



Characterisation of Kraft lignin separated by gradient acid precipitation



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ABSTRACT

Lignin is considered the second most abundant organic natural compound in the world. Lignin represents a promising source of new materials based on renewable resources. Currently the lignin is used in low value-added applications and for energy production and chemical recycling for industrial process itself in production of pulp and paper. Only a small amount is being used for other applications. The aim of this study was to analyze the effects of precipitation on the composition of lignin extracted from black liquor produced in industry CMPC Celulose Riograndense. For this, lignins were precipitated at different pH conditions, with various acids. Obtained lignin samples were characterized by various analytical techniques such as FTIR, GPC, HPLC and XRD. Furthermore, the quantity of carboxyl groups was also analyzed. The antioxidant activity of the analyzed lignins was evaluated by the radical ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay. Total phenolic content was evaluated by Folin-Ciocalteu method while the antioxidant potential of lignins was measured by DPPH. The lignins showed a variable composition according to the acid used for its precipitation; the lignin precipitated with sulphuric acid presented Na₂SO₄ salts while with hydrochloric acid, NaCl was found. In addition, a change in lignin composition was observed at different pH.

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1. Introduction

After cellulose, lignin is the organic component most abundant in the environment. The annual production of lignin on earth has been estimated in the range of 5–36 × 10⁸ tons (Lin and Dence, 1992; Gellerstedt and Henriksson, 2008). Lignin is an amorphous polyphenolic material created through enzymatic polymerisation of three monomers, coniferyl, sinapyl and *p*-coumaryl alcohol. Maziero et al. (2012) affirmed that the monomers forming lignin (*p*-hydroxyphenyl (H), guaiacyl alcohol (G) and syringyl alcohol (S)) differ only in the degree of substitution on the phenolic ring. Lignins are a promissory source of new materials; Jin et al. (1990) used the lignin as a phenolic resin, Park et al. (2008) as a substitute of phenol in phenol formaldehyde (PF) and Boeriu et al. (2004) as an emulsifier. Toledano et al. (2012) reported the potential use of fractionated lignins by selective precipitation as a dispersant or as a chelating agent. According to Rojas and Salager (1994), the dispersant activity of lignin increases as the pH used for its precipitation. Otherwise, due to the high content of diverse functional

groups (phenolic and aliphatic hydroxyls, carbonyls, carboxyls, etc.) and its phenylpropanoic structure, lignin can serve as a neutralizer or inhibitor in oxidation processes, stabilizing reactions induced by oxygen radicals and their derived species (García et al., 2010). Nevertheless, this antiradical activity greatly depends on lignocellulosic material from which lignin is obtained, the method used for its extraction, and the treatments applied during its isolation and purification.

Technical lignins are obtained from industrial process; Kraft pulping is by far the most important industrial method for chemical pulps production (Gellerstedt et al., 2004), and many tons of lignin are generated as by-product. Kraft process is characterised for accepting any type of wood and forest species, and chemical products from inorganic process can be recovered and reused efficiently. Kraft pulp represents approximately 90% of chemical pulp, and about 130 million tons of cellulose per year in the world (Tran and Vakkilainen, 2007). However, volume of residues generated during the pulp production is preoccupant for the environment because currently the finality of these residues is for recycling the chemical agents and for energy production to supply the industry.

New researches are needed to increase utilisation of the lignin as a raw material. According to Lora and Glasser (2002), only a small quantity (<2%) of the lignin worldwide produced is used

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in different applications for production of value-add, renewable and eco-friendly materials. In this study, Kraft lignin produced by CMPC-Celulose Riograndense pulp and paper industry was precipitated at different pH in order to analyze the effect of the process and the precipitation technique on lignin composition. Changes on composition were determined using ATR-IR, XRD, HPLC and GPC. The carboxyl groups and antioxidant power of lignin were measured by ABTS and Folin–Ciocalteu techniques. Lignin composition recovered with this study would be useful in order to evaluate its potential for further applications like source of bulk chemicals by its depolymerisation.

