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Artigos

Structural characterization of the stem cell wall lignin of *Euterpe oleracea*

Caracterização estrutural da lignina da parede celular de *Euterpe oleracea*

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ABSTRACT

This paper aimed to characterize the lignin in the cell wall of *Euterpe oleracea* Mart. Samples from two regions of the base of the palm were acquired: outer and inner regions (OR and IR, respectively). The extraction with organic solvents and the treatment with 1% NaOH were carried out. Holocellulose, α-cellulose and lignin content were quantified, the latter also isolated by the Björkman method for the structural characterization using ^{1}H and ^{13}C NMR in the liquid state, as well as spectroscopic (FTIR) and chromatographic analysis. The extracted stem had 10.33% (OR) and 15.66% (IR) of the lignin. After the treatment with 1% NaOH, a reduction in these values was observed, and it was also possible to confirm this result through the decrease in the intensities of the signals referring to the functional groups linked to lignin and hemicellulose. The spectroscopic and chromatographic analyzes revealed GS-type lignin incorporated with *p*-hydroxybenzoic and protocatechuic acid in both samples. The main structural units present in the lignin were also observed, such as β-O-4 bond, β-O-4' Y-acetyl bond, guaiacyl unit, syringyl unit and *p*-hydroxybenzoate.

Keywords: Açaí; Benzoic acid derivatives; Monolignols; Spectroscopic analyses

RESUMO

Este trabalho teve como objetivo caracterizar a lignina na parede celular de *Euterpe oleracea* Mart. Foram adquiridas amostras de duas regiões da base da palmeira: região externa e região interna (OR e IR, respectivamente). A extração com solventes orgânicos e o tratamento com NaOH 1% foram realizados. O conteúdo de holocelulose, α-celulose e lignina foi quantificado, esta última também isolada pelo método de Björkman para a caracterização estrutural utilizando RMN de ^{1}H e ^{13}C no estado líquido, assim como análises espectroscópicas (FTIR) e cromatográficas. O caule extraído apresentou 10,33% (OR) e 15,66% (IR) de lignina. Após tratamento com NaOH 1%, observou-se uma redução desses valores, e foi possível confirmar esse resultado também através da diminuição nas intensidades dos sinais referentes aos grupos funcionais ligados à lignina e hemicelulose. As análises espectroscópicas e cromatográficas revelaram lignina do tipo GS incorporada com ácido *p*-hidroxibenzoico e protocatecuico em ambas as amostras. Também foram observadas as principais unidades estruturais presentes na lignina como ligação β-O-4, β-O-4' Y-acetiladas, spiro-dienona, unidade guaiacila, unidade siringila e *p*-hidroxibenzoato.

Palavras-chave: Açaí; Derivados de ácido benzoico; Monolignóis; Análises espectroscópicas

1 INTRODUCTION

The cell wall is a multimolecular system made up of cellulose, hemicellulose, lignin and *p*-hydroxycinnamic acids that act as anchors in this system. Lignin has been defined as a macromolecule of phenylpropanoid origin, constituted mainly by the subunits of *p*-coumaryl, coniferyl and sinapyl alcohols that encrustate the cell wall of plants, which form the *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) lignins by the combinatorial coupling of these subunits. Moreover, various monolignol conjugates have also been shown to participate in lignification (DIXIT; NAIR, 2014). For example, in some group of plants, γ-*p*-coumaroylated monolignols are produced and incorporated into the growing lignin polymer, particularly at advanced maturity. Conversely, *p*-hydroxybenzoylated lignins are found on others (RENCORET *et al*., 2013).

Although extensively investigated, the complex and irregular structure of the lignin is still not completely elucidated. Various spectroscopic and chromatographic methods such as infrared, ultraviolet-visible, Raman spectroscopy, nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) have been applied to analyze the lignin structure, even the lignin-carbohydrate complex (LCC).

In the last years, the monocot cell wall composition has received considerable attention from researchers who have aimed not only to improve lignin extraction processes, but also to elucidate the lignin structure, characterize the chemical reactivity, properties and the develop new applications. There is an increased interest in vegetal fibers from different segments of industry due to a high demand for new biomass resources. Some of the materials that have received increased research attention include palm leaves (HEGAZY; AREF, 2010), date palm branches (NEMLI; KALAYCIOĞLU; ALP, 2001) and wastes of oil palm fruit (SHINOJ *et al*., 2011; TRANBARGER *et al*., 2011). In countries where those materials have adequate supply, these results indicate that the usage of these resources is sustainable.

