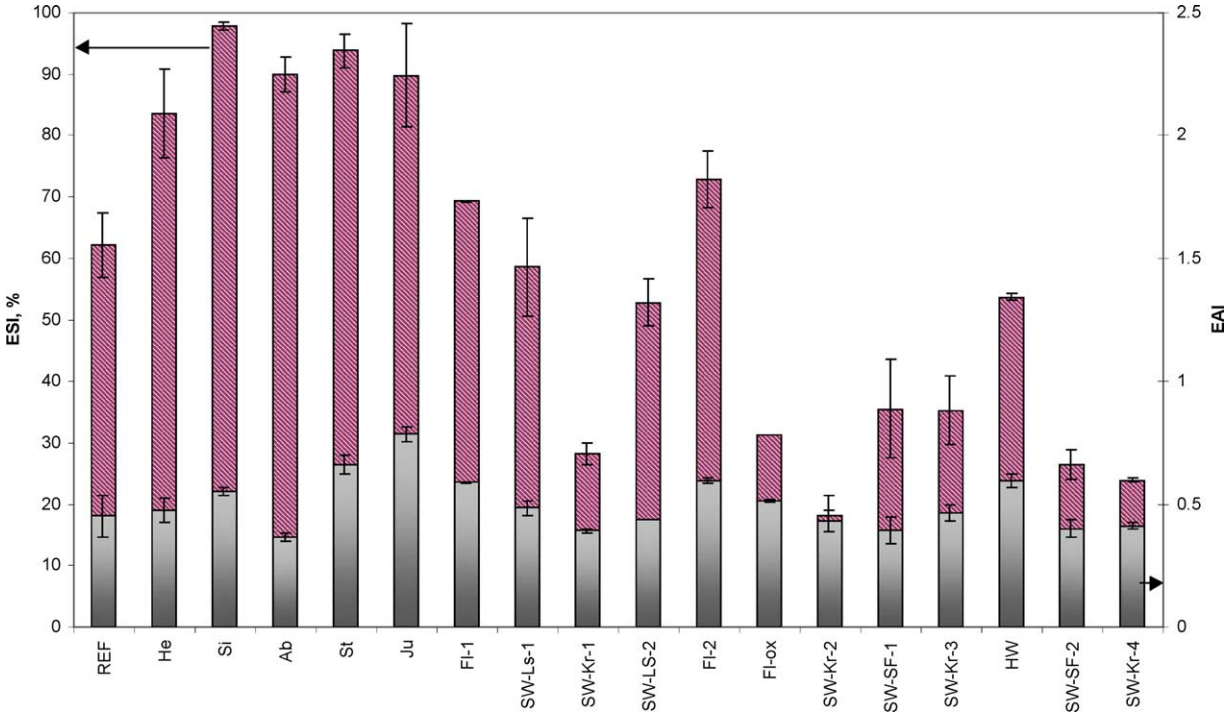


Fig. 4. Effect of lignins on the formation and stabilisation of water-in-oil emulsion (▨) EAI; (■) ESI.

discrimination between samples were identified by the analysis of the loading plot, and they are listed in Table 2, in decreasing order of intensity.

The classification of lignin samples according to their loadings on PC1 and PC2 is shown in Fig. 5. An unequivocal classification of lignin samples according to their botanical origin (implicitly related to the composition in monolignol residues) is obtained. Lignins isolated from hardwood and herbal crops, build up primarily from G and S rings, have negative loadings on the first PC, and are clearly separated from the softwood lignosulfonates and kraft lignins (G rings), which show positive loadings on PC1 (Fig. 5).