2. Materials and methods

2.1. Lignin precipitation

The acid precipitation of lignins from black liquor from Kraft pulp of CMPC-Celulose Riogrande pulp and paper industry located in Southern Brazil (30°08'56.85"S 51°18'49.51"O) was performed at different pH (2, 4 and 6) using a method described by [Alriols et al., 2009](#). To achieve this, sulphuric acid (98%, w/w) and hydrochloric acid (37%, w/w) were used. Samples were referenced as follows: lignin precipitated with sulphuric acid at pH 2 LKS 2, at pH 4 LKS 4 and at pH 6 LKS 6 while lignin samples precipitated with hydrochloric acid LKH 2, LKH 4 and LKH 6.

2.2. Composition of lignins

Yield of Kraft lignin (g/L) was determined by gravimetric method, based on total dry solid of black liquor. Analysis of main sugars content was performed in HPLC equipment using the filtrated liquid obtained from Klason lignin measurements (TAPPIT222 om-98). Insoluble lignin content was measured using a method described by [Toledano et al. \(2012\)](#). Acid-soluble lignin was measured in a Jasco V-630 spectrophotometer UV–vis. To achieve this, an aqueous solution containing H₂SO₄ 1 M of the filtrated material previously obtained was done until the absorption of the samples was between 0.1 and 0.8, at 205 nm.

The antioxidant power of lignins samples as the lignin capacity to reduce ABTS radical was measured through spectrophotometric method described by [García et al. \(2012\)](#) using a UV–vis spectrophotometer.

2.3. Carboxyl groups and antioxidant power of lignin

Carboxyl groups were determined by aqueous titration as described by [Toledano et al. \(2012\)](#). The antioxidant activity in Kraft lignins was determined using a method described by [Dizhbite et al. \(2004\)](#). Spectrophotometric method based on the use of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) using a spectrophotometer Jasco V-630. Extracted samples dissolved in dioxane/water (90:10, v/v) at a concentration of 1 g/L, the 0.1 mL were mixed with

3.9 mL of a 6×10^{-5} M DPPH solution, and the absorbance at 518 nm of the mixture was measured at different times, 15 min and 30 min.

The total phenols content of lignins samples were determined using a method described by [García et al. \(2012\)](#). To achieve this, a volume of 2.5 mL Folin–Ciocalteu reactive and 5 mL of 20% Na₂CO₃ solution was mixed with 0.5 mL of lignin solution (20 mg in 10 mL of DMSO). The mixture was kept for 30 min at 40 °C before measuring the absorbance at 750 nm. The intensity of blue colour was measured at 750 nm in a UV–vis spectrophotometer, and the total phenols content was determined using a standard curve with gallic acid solutions.

2.4. Molecular weight distribution

Molecular weight of lignins was analyzed through gel permeation chromatography (GPC) technique using a JASCO instrument equipped with an interface (LC-NetII/ADC) and a refractive index detector (RI-2031Plus). Two PolarGel-M columns (300 × 7.5 mm²) and PolarGel-M guard (50 × 7.5 mm²) were employed. Dimethylformamide + 0.1% lithium bromide was the eluent. The flow rate was 0.7 mL/min and the analyses were carried out at 40 °C. Calibration was made using polystyrene standards (Sigma-Aldrich) ranging from 70,000 to 266 g/mol.

2.5. X-ray diffraction

X-ray diffraction was performed in order to study the crystallinity of lignins. For this propose, a Shimadzu XRD-6000 X-ray diffractometer equipped with a copper anode lamp ($\lambda = 0.154$ nm) was used.

2.6. Infrared spectroscopy (ATR-IR)

The attenuated total reflectance infrared spectroscopy (ATR-IR) by direct transmittance in a single-reflection ATR System was performed in the lignins. The equipment was configured for 32 scans in a range of 4000–600 cm⁻¹ and resolution of 4 cm⁻¹. Moreover, the salts presented in the lignins sample were precipitated after addition of 25 mL of absolute ethanol in 0.5 g of lignin, constant agitation for 24 h, filtration and drying. The precipitated material was analyzed by ATR-IR and was compared with the commercial salts of Na₂SO₄ and NaCl.

3. Results and discussion

3.1. Composition and characterisation of lignins

[Table 1](#) shows the results for analysis of lignin content and elemental sugars of lignins precipitated with different pH. The lignin precipitation could be explained by its colloidal behaviour. [Marton \(1964\)](#) found that the lignin behaves as a hydrocolloid that is precipitated with low pH due to the effect of protonation of acid groups in the lignin.