Knowledge about palm lignification may expand future pathways for an improved stem utilization, as well as higher value-added products. In this context, the palm stems that are often discarded in favor of other products may become an important raw material for a variety of purposes. The lack of information on the chemical composition, especially on palm lignin, makes this management an obstacle. Emphasis on cell wall lignin occurs due to the limiting factor that it imposes as a source of energy and nutrition for ruminants, as well as hindering the separation of the fiber cells, which is the main purpose of pulping.

Among the palms, *Euterpe oleracea* Mart., known as Açaí, is considered an important raw material for commercialization of its fruits, especially in the northern region of Brazil. This species, which belongs to the Arecaceae family, is native from the Amazon River basin, grows in groups of up to 20 stems and can produce up to 120 kg of fruits per year or even more under forest management system (OLIVEIRA; SCHWARTZ, 2018). It is characterized by being a fast growing palm tree, with the highest stems reaching 20 to 25 meters. The root system is called a pneumatophore, which allows it to adapt to seasonal flooding. The flowers are unisexual grouped in composite panicles, being from yellow brown to purple and the flowering occurs throughout the year with its peak between February and July (AMSELLEM-LAUFER, 2015).

From the Açaí palm, everything can be used: fruits and hearts of palm as food; dry, clean and polished stones/kernels in handicrafts (handmade jewelry); straws in house roofing and woven fibers for domestic use; the trunk, in the form of slats and rafters, on stilt houses; the new roots as a vermifuge; and trusses as garden brooms (TAVARES; HOMMA, 2015).

The aim of this paper was to characterize the lignin and phenolic acids, both constituents of the cell wall, in order to study the crosslinks between lignin and the carbohydrate-phenolic acid complex in the stem of *Euterpe oleracea*. To do so, we performed spectroscopic techniques such as infrared and nuclear magnetic resonance (NMR) and the chromatographic analysis for the structural characterization *of Euterpe oleracea* lignin.

2 MATERIALS AND METHODS

For the analyses, three 15 year-old specimens of *Euterpe oleracea* were collected in Paraty, Rio de Janeiro state, Brazil. They were processed and registered in the wood collection of the Forest Product Department, Federal Rural University of Rio de Janeiro, under the following registration numbers: tree 1 - 7639; tree 2 - 7640; tree 3 - 7641. The samples were taken from the base of the palm tree from two regions: outer region (OR - closer to the outermost peripheral region) and inner region (IR – closer to the center of the disc) for studying the lignin chemistry.

2.1 Preparation of the samples

Fragments from the outer and inner regions of *Euterpe oleracea* stem were air-dried and ground in a Wiley-type mill. Then, the material was sieved and the particles were extracted with cyclohexane, ethyl acetate and methanol in a soxhlet extractor for a period of 24 hours for each solvent (ABREU *et al*., 2006). Afterwards, a fraction of the extracted material was treated with 1% NaOH (TECHNICAL ASSOCIATION OF THE PULP AND PAPER INDUSTRY, 1979), totaling four treatments: extracted OR sample, extracted and 1% NaOH treated OR sample, extracted IR sample, extracted and 1% NaOH treated IR sample.

2.2 Chemical analysis

The material obtained from OR and IR samples were used for determination of lignin (Klason method), holocellulose (Chlorination) and α-cellulose (NaOH method), as described by Abreu *et al*. (2006). For qualitative analysis, the lignin was obtained using the Björkman method.

2.3 Infrared Spectroscopy

Infrared spectroscopy analyses were performed on milled stem and lignin powder of *Euterpe oleracea*. FT-IR spectra were recorded with a VARIAN 640-IR FT-IR spectrometer. Each sample was obtained in the 400 – 4000 cm^{-1} range, 128 scans and at a resolution of 4 cm^{-1}. The relative absorbances were calculated for the main absorption signals in order to compare the changes in functional groups with the changes in spectra after the alkaline treatment. The bands were normalized as the ratio of each absorbance to the absorbance at 1514 cm^{-1} according to the methodology followed by Vallejos *et al*. (2011).

2.4 ^{1}H and ^{13}C Nuclear magnetic resonance (NMR) of *Euterpe oleracea* lignin

The structural elucidation of the OR and IR lignin samples of the *Euterpe oleracea* was performed by nuclear magnetic resonance (NMR) for the spectroscopic technique one and two-dimensional carbon atoms (^{13}C NMR) and protons (^{1}H NMR). The acetylated lignin of OR and IR samples were dissolved in $CDCl_3$ (deuterated chloroform) and the ^{1}H and ^{13}C NMR spectra were recorded on Bruker Advance II 400 MHz device.