The model allows further discrimination between samples according to their chemical composition (lignin content, type and amount of impurities, type and concentration of functional chemical groups). Lignins isolated from hardwood, abaca, sisal, jute and wheat straw are correlated and form one class, having negative loadings on both PC1 and PC2. All these samples contain similar amounts of phenolic hydroxyl groups (see Table 1). The HW sample consists of high amount of lignin (96.8%), and this suggests that all other lignins grouped in this class are also characterised by high lignin content. They differ from lignins from flax and hemp, which have negative

Table 2
Loadings of IR frequency data on PC1 and PC2

	IR vibration	
	Positive loadings	Negative loadings
PC1	2851, 2917, 1115, 1429, 1522, 1549, 1730, 1710, 1329, 985, 838	2830, 2868, 1572, 1140, 2940, 2897, 1497, 1408, 1350, 1261, 1040
PC2	1110, 2830, 833, 2868, 2895, 906, 1242, 1329, 1612, 1526, 1180	2917, 2849, 1705, 1265, 1138, 1080, 1030m 1570, 927, 854, 817

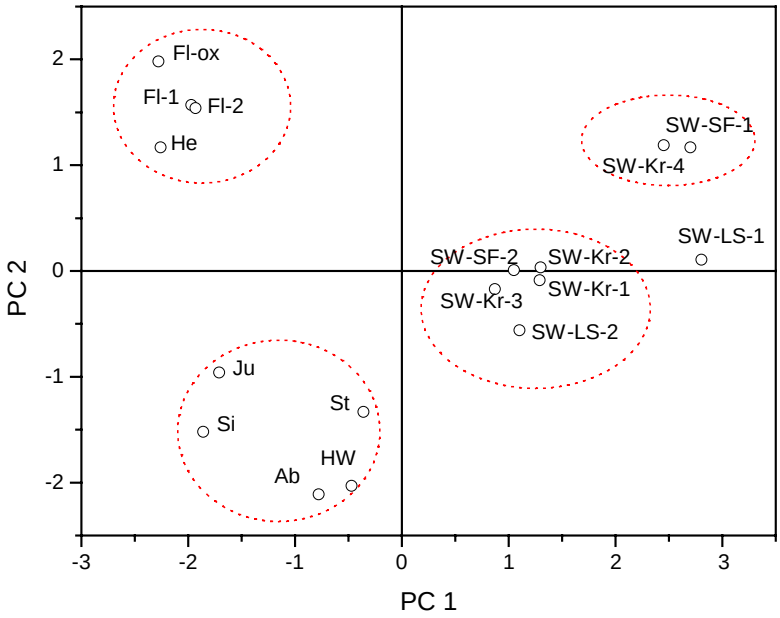


Fig. 5. Plot of the loadings of lignin samples on PC1 and PC2.

loadings on PC1 and positive loadings on PC2. These samples contain about 87–92% lignin and have a high content of fatty acid impurities (high loadings on the aliphatic methylene and carboxyl spectral regions of both PC1 and PC2). Flax samples 1 and 2, obtained from different batches using the same processing condition (e.g. alkaline extraction), are classified as very similar, while sample Fl-ox, which was modified after extraction, is still classified as a flax lignin.

Most softwood lignosulfonates and kraft lignins are similar as related to their loadings on PC1 but are discriminated according to the loadings on PC2. Samples SW-Kr-1, SW-LS-2, SW-Kr-2, SW-Kr-3 and SW-SF-2 have positive loadings on both PC1 and low negative loadings on PC2 are grouped in one class. These samples consist of relatively high amount of lignin (92–99%). Samples SW-Kr-4 (80.3% lignin), the sulphur-free softwood lignin (SW-SF-1), which contains high amount of ash (approximately 40%) and sample SW-LS-1, which is a modified sodium lignosulphonate derived from fermented spruce wood sulphite liquor and contains, according to the analysis of IR data and the chemical analysis, a relatively high amount of carboxylic groups, are not assigned to the group.