Table 1
Chemical characterisation of lignins from different pH.

Parameter (%)	LKS 2	LKS 4	LKS 6	LKH 2	LKH 4	LKH 6
Glucose	0.0027 ± 0.0001	0.0019 ± 0.0003	0.0013 ± 0.0005	0.0008 ± 0.0003	0.0021 ± 0.0002	0.0027 ± 0.0001
Xylose	0.027 ± 0.0023	0.0314 ± 0.0004	0.0104 ± 0.00009	0.00066 ± 0.0001	0.0175 ± 0.0001	0.0219 ± 0.0006
Arabinose	ND	0.0006 ± 0.000072	ND	ND	ND	ND
Insoluble lignin	40.93 ± 0.79	41.97 ± 2.09	26.88 ± 3.05	57.11 ± 1.07	30.02 ± 3.33	35.59 ± 7.17
Soluble lignin	1.59 ± 0.20	2.16 ± 0.09	1.49 ± 0.03	1.90 ± 0.11	2.07 ± 0.10	1.71 ± 0.62
Total lignin	42.52 ± 0.91	44.10 ± 2.06	28.38 ± 3.09	59.01 ± 1.18	32.12 ± 3.24	37.30 ± 6.78
Total salts	57.45 ± 0.99	55.83 ± 2.18	71.62 ± 3.08	40.99 ± 1.18	67.89 ± 3.43	62.67 ± 7.79
Yield of solids (g/L)	100.01	123.23	107.41	75.63	93.61	78.13

ND= no detectable.

Table 2

Average values of carboxyl groups, ABTS inhibition and DPPH inhibition for the lignins precipitated with different pH.

	Carboxyl groups (%)	GAE ^a (%)	ABTS inhibition (%)	DPPH inhibition (%)15 min	DPPH inhibition (%) 30 min
LKS 2	0.2010	29.61 ± 1.61	99.21 ± 0.31	48.89 ± 6.39	54.76 ± 5.32
LKS 4	0.1455	26.67 ± 0.50	98.94 ± 0.30	57.58 ± 6.39	61.91 ± 4.69
LKS 6	0.1108	19.95 ± 0.74	98.63 ± 0.32	60.85 ± 1.25	63.05 ± 0.57
LKH 2	0.1931	36.14 ± 4.34	98.92 ± 0.42	60.02 ± 10.25	72.55 ± 3.21
LKH 4	0.1238	23.49 ± 0.04	99.47 ± 0.13	61.11 ± 12.25	68.87 ± 3.81
LKH 6	0.1217	39.20 ± 1.97	94.80 ± 2.03	54.23 ± 8.58	60.77 ± 8.67

^a Gallic acid equivalent.

The predominant elemental sugars in lignins were xylose and glucose while arabinose was the minor component. LKS 4 showed the highest content of insoluble lignin precipitated with sulphuric acid. On the other hand, LKH 2, which was precipitated with hydrochloric acid, presented the best results for soluble lignin, insoluble lignin and total lignin. Moreover, this lignin showed the lowest content of sugars in relation to the other lignins. As it can be observed in Table 1, the solid with the best yield in lignins showed the highest content of salts, such as LKS 6. This could be explained by the formation of salts during the precipitation process. In addition, the quantity of solids recovered at pH 4 was higher regardless the acid used for the precipitation. This behaviour was unexpected and contrary to what is found in the literature. At this pH, almost all the lignin has precipitated. Moreover, the salts and hemicelluloses also have precipitated. However, at lower pH some re-dissolution could occur and the colloids that were instable at pH 4 could become stable at pH 2.

LKH 2 presented the highest purity, which could be related to the precipitation technique. The process conditions with hydrochloric acid were more drastic and effective than the process conditions with sulphuric acid because the first allows separating a big quantity of lignin from the black liquor. According to García et al. (2009), recovered lignin content varies as a function of pH. This way, it is possible to obtain 20% of lignin when black liquor is acidified until pH 6–5 and as far as 80% when precipitation is performed close to pH 2.

As it can be observed in Table 1, LKH 2 presented the lowest yield in lignin per liter of black liquor precipitated while LKS 4 showed the highest yield in lignin. However, this yield in lignin not refers to quality of precipitated lignin, since LKS 4 presented ~44% of purity, which results in a high quantity of lignin but with low quality.