2.5 *HPLC analysis*

The extraction of phenolic acids from *Euterpe oleracea* stem was carried out as previously described by Geetha *et al*. (2011). In addition to the stem samples, the extracts obtained with ethyl acetate and methanol were also analyzed. The obtained solution was concentrated to dryness and the resulting residue was diluted with methanol for the chromatographic analysis.

The analyses were performed on HPLC (Shimadzu HPLC) with two LC-20AT pumps, PDA detector (Shimadzu), CTO-20A column furnace (Shimadzu) at 27°C and a DGU-20A3 controller system. A Phenomenex Luna C18 column (25 cm x 4.0 mm, 5 µm) was used. The samples were analyzed in an eluent system consisting of two solvents: solvent A (65% H_2O: 1% AcOH) and solvent B (35% MeOH).

A gradient program was adopted, starting with 80% solvent B for 2 min, 92% Solvent B for 4 min, and 35% Solvent B for 2 min. The flow rate was maintained at 1 ml/min and the injection volume was 20 µL. The wavelength for the detection was 200-500 nm and the column temperature was maintained at 27°C.

3 RESULTS AND DISCUSSION

The chemical composition of the samples from the outer region (OR) and the inner region (IR) is presented in Table 1. Cellulose, hemicellulose and lignin content correspond to 90% of the total mass of the stem, whereas the remaining 10% are composed of extractives. Holocellulose and α-cellulose contents of the OR and IR samples of the stem had similar average values between them. However, these results were superior to those found in several wood species and monocots.

Table 1 – Holocellulose and lignin content of the different samples of *Euterpe oleracea* stem

Sample	Holocellulose (%)	α-Cellulose (%)	Lignin (%)
OR Extracted	74,4	64,08	10,33
OR Extracted + NaOH 1%	80,48	64,92	8,5
IR Extracted	74,42	63,08	15,66
IR Extracted + NaOH 1%	79,15	68,17	11,33

Source: Authors (2017)

The extracted OR and IR samples had lignin content of 10.33% and 15.66%, respectively, which could be attributed to the maturation stage. The values were lower than other biomasses, such as Babassu endocarp (27.9%), giant bamboo (22.7%),

Eucalyptus grandis wood (23.9%) and *Pinus oocarpa* (25.1%) obtained by Silva, Barrichelo and Brito (1986), Marinho *et al*. (2012), Pereira *et al*. (2000) and Moraes, Nascimento and Melo (2005), respectively.

After the treatment with 1% NaOH, an increase of 6.08% and 4.73% (OR and IR, respectively) in holocellulose content occurred, but there was a reduction in the lignin content, resulting in the dissolution of 1.83% and 4.33% of OR and IR lignin, respectively, which allows to assume that part of the material was solubilized. This fact might be related to the lignin bonds of IR samples, which are relatively more labile between lignin monomers or between lignin and polysaccharides.

