ISOLATION AND CHARACTERIZATION OF LIGNIN FROM *STIPA TENACISSIMA L.* AND *PHOENIX DACTYLIFERA*

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This paper is devoted to the isolation of lignin from *Stipa tenacissima L.* and *Phoenix dactylifera* by three different processes, namely, CIMV, MILOX and Acetosolv, and its characterization. The Acetosolv process was found to be the most efficient cooking process, as deduced from the combined severity values and the pulp yield. The UV spectroscopy revealed that the lignin obtained from *Phoenix dactylifera* is more pure than that arising from *Stipa tenacissima L.* In fact, the latter presents higher amounts of carbohydrate impurities. Also, several techniques were used in order to characterize the obtained lignin from the two raw materials, namely: ^{13}C-NMR and FTIR spectroscopy. Moreover, the chemical study of the structure of lignin isolated from *Stipa tenacissima L.* demonstrated that it is composed of HGS type and characterized by a high content of syringyl units. Instead, the lignin isolated from *Phoenix dactylifera* presents a higher content of the guaiacyl units.

Keywords: *Stipa tenacissima L.*, *Phoenix dactylifera*, lignin, NMR, UV spectroscopy

INTRODUCTION

Lignin is a renewable material obtained in huge quantities as a by-product of the pulp industry.[1-3] Until now, it has been mainly used as a fuel and only a small amount is isolated and commercialized. Nevertheless, worldwide, this amount is estimated at 2% and corresponds to 1 million tons per year.[4-6] Additionally, based on its interesting functionalities and properties, lignin offers a perspective for higher value-added applications. The interest in developing lignin-based applications is nowadays driven by three important factors, namely (i) the availability of new lignin sources, such as, sulfur-free, organosolv, steam explosion lignins, etc. (ii) the growing interest in the biorefinery concept, where lignin valorization is becoming relevant, because it is the only bio-sourced molecule containing aromatic moieties, and (iii) the approach of sustainable chemistry, where green processes and bio-based products are in focus.

However, the most difficult step of obtaining lignin residue is the isolation approach.[7-8] In fact, the choice of the lignin isolation method depends on many parameters, such as the process, the raw material (wood, annual plant, non-wood), etc. This is the reason why a large number of publications and patents aiming at studying the isolation of lignin from annual plants or/and agricultural wastes is available in the literature.[4,9-13]

In Tunisia, the date palm (*Phenix dactylifera*) is one of the most cultivated plants and more than 4 million palms cover about 32 thousand hectares, as reported by the Tunisian Ministry of Agriculture. This culture produces a huge amount of date palm rachises, which are left on the soil to biodegrade thus fertilising it and/or are incinerated for energy recovery purposes.[14-16] This plant presents a high amount of lignin as described previously and has not been extensively studied.[15] On the other hand, *Stipa tenacissima* is a widely available lignocellulosic biomass in Tunisia, which occupies more than 400 thousand hectares distributed especially in Kasserine (80%), Sidi Bouzid, Gafsa (19%), and Kairouan (1%).This annual plant is an important source for

the Tunisian pulping industry, since it contains about 70% holocellulose and 25% lignin.[17] It is easy to cook using the soda process (13 minutes at 160 °C), which is suitable for annual plants. In most cases, after cooking, the dissolved lignin (about 25% w/w with respect to the initial oven dried (o.d.) raw material) is burned for energy recovery.

To the best of our knowledge, there are no data reporting the investigation of the isolation and the characterization of lignin from *Stipa tenacissima* and *Phoenix dactylifera*. In this study, three different delignification processes were established to isolate different lignins from the two materials, namely: CIMV, MILOX and Acetosolv. Then, the obtained compounds were carefully characterized following common standards.