3.2. Carboxyl groups and antioxidant power

As it can be seen in Table 2 carboxyl groups were detected in low quantities. Amongst the lignins, LKS 2 and LKH 2 showed the highest contents. Table 2 shows the antioxidant power of Kraft lignins, which is influenced by the type of process for its obtaining. The conditions of lignins precipitation affect the total phenols content. As it can be observed, both lignins precipitated with sulphuric acid and chloridric acid did not show a tendency and the highest total phenols content was found for lignin with pH 6. According to García et al. (2012), lignins in acid conditions present higher percentage of total phenols because have, in general, lower impurities content and higher content of phenolic compounds. Nevertheless, Norgren et al. (2001) stated that Kraft lignin is soluble in alkaline solution due to brittleness of phenolic groups presented on its composition. This occurs as a function of dissociation of phenolic groups due to the driving force of the mixture and mainly to increase of entropy of ions. Therefore, increase of pH and characteristics of the environment results in high instability of phenolic groups. LKS 6 showed the phenolic groups less accessible, which result in a low phenols content.

The total phenols content reported in Table 2 did not show linear correlation with pH values; the same behaviour was observed by Faustino et al. (2010).

Lignins could oxidise from ABTS to ABTS+ due to their reducing potential, which result in a colour change (blue to green colour). The studied Kraft lignin samples did not present a clear tendency as a function of pH variation because antioxidant capacity in relation to ABTS radical does not significantly change with the pH. These results are similar to those found by García et al. (2010).

Percentage of elimination of DPPH radicals for different lignins was evaluated at 15 and 30 min. Velocity of DPPH radicals elimination decreased with increasing time of analysis. In the first 15 min, the elimination was fast with a high capacity of elimination of DPPH radicals (48–61%). Pękal et al. (2011) observed similar behaviour, in which DPPH radicals decreased in the first minutes followed by a low elimination of the reactive. Lignins with a low percentage of phenols showed the highest elimination of DPPH radicals, which was also observed by Pękal et al. (2011). Moreover, pH did not influence the total phenols content.

The capacity of elimination of DPPH radicals was 50% lower than the capacity of elimination of ABTS radicals. According to Huang et al. (2005) and Arshanita et al. (2013), this behaviour is related to the mechanisms of radicals catching for the lignins. Moreover, according to López-Alarcón and Denicola (2013), a low correlation between DPPH and ABTS tests is observed due to the dependence of the index in relation to the experimental conditions and complexity of mechanisms of reaction.

3.3. Molecular weight distribution

The results of gel permeation chromatography (GPC) showed that weight-average (M_w) of the lignins were more distinct and showing values between 3522 and 6760 g/mol (Table 3). LKH 6 showed the highest value of M_w while LKS 2 presents the lowest value. In general, at higher pH the fraction of lignin that precipitates has higher M_w as this fraction is easier to destabilize. Moreover, the proportion of hemicelluloses that precipitates is higher at higher pH what makes the M_w of the solid precipitated to be higher.

On the other hand, similar distribution for the polydispersity (M_w/M_n), which remained between 5.06 and 6.73, was observed. It can also be observed that M_w of LKH lignins is higher than LKS lignins and in both cases the lowest M_w lignin was obtained at pH 2, and this lignin has also the lowest polydispersity

Table 3Average values of M_w (weight-average), M_n (number-average) and M_w/M_n (polydispersity) of the precipitated lignins.

	M_w	M_n	M_w/M_n
LKS 2	3522	695	5.06
LKS 4	5741	852	6.73
LKS 6	4575	750	6.10
LKH 2	4993	866	5.76
LKH 4	5798	895	6.47
LKH 6	6760	1054	6.41