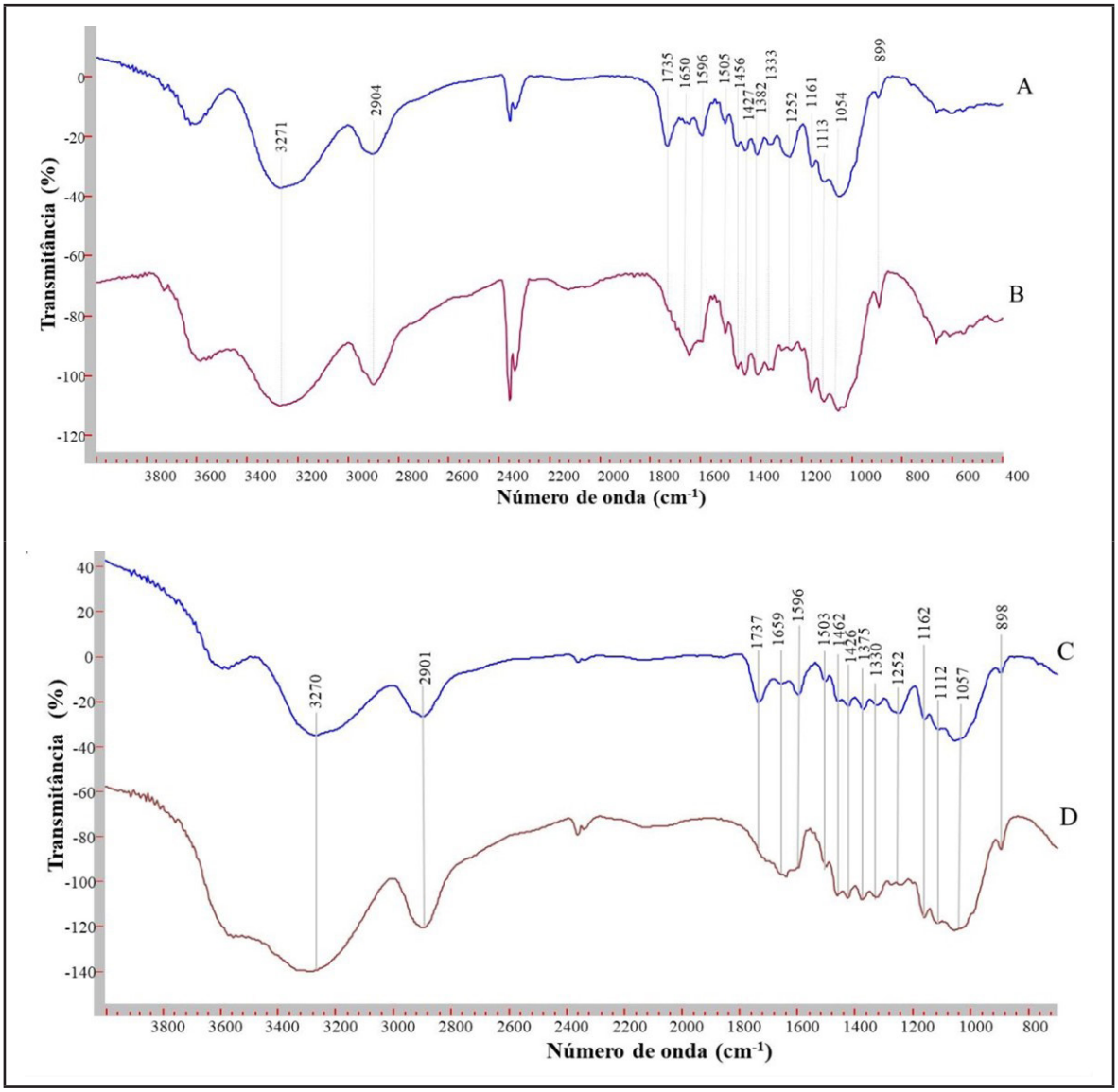
These results can be explained by the alkaline treatment that promotes the solubilization of hemicelluloses by the rupture and breaking of hydrogen bonds. Xiao, Sun and Sun (2001) state that these substances, when bound by ester groups, can be broken by a base. They also said that the high solubility of lignin and hemicelluloses can also be attributed to the breakdown of ester bonds between hydroxycinnamic acids, such as *p*-coumaric and ferulic acids. In *Euterpe oleracea* stem, when analyzed by spectroscopic technique (infrared and ^{1}H and ^{13}C NMR) and by HPLC-DAD, we managed to observe the presence of the *p*-hydroxybenzoic acid and protocatechuic acid, forming links between them and the lignin.

3.1 Infrared spectroscopy (FTIR) analysis of *Euterpe oleracea* stem

In this study, the samples extracted and treated with 1% NaOH of *Euterpe oleracea* (OR and IR) were submitted to FTIR analysis to identify the functional groups of lignocellulosic biomass and the changes caused by the pre-treatments. The spectra were similar, showing important regions of absorption of samples. The characterization of the absorption signals of the samples was performed mainly qualitatively by the comparison with the literature data, as described by, Xu *et al*. (2013) and Latif *et al*. (2015).

Spectra of both extracted OR and IR regions were similar and presented a strong stretching O-H signal in 3271-3270 cm^{-1}. The absorbance at 2904-2850 cm^{-1} was due to a strong C-H stretching in aromatic methoxy groups and in methyl and methylene groups in the side chains of lignin. We also observed a signal in 1737-1735 cm^{-1} in spectra attributed to ester C=O stretching certainly derived of acetyl groups linked to hemicelluloses (Figure 1).