EXPERIMENTAL

Materials

The *Stipa tenacissima L.* (AL) and *Phoenix dactylifera* (Pal) used in this work were collected from Gafsa (August 2011). They were washed, rinsed with distilled water, in order to eliminate sand and contamination, then dried under atmospheric conditions. The obtained materials were milled and sieved to particle sizes in the range between 200 µm and 1 mm. Prior to isolation of lignin, the dried powder of AL and Pal was dewaxed with ethanol-toluene (1:2, v/v) extraction, using a Soxhlet apparatus for 6 h, and then air dried.

Fractional isolation of lignin

Figure 1 displays the isolation pathways leading to the preparation of different lignins from the two studied raw materials.

During this study, three different processes, namely, CIMV, MILOX and Acetosolv, were carried out to extract various fractions of lignin from (AL) and (Pal). Since these processes are not very common, a brief description of each one of them is given below.

- Milox process.[18] The extraction involves the use of peroxyformic and formic acids. This technique consists in forming peroxyformic acid by reacting formic acid with hydrogen peroxide. The protic character of formic acid is sufficient to break the bonds between lignin and polysaccharides. The implementation of this method allows the separation of lignin at 107 °C, under atmospheric pressure during 4 to 5 hours. Then, a bleaching step is carried out to eliminate the residual lignin from the cellulose fibers. Finally, a filtration step is performed in order to obtain the black liquor solutions, from which lignin samples were precipitated. All the lignins prepared using the Milox process were obtained by repeating the experimental procedure at least three times.

- Acetosolv process.[19,20] The Acetosolv method consists in extracting lignin by treating the raw material by acetic acid (90%) containing 0.1% hydrochloric acid. As described in the case of the Milox process, peroxyacetic acid is also formed in this process. The reaction is conducted under atmospheric pressure at 80 °C during 5 hours. The prepared lignin is also precipitated in water and the experiment was repeated at least three times.

- CIMV process.[21,22] CIMV is derived from the Formacell process, which uses acetic acid/formic acid and water in various ratios at high temperature and under different pressures. The difference between this process and the ones described before is that this method can offer a high lignin quantity. The CIMV process uses the same reagent as Formacell process at low temperature. The lignin extraction is accomplished at a temperature range between 105 and 110 °C, under atmospheric pressure, during 3 h, using a 30/55/15 v/v/v ratio of acetic acid/formic acid/water. In fact, in this process, acetic acid is a solvent for lignin and hemicelluloses, whereas formic acid is the chemical agent that plays the role of catalyst to break the ether and ester bonds between polysaccharide and lignin. The addition of water allows the elimination of hemicelluloses and lignin. The precipitation and isolation of the lignin samples is performed by filtration. The experimental tests were repeated at least three times.

Characterization of the ligninfractions

FTIR analysis

FTIR spectra of the different lignins were collected on a FTIR spectrophotometer (Bruker), by preparing KBr pellets using a lignin concentration of 1% (w/w). Spectra were recorded between 400 and 4000 cm^{-1} at a resolution of 4 cm^{-1}. The recorded spectra were the average of sixteen scans. To avoid pellet moisture contamination, the following procedure was followed: (i) the lignin fraction and KBr were left during 24 hours at 40 °C under reduced pressure before pellet preparation; (ii) pellets were subjected to the same conditions during 12 hours before being submitted to FTIR analyses.

NMR analysis

^{13}C-NMR samples were prepared by dissolving 300 mg of lignin (acetylated or non-acetylated) in 4 mL of dimethyl sulfoxide-d_6 (DMSO-d_6, 99.8%), using a Bruker AV III 400 MHz NMR spectrometer. The acetylation reaction was performed without solvent, using a weight ratio of acetic anhydride to lignin of 2:1 and 1-methyl imidazole as catalyst (0.05 mL per g lignin). The reaction was conducted at 50 °C, under a stream of nitrogen and vigorous stirring during one night. The reaction was then quenched with ethyl ether (100 mL) and the ensuing mixture was washed five times with deionised water (50 mL for each time). Cyclohexane (100 mL) was finally added to the ether

phase to precipitate the lignin derivative. The lignin samples were recovered by filtration and then freeze dried. The final samples were stored in a desiccator at room temperature.