3.6. Relationship between FT-IR spectra and chemical and functional properties

PLS multivariate regression analysis was used to quantify the relationship between the infrared spectral data and the amount of lignin, carboxyl and phenolic hydroxyl groups as well as antioxidant and emulsifying properties of the lignin samples. PLS-1 models were built using calibration sets consisting of all reference samples (see Table 3), with full cross-validation as statistical validation method. The PLS models were optimised by removal of non-significant spectral regions. Accordingly, the spectra were truncated to include only the frequency range between 720 and 1800 cm^{-1}, 2700 and 3120 cm^{-1}, and 3460 and 3740 cm^{-1}. The statistical results of the PLS calibration models are given in Table 3. A small number of factors (e.g. principal components) were needed to obtain models with high correlation coefficients, ranging between 0.9 and 0.98, which explained more than 90% of the *y*-variance. Only for the amount of carboxyl group in lignin samples a regression model was obtained with a low correlation ($R_{calib}{}^2 = 0.77$) and a high predictive error ($RMSEP_{calib} = 0.42$), which explained less than 70% of the variance in the sample

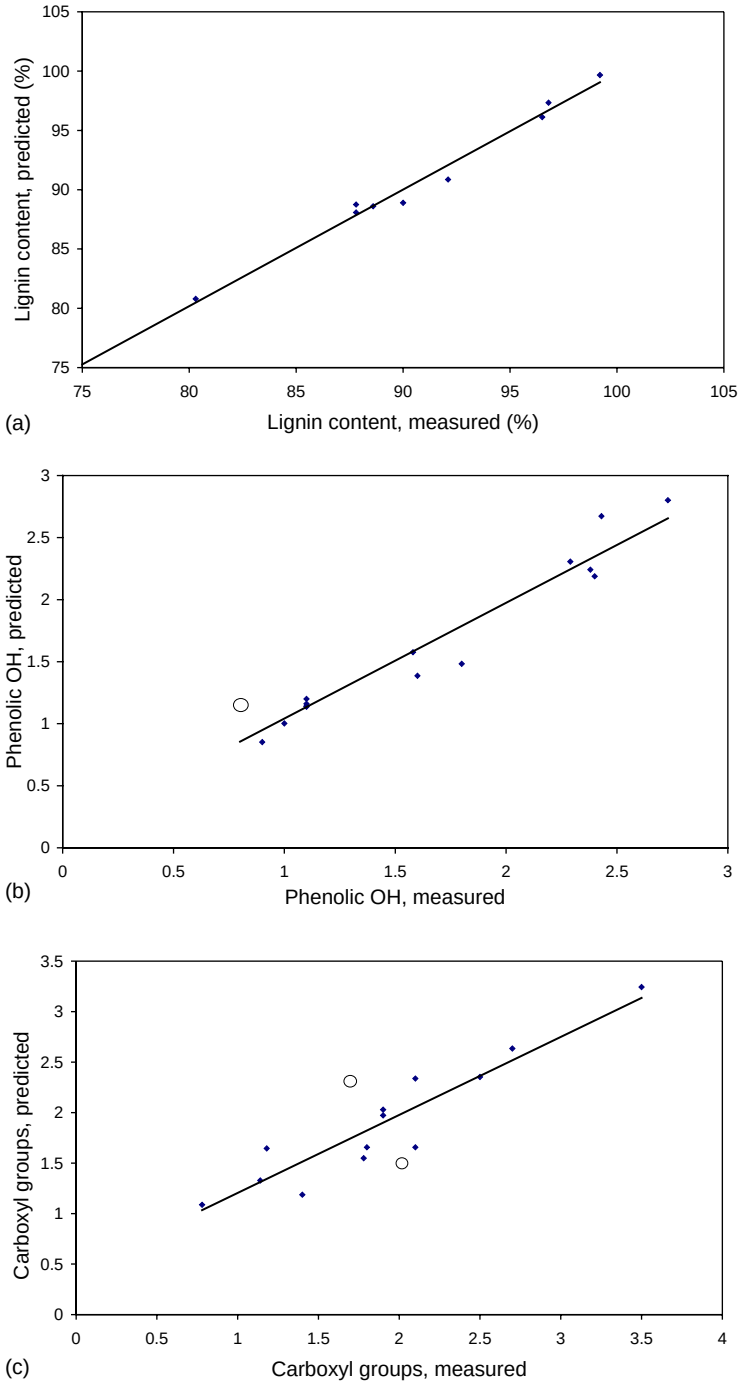
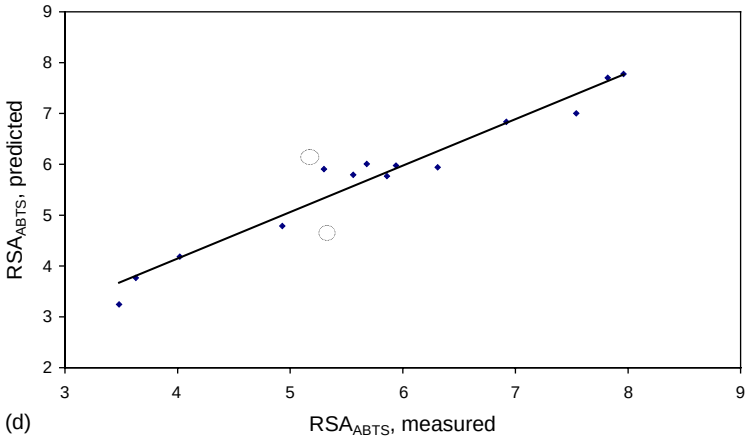
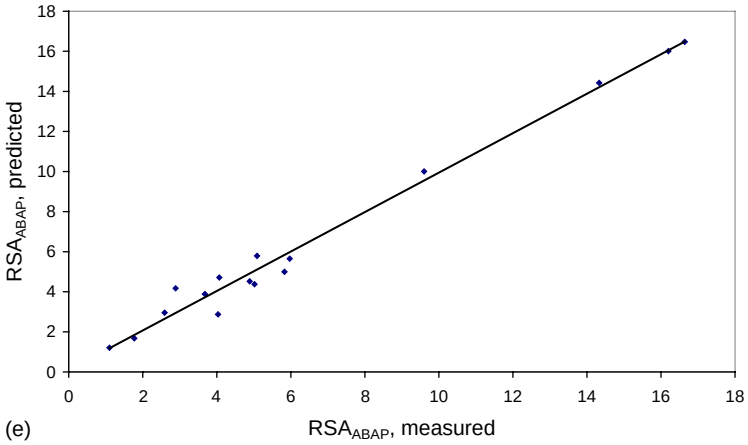