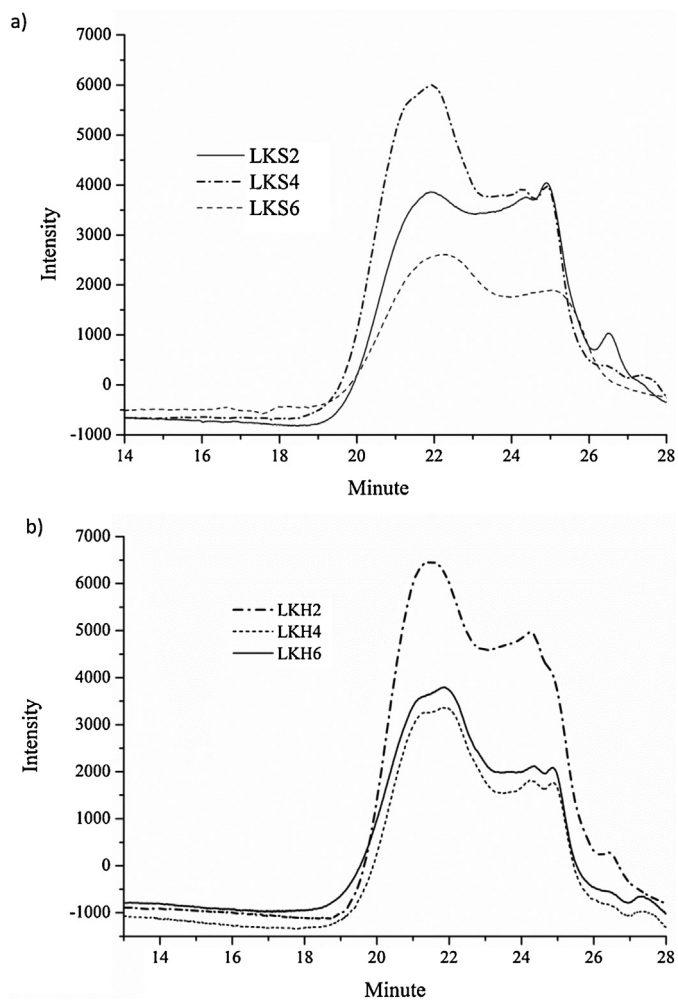


Fig. 1. Gel permeation chromatography analysis of Kraft lignin precipitated with different pH. A. Kraft lignin precipitated with H_2SO_4 . B. Kraft lignin precipitated with NaCl.

As it can be observed in Fig. 1A, otherwise, LKS 6, which presented the lowest purity (28.38% of total lignin), showed the lowest intensity of peaks due to the high concentration of salts.

3.4. X-ray diffraction

Crystalline phase in Kraft lignins precipitated with sulphuric acid was detected (Fig. 2A) due to the formation of Na_2SO_4 salts as described in Card number 37-1465 of Joint Committee on Powder Diffraction Standards (JCPDS). Moreover, elemental-S was detected in LKS 2 as described by JCPDS. Addition of acid in the sample results in an unstable environment and, consequently, in the precipitation of other types of salts.

Likewise, crystalline phase in the lignins precipitated with hydrochloric acid was observed, which was specifically related to sodium chloride (NaCl) (Fig. 2B). Moreover, interferences in some regions without significant peaks were detected, proving that this material has an amorphous characteristic (González et al., 2007; Buranov and Mazza, 2008; Lu et al., 2012)

3.5. Infrared spectroscopy (ATR-IR)

As it can be observed in Fig. 3, lignins showed a similar spectrum with slight variations in some peaks. In general, all the spectra presented characteristic peaks of lignin, such as at 3300 cm^{-1} referred to OH stretching, which was lower in LKS 4 at $2950\text{--}2800\text{ cm}^{-1}$