UV analysis

The UV spectra of the various lignins were recorded on an ultraviolet/visible spectrophotometer (Tech comp, UV 2300). The different fractions of lignin samples (5 mg) were dissolved in 95% (v/v) dioxane aqueous solution (10 mL). About 1 mL aliquot was diluted to 10 mL with 50% (v/v) dioxane aqueous solution and the absorbance between 250 and 400 nm was recorded and measured. The measurement tests were performed at least five times.

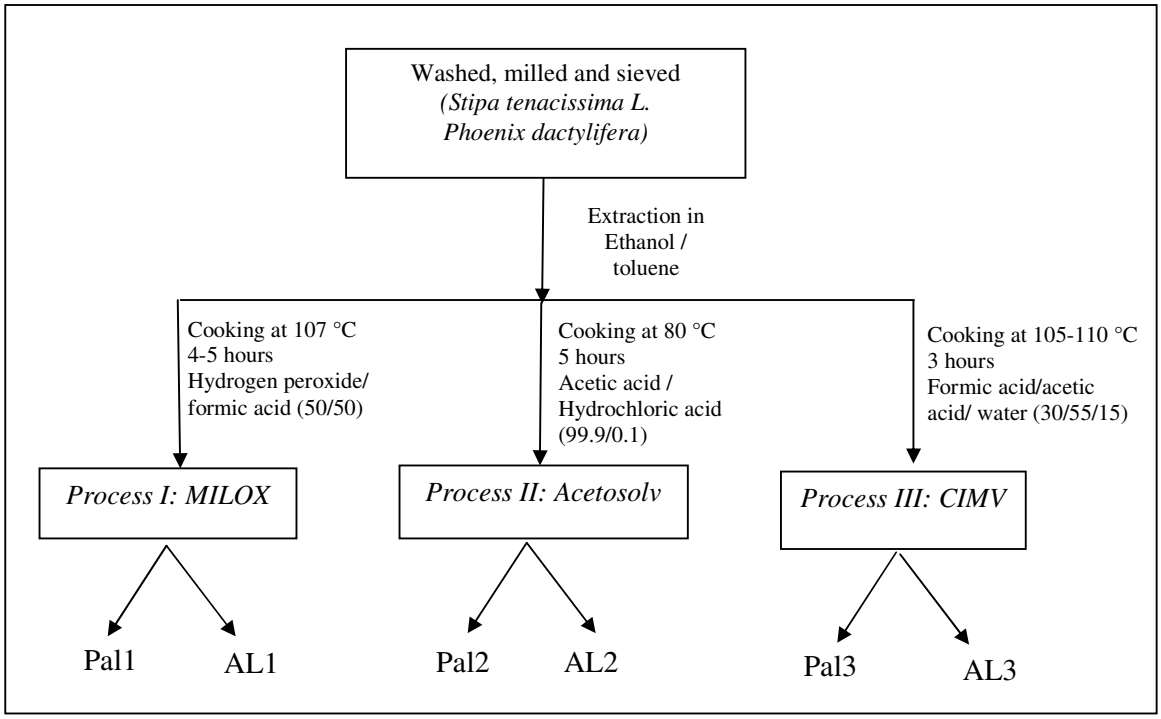

Figure 1: Treatment of samples

Combined severity

Due to the pre-treatment being performed under acidic conditions, the effects of pH were taken into consideration by the combined severity,[23] as follows:

Combined severity (CS) = $\log(R_0) - pH$ (1)

where:

$$\log(R_0) = 0.67 \times \log(t.e^{(T-T_{Ref})}); \quad (2)$$

T_{Ref}= 25°C

The combined severity correlates the reaction efficiency with the reaction time, temperature and pH.