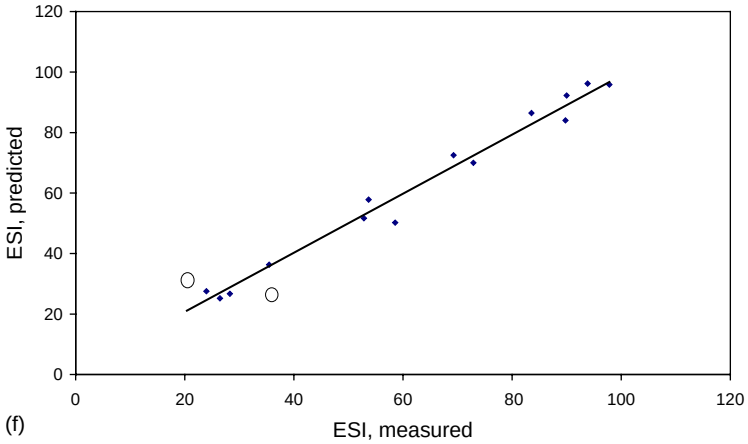
Fig. 6. Regression lines for (a) lignin content; (b) phenolic OH; (c) carboxylic groups; (d) RSA_{ABTS}; (e) RSA_{ABAP}; and (f) ESI.



(d)



(e)



(f)

Fig. 6. (*Continued*).