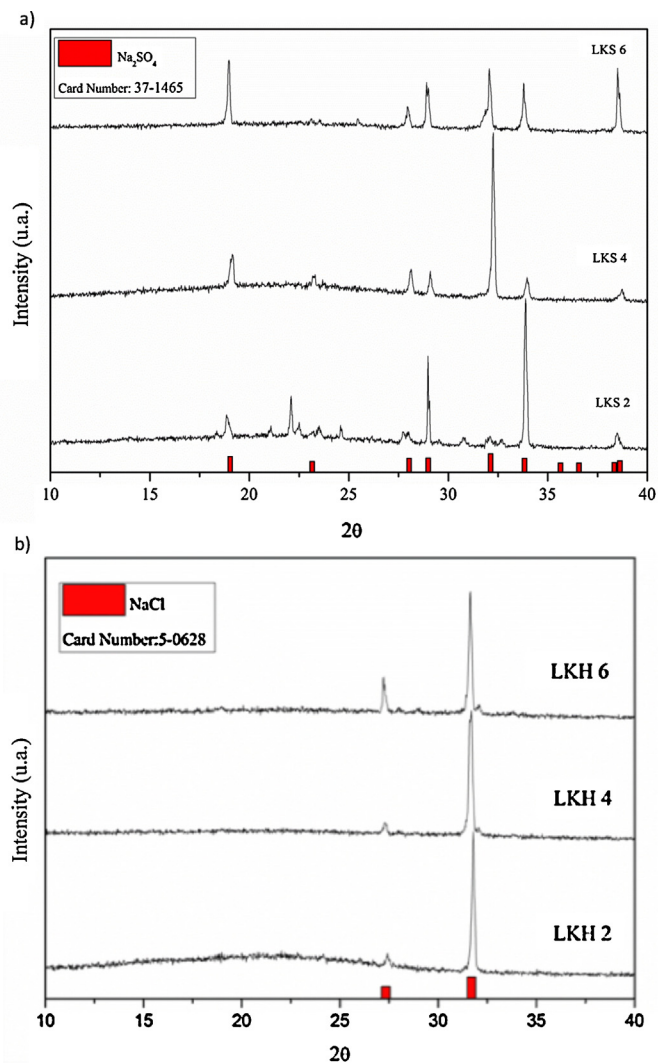


Fig. 2. X-ray diffraction spectra of Kraft lignin precipitated with different pH. A. Kraft lignin precipitated with H_2SO_4 . B. Kraft lignin precipitated with HCl.

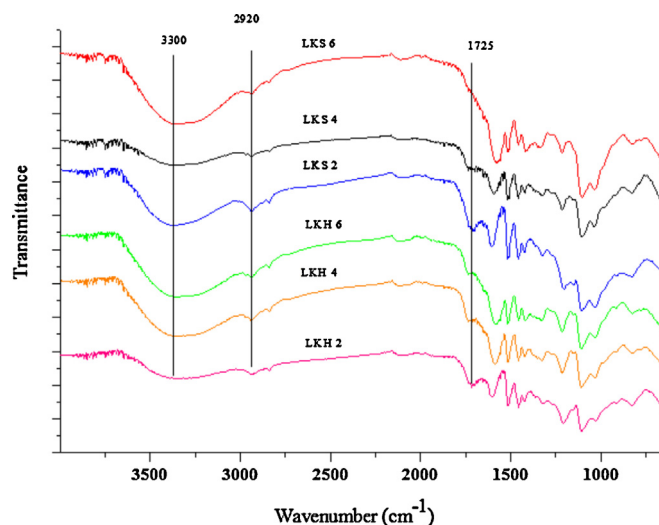


Fig. 3. ATR-IR spectra of Kraft lignin precipitated with different pH.