RESULTS AND DISCUSSION

Lignin extraction efficiency

Table 1 reports the yield of the extracted lignin. The percent of lignin recovered for all the six obtained fractions (Pal1, Pal2, Pal3, AL1, AL2 and AL3) has been calculated with respect to the percentage of Klason lignin, which was previously established at 17% and 23%, for Pal and AL, respectively. It is obvious that the use of different processes played an important role in lignin extraction. In the case of *Phoenix dactylifera*, the maximum lignin extraction using the CIMV process was found to be relatively high, i.e. 65, 82 and 94% for Pal1, Pal2 and Pal3, respectively. However, in the case of *Stipa tenacissima L.*, the extraction efficiency associated with the Acetosolv and CIMV processes was better than that for Milox, and reached 65%. Thus, this part of the study indicates clearly the effect of the process on lignin isolation.[6]

Table 1
Yield determination of isolated Klason lignins

	Phoenix dactylifera			*Stipa tenacissima L.*		
Process	Milox	Acetosolv	CIMV	Milox	Acetosolv	CIMV
Designation	Pal1	Pal2	Pal 3	AL1	AL2	AL3
Kalson lignin (g)	1.7	1.7	1.7	2.3	2.3	2.3
Extracted lignin (g)	1.11	1.40	1.60	0.92	1.50	1.43
Yield (%)	65	82	94	40	65	62

Each measurement is replicated 3 times; the deviation between the experimental values does not exceed 5%

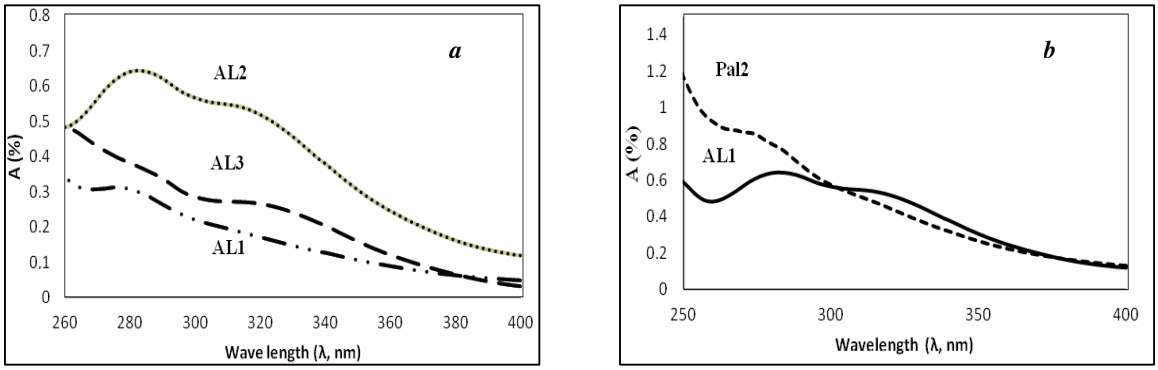

Figure 2: UV spectra of (a) AL1, AL2 and AL3 lignin and (b) AL2 and Pal2 lignin

Purity of extracted lignin

Dioxane-water solutions of the six acid-insoluble lignin fractions having the same concentration were prepared and subjected to UV-vis absorption measurements at λ= 250-400 nm. The intensity of the absorbance is related to the level of lignin concentration, which gives an indication of its level of purity. Figure 2a represents the spectrum of acid-insoluble AL lignin fractions, isolated by the three processes. The spectra present a typical profile of lignin in the zone between 270 nm and 320 nm. In particular, two characteristic peaks are present: the maximum absorption at 280 nm, which originates from non-conjugated phenolic groups in the lignin, and the second characteristic region of lignin absorption at around 318 nm, which is usually assigned to the presence of both ferulic and p-coumaric acids.[24,25] Interestingly, the same curve was observed in the case of Pal samples (Fig. 2b). Therefore, it can be also deduced that the high absorption coefficient was attributed to the AL2 fraction, suggesting that the most pure lignin preparation can be obtained when the lignin is extracted by the Acetosolv process.