Table 3
Results of partial least-squares regression between chemical and functional characteristics of lignins and their FT-IR spectra

Statistical information	Values					
Range of variation	64.7–99.2	0.8–2.8	0.7–3.5	3.6–7.9	1.1–16.6	20–97
Number of samples calibration set	10	16[a]	16[a]	17	17	17
Aim of analysis	Calibration					
Calibration						
R_{calib}^2	0.98	0.92	0.77	0.9	0.92	0.96
$RMSEP_{calib}$	0.71	0.26	0.42	0.38	0.59	4.74
Cross-validation						
R_{valid}^2	0.88	0.81	0.54	0.8	0.8	0.88
$RMSEP_{valid}$	1.26	0.36	0.6	0.67	0.96	9.04
Number of PCs	3	4	2	5	5	4
Outliers	1	1	1	1	1	–
RAA (%)	5.4	10.5	22.5	6.8	9.5	8.4

R_{calib}^2 an R_{valid}^2 are the squared correlation coefficient for the calibration and validation, respectively; $RMSEP_{calib}$ and $RMSEP_{valid}$ is the root mean square error of prediction for calibration and validation, respectively; outliers are number of samples/spectra that deviate from the model and are consequently not used in the final model; RAA is the relative analytical accuracy, calculated as (RMSEP/mean value of the measurement) × 100. *X*-variables: FT-IR spectral data; range: 720 – 1800 cm^{-1}, 2700-3120 cm^{-1} and 3460 – 3740 cm^{-1}; *Y*-variables: lignin content, phenolic OH, COOH, RSA_{ABTS}, RSA_{ABAP}, ESI.

[a] Sample SW-SF1 was not included in the calibration set.

set. The predictive error of the models expressed as RMSEP is analytical acceptable for the prediction of lignin content, phenolic OH, RSA_{ABTS}, RSA_{ABAP} and ESI, corresponding to a relative analytical accuracy lower than 10% (see Table 3). To illustrate the predictive ability of the models developed, examples of the regression lines of the cross-validated PLS-1 models for each parameter are shown in Fig. 6.

The lignin-PLS calibration model developed was used to estimate the lignin content of samples 1–5, 7, 9 and 13, based on the recorded infrared spectra. The following results were obtained: He: 83.8%, Si: 91.8%, Ab: 88.5%, St: 91.3%, Ju: 96.7%, SW-LS-1: 70.2%, SW-LS-2: 78.2%. For sample SW-SF-1, the infrared-based PLS model predicted a lignin content of 66.8%, very close to the amount experimentally determined (64.7%).

These results indicate that PLS models based on infrared spectra can be used for the quantitative characterisation of lignin samples. The models developed in this study are based on a relatively small number of samples, and they can be regarded as a first indication about the applicability of infrared spectroscopy and chemometrics for the chemical and functional characterisation of lignins. Extension of the calibration models with a larger number of samples covering as much as possible the variability of the lignin population and validation of the models with independent sample sets is necessary to develop robust calibration models.

Quantitative evaluation of IR spectra and their correlation with wet chemistry data has been reported previously (Abott et al., 1988; Faix, 1992; Owen and Thomas, 1989; Schultz et al., 1985; Schultz and Glasser, 1986), but the correlation with functional properties of lignins is, to our knowledge, reported now for the first time.

4. Conclusions

The results presented in this study show that FT-IR spectra are an important source of information for a quick qualitative and quantitative characterisation of the chemical structure and functional properties of lignins. PCA analysis allows classification of lignin materials with respect to botanical origin, pulp processing and modification treatments. The PLS models developed allow an accurate determination of the concentration of lignin polymers and phenolic hydroxyl groups as well as a good prediction of the antioxidant and emulsion-stabilising properties of lignin based materials.

In conclusion, the results presented in this article strongly suggest that Fourier transform infrared

spectroscopy combined with chemometrics can be used as a fast and reliable non-destructive technique for characterisation and quality control of lignin based materials.

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