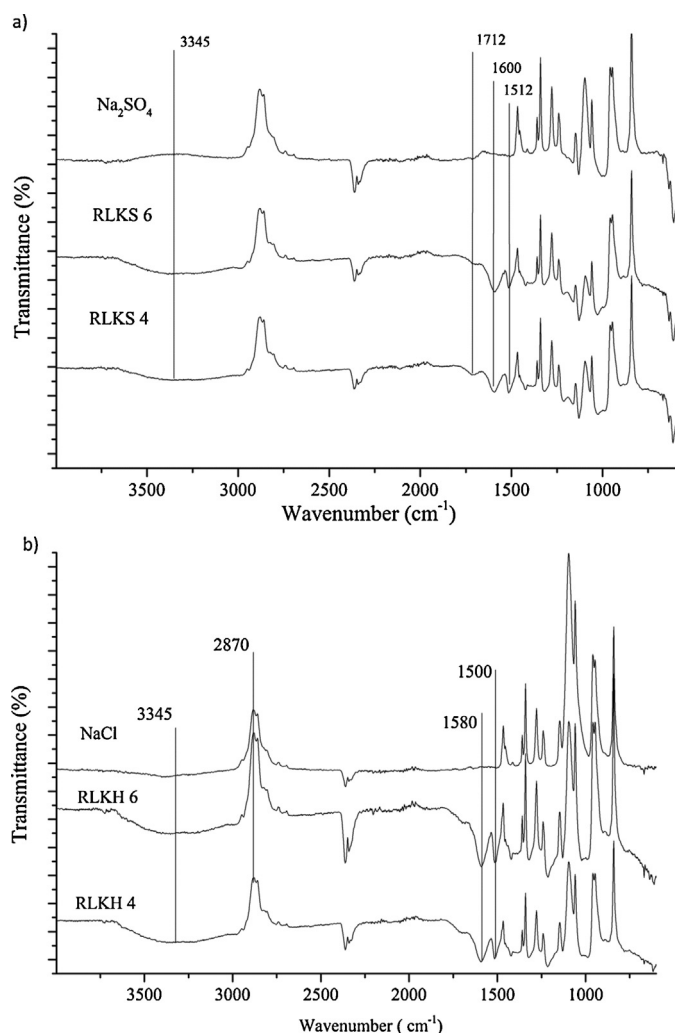


Fig. 4. ATR-IR spectra of commercial salts of Na_2SO_4 , NaCl and Kraft lignin precipitated with different pH. A. The residue RLKS 4, RLKS 6 of Kraft lignin precipitated with H_2SO_4 and Na_2SO_4 salt. B. The residue RLKH 4 and RLKH 6 of Kraft lignin precipitated with HCl and NaCl salt.

(C–H extending in methylene groups presented in different quantities). The peak at $1730\text{--}1712\text{ cm}^{-1}$, which is related to conjugated carboxyl groups, in LKS 6 was lower than those observed for other lignins; according to Cademartori et al. (2013) carboxyl groups are the most abundant functional groups in hemicellulose. Moreover, vibration of aromatic structures (1515 and 1438 cm^{-1}), syringyl (1110 and 1330 cm^{-1}) and units of guaiacyl (1023 cm^{-1}) can be observed in all samples.

Spectra of residues precipitated after washing Kraft lignins with ethanol are shown in Fig. 4. Kraft lignins precipitated at pH 2 did not produce a sufficient quantity of salts for infrared spectroscopy analysis so only residues from lignins at pH 4 and 6 are presented.

In general, chemical structures of the residues from lignins precipitated with sulphuric were similar to Na_2SO_4 . According to Prado et al. (2012), formation of sodium sulphate occurs when lignin contain sodium and is precipitated with sulphuric acid.

Residues of LKS 6 and LKS 4 showed peaks characteristic to lignin at 3300 cm^{-1} referred to OH stretching, at 1712 cm^{-1} due to the presence of conjugated carboxyl groups, in which peak intensity in LKS 6 was lower than the intensity found in LKS 4. Peaks at $1600\text{--}1500\text{ cm}^{-1}$ referred to aromatic skeletal vibration and peaks at 1110 cm^{-1} related to asymmetric stretching of SO_4 groups were observed in both samples.

Chemical structures of residues of LKH 6 and LKH 4 were similar to NaCl salt structure, in the band of 2870 cm^{-1} regarding the presence of NaCl . As observed for spectra of LKS, LKH6 and LKH 4 spectra showed characteristic peaks of lignin, such as at 3345 cm^{-1} (OH stretching), 1712 cm^{-1} (presence of conjugated carboxyl groups) and $1600\text{--}1500\text{ cm}^{-1}$ (aromatic skeletal vibration).

In this study, gradient acid Kraft lignin precipitation was studied with the aim to determine the most economical and easy method in order to obtain suitable the lignin to be used as a source of new products. Through selective precipitation is possible to obtain lignin in more or less pure form, keeping the main structure of phenolic groups, primary and secondary alcohols, methoxyl groups, carboxylates, and carbonyls.

4. Conclusions

In order to determine the best precipitation method with different acids is necessary to consider several factors, first the quality of the product we are producing, then the yield on the methods. Taking into account these two factors can be observed that the sample that had the best quality (LKH 2) which was precipitated with hydrochloric acid did not present the best yield in quantity of lignin precipitated. It showed the best results for, soluble and insoluble lignin percentage and had lowest salt content in relation to the other lignins. In the GPC was possible to observe a similar polydispersity (M_w/M_n) regardless the pH or the acid used for the precipitation. However, lignins precipitated with HCl had higher M_w than H_2SO_4 ones. The method of X-ray diffraction is a good method to observe the nature of the sample contamination lignin, since because the amorphous nature of the material, not shown diffractogram peaks represent the crystalline portion, which show that the sample has salts, because contamination could be determined by FTIR bands representing the salts found in the precipitated lignin samples with different acids.

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