On the other hand, Figure 2b shows that *Phoenix dactylifera* lignin is of higher purity than that isolated from *Stipa tenacissima L.* (the lowest absorption coefficient of AL2 fraction). These results indicate the presence of some bound hemicelluloses and other non-lignin materials to lignin macromolecules.

Combined severity (CS)

The combined severity of all prepared lignins (Pal1, Pal2, Pal3, AL1, AL2 and AL3) was determined and the obtained results are shown in Figure 3. As described before, the combined severity value[26] strongly depends on the used process and is a function of the treatment temperature and the pH of the medium. The CS varied from 7.5 to 11.7 and the lowest CS value corresponded to AL2 and Pal2 samples associated to lignin isolated by the Acetosolv process, as previously reported for other raw materials.[27,28]

Thus, Acetosolv can be considered as the best process for lignin extraction, at least regarding *Stipa tenacissima L.* and *Phoenix dactylifera* lignocellulosics, both in terms of yield and purity.

FT-IR analysis

The infrared spectra of Pal2 and AL2 lignin samples obtained by the Acetosolv process are presented in Figure 4a and the corresponding band assignments are given in Table 2. All lignin

samples presented a broad band attributed to OH stretching (3412-3460 cm^{-1}), and peaks corresponding to the C-H stretching of methyl and methylene group (2842-3000 cm^{-1}) and methyl group of methoxyl (2689-2880 cm^{-1}). The most characteristic vibrations of lignin correspond to those of aromatic rings at approximately 1600 cm^{-1}, 1513 cm^{-1} and 1420 cm^{-1}. These bands were present in the spectra of AL2 and Pal2 samples, although with different intensities.

The differences among lignin samples became more evident in the region between 1750 and 1625 cm^{-1}, corresponding to the C=O stretching of carbonyl moieties and in the region below 1430 cm^{-1}. It can be noticed that the spectrum also shows similar features with those observed for hardwoods. The band assignments were inspired by literature data.[29-33] The IR absorption peak intensity of hardwoods is as follows: I(1455-1465) > I(1500-1515), I(1220-1230) > I(1266-1270) and I(1121-1125) > I(1030-1040). In the case of Pal2, the IR absorption peak intensity is as follows: I1465 > I1511, I1225 > I1272 and I1125 > I1037. Whereas in the case of AL2 lignin, the IR absorption peak intensity is the following: I1461 < I1517, I1247 > I1270 and I1125 < I1037, indicating, as expected, that this lignin originates from annual plants.

Figure 4b shows the spectral region below 1430 cm^{-1} and displays much more complex spectral behaviour, due to the contributions of various vibration modes. Nevertheless, it is possible to identify the characteristic bands of syringyl (S), guaiacyl (G) and p-hydroxyphenyl (HGS) units, which confirms the type of the most relevant units in each lignin sample.

The presence of HSG units in two spectra is also evident from the high intensity of the bands at 1170 cm^{-1} and 840 cm^{-1}. The strong intensities of the bands at 1334, 1231, 1125 and 1462 cm^{-1} are associated with syringyl structures in lignin macromolecules. In fact, these bands are much stronger in the Pal2 spectrum, indicating that the Pal2 skeleton contains more S units.[34] The relatively weak intensity of the band at 1039 cm^{-1} in AL2 spectrum and that at 1045 cm^{-1} in Pal2 counterpart corresponds to the guaiacyl units, which confirms that the two lignins are of the HGS type.[29] Finally, it can be mentioned that the absorbances near 1000, 1125, 1040, 1095 cm^{-1} are typical of xylem units.

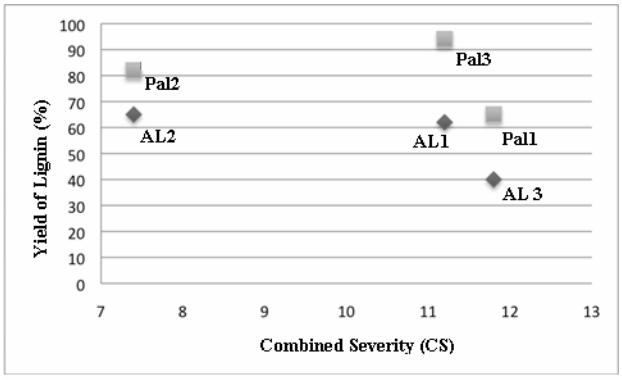

Figure 3: Evolution of lignin content as a function of combined severity (CS)

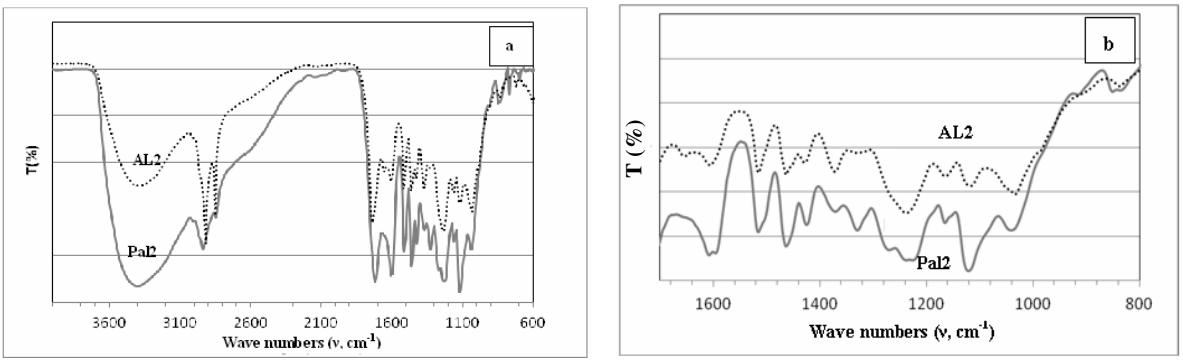

Figure 4: FTIR spectra of *Stipa tenacissima L.* and *Phoenix dactylifera* lignins

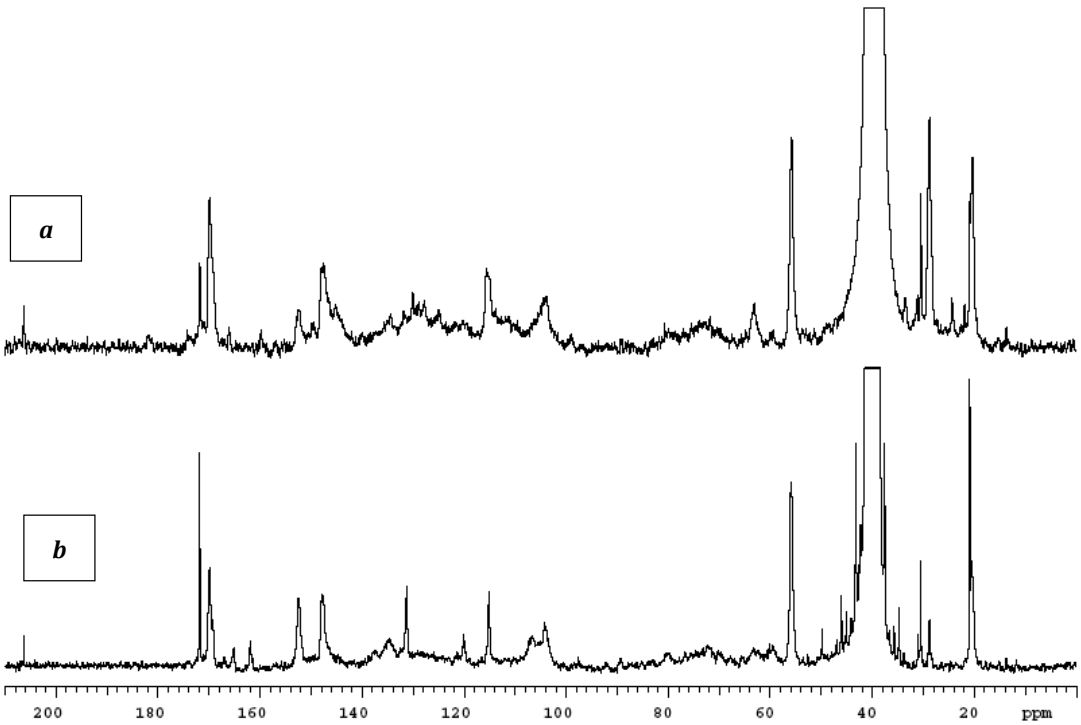

Figure 5: ^{13}C-NMR spectra of (a) *Stipa tenacissima L.* and (b) *Phoenix dactylifera* lignins

NMR analysis

The ^{13}C-NMR spectrum of the lignin isolated using the Acetosolv process was recorded, as shown in Figure 5. Carbonyls acetylated fractions are calculated by integrating the signal in the chemical shifts region between 168.5 and 172 ppm. In fact, the chemical shifts region at 169.4-170.3 ppm is attributed to the aromatic acetyl carbons, whereas that ranging from 170.3 to 172 ppm is assigned to secondary and primary aliphatic acetyl moieties. These integrals are normalized to that of C6 units, which is observed at 99.6-159.7 ppm.[24] Therefore, it can be deduced that the two lignins are acetylated during the cooking process. The signal of C-γ in β-O-4 linkages of guaiacyl and syringyl groups appears in two positions, 59.6 and 63.1 ppm for non-acetylated and acetylated lignins, respectively. These signals are stronger in the case of AL2 lignin, compared to that associated to Pal2.

Table 3 summarizes the data concerning acetylated lignins and shows that secondary aliphatic carbonyls of AL2 are the most sensitive to acetic acid. From the ^{13}C-NMR spectrum, it can be observed that the most striking characteristic is the absence of typical polysaccharide signals, which can appear between 57 and 103 ppm. This proves the efficiency of lignin extraction using the Acetosolv process.[35] The signal from 104.24 ppm to 165.16 ppm is associated with the aromatic part of lignin. The syringyl (S) units were identified with the signals at 152.4 ppm (C-3 /C-5: S); 147.9 ppm (C-3/C-5: S non-etherified); 137.3 ppm (C-4: S etherified); 134.6 ppm (C-1: S etherified 134.3 ppm for AL2), 131.2 ppm (C-1: S non-etherified 131.9 for AL2) and 104.2 ppm (C-2/C-6 S 103.9 for AL2.[35] Guaiacyl (G) units gave signals at 147.5 ppm (C-4 G); 134.3 (C-1 G etherified) and 115 ppm (C-5 G); 149.4 ppm (C-3 G for AL2) and 145.1 ppm (C-4 G non-etherified AL2). The p-hydroxyphenyl (H) units appeared as a weak signal at 120 ppm (C-2/C-6, H). These signals confirmed that the lignin fraction could be assigned as HGS-lignin.

The assignment bands of p-hydroxybenzoic acid were attributed to signals at 161.9, 131.6 and 115.3 ppm for Pal2 and AL2. Moreover, the furelicacid was shown only for AL2 at 143.4 ppm (etherified) and 122 ppm (non- etherified).

Table 2
Assignments of FT-IR absorption bands (cm^{-1})

Lignin sample		References	
Absorption bands (cm^{-1})		Absorption bands (cm^{-1})	Assignments
AL2	Pal2		
3442	3445	3460-3412	OH stretching
2916	2930	3000-2842	C-H stretching in CH_3 or CH_2groups
2845	2850	2880-2689	C-H vibration of methyl group of methoxyl
-	2708	1738-1709	C=O stretching in unconjugated ketones, carbonyls and in ester groups
1740	1730	1675-1655	C=O stretching in conjugated p-substituent carbonyl and carboxyl
1680	-	1605-1593	Aromatic skeletal vibrations and C=O stretching ring
1517	1511	1515-1505	Aromatic skeletal vibration
1461	1465	1470-1460	C-H deformation asymmetric in CH_3 and CH_2
1425	1422	1430-1422	Aromatic skeletal vibration with C-H in-plane deformation
-	-	1370-1365	Aliphatic C-H stretching in CH_3; phenolic OH
1336	1334	1330-1325	Syringyl ring breathing with C=O stretching
1270	1272	1270-1266	C-C, C-O and C=O stretching, guaiacyl ring and C=O stretching
1230	1230	1230-1221	Guaiacyl ring breathing with C=O stretching
1147	1149	1140	Aromatic C-H in-plane deformation; typical of guaiacyl units
1125	1125	1128-1125	Aromatic C-H in-plane deformation typical ofsyringyl units; secondary alcohols and C=O stretching
1095	1090	1086	C-H deformation in secondary alcohols and aromatic ethers
1037	1037	1035-1030	Aromatic C-H in-plane deformation, guaicyl; C-O deformation in primary alcohols; C=O stretching
925	-	925-915	Aromatic C-H out-of-plane deformation
854	856	858-853	C-H out-of-plane in position 2,5 and 6 of guaiacyl units
-	840	835	C-H out-of-plane in position 2 and 6of syringyl, and all p-hydroxyphenyl units
-	-	815	C-H out-of-plane in position 2,5and 6 of guaicyl units

Table 3
Quantification of acetylated carboxyl groups of lignin obtained from *Stipa tenacissima L.* and *Phoenix dactylifera*

	Aromatic acetyl	Secondary aliphatic acetyl	Primary aliphatic acetyl
Pal2	0.04	0.08	0.05
AL2	0.02	1.04	0.04

The methoxyl groups of G and S were noticed at 55.9 ppm and 55.8 ppm for AL2 and Pal2, respectively. Furthermore, the ^{13}C-NMR spectra also indicated that β-O-4 are the major linkages: thus, in the case of Pal2, two peaks were noticed at 72.3 ppm (C-α) and 60.1 ppm (C-γ). However, for Al2, these two signals appeared at 71.7 ppm (C-α) and 59.3 ppm (C-γ). Moreover, the bands corresponding to β-β' (54.1 ppm), β-5 (53.4 ppm) and β-1 (77 ppm) linkages were detected with much lesser intensity. Therefore, it can be concluded that AL2 and Pal2 lignins were characterized by a high amount of (C-C) and β-O-4 bonds, respectively.[36]

CONCLUSION

Three processes, namely, CIMV, MILOX and Acetosolv, were investigated to extract the lignin from *Stipa tenacissima L.* and *Phoenix dactylifera*. The amount of lignin recovered depended on the experimental conditions used. The CIMV process gave a high lignin yield for each plant. The percent of lignin recovered, as well as the combined severity (CS) values, showed that the Acetosolv method is the most efficient and yielded the highest purity lignin. The analyses by FTIR and ^{13}C-NMR of the two lignin fractions isolated from *Stipa tenacissima L.* (AL2) and *Phoenix dactylifera* (Pal2) confirmed that both are of the HGS type, characterized by a low amount of sugars and xylose. Moreover, the quantification of lignin carboxyl groups showed that, during the cooking process, secondary aliphatic alcohols of AL2 are more acetylated than those arising from the Pal2 sample. Finally, it can be mentioned that the lignin fraction contained some amount of p-coumaric acid and ferulic acid, which differs significantly from those recovered from softwood and hardwood.

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