Figure 1 – Infrared spectra of the extracted samples, extracted and treated with 1% NaOH, respectively, from the outer (OR) and inner (IR) regions of the stem of *Euterpe oleracea*



Source: Authors (2017)

In where: (A) Extracted outer region; (B) Extracted and treated with 1% NaOH outer region; (C) extracted inner region and (D) Extracted and treated with 1% NaOH inner region.

Signals from 1600 to 1450 cm^{-1} were related to vibration of the lignin aromatic skeleton. Signals at 1596, 1505-1503, 1427-1426 cm^{-1} arise from the vibrations of the lignin aromatic skeleton (-C = CC- and -C = CC = C- or binding of lignin monomers β-1, β -β, 5-5, and β-5). The absorption at 1462-1456 cm^{-1} was attributed to the asymmetric angular deformation of the methyl and methylene CHs groups. These signals can be seen more clearly after the lignin isolation by the Björkman method of *Euterpe oleracea* samples (OR and IR).

The signal at 1382-1375 cm^{-1} was related to C-H bending of CH_3 cellulose and hemicellulose groups, whilst 1225 cm^{-1} was assigned to C-O stretching of the phenolic hydroxyl group present in lignin. Other lignin-related signals observed in the spectra were at 1272 cm^{-1} for C=O-linked guaiacyl propane units (G) and 1329-1328 cm^{-1} for the increased propane syringyl units (S) linked to the condensed G-ring (i.e., G-ring substituted at position 5) (FAIX, 1991).

The Infrared Spectra Classification System of Lignin proposed by Faix (1991) allows us to assume that the Björkman lignin spectra from the OR and IR samples of *Euterpe oleracea* belong to the guaiacyl-syringyl (GS) type due to the following characteristics: the absorbance at 1462 cm^{-1} is more intense than the absorbance of the reference signal at 1505-1503 cm^{-1}; the signal at 1329-1328 cm^{-1} is more intense than the signal at 1272 cm^{-1}; the low signal at 1272 cm^{-1} (sometimes only shoulder-like peak appears); the high signal at 1125 cm^{-1} and the pronounced signal at 835 cm^{-1}.

In order to analyze differences in specimens of *Euterpe oleracea* stem samples after 1% NaOH treatment, the signals were normalized. This was done using the ratio of the absorption intensity of each signal to the signal absorption intensity of 1505-1503 cm^{-1} for OR and IR samples, respectively. This signal corresponds to the aromatic ring stretch, selected as an internal reference for its relatively constant intensity.

Most of the characteristic absorption of lignin and hemicellulose signals in samples treated with 1% NaOH were significantly weakened when compared to signals of extracted samples from *Euterpe oleracea* stem, suggesting that lignin and hemicellulose were partially degraded during the pre-treatment (Figure 1).

The most noteworthy changes that occurred in the samples after 1% NaOH treatment were the disappearance of 1737-1736 cm^{-1} peaks in both OR and IR and reduction of intensity in 1162-1161 cm^{-1} (asymmetric C-O stretching of ester groups) and 1059-1053 cm^{-1} (CO, C-C, C-OH stretching) also in both OR and IR samples (Figure 1). This result allows us to understand that a saponification reaction occurred during alkaline pre-treatment, breaking the ester bond between lignin and carbohydrates and releases cellulose from the lignin encapsulation, making the cellulose more exposed (HE *et al*., 2008).

The mechanism of alkaline hydrolysis occurs by saponification of intermolecular ester bonds, which crosslink xylan hemicelluloses and other components. For example, lignin and other hemicellulose. Durot, Gaudard and Kurek (2003) point out that alkaline treatment may release some cellulose and hemicellulose by the hydrolysis of the ester bonds between lignin and cellulose or hemicellulose.

3.2 ^{1}H and ^{13}C NMR analysis of the lignin solution in *Euterpe oleracea*

The ^{1}H NMR spectra of the *Euterpe oleracea* acetylated lignin (OR and IR) samples revealed to be similar and were compared to the literature data, which frequencies are grouped in the main regions of the lignin spectrum. Signals between 6.0 and 8.0 ppm are assigned to aromatic ring hydrogens in G and S units, where signals at 6.7 and 6.8 ppm represent aromatic hydrogens in G units, while signals in 6.6 ppm originate from units S (FAIX; GRUNWALD; BEINHOFF, 1992).

The higher intensity at δ_H 6.60 in comparison to δ_H 6.90 suggests that lignin was composed of a relatively higher proportion of syringyl than guaiacyl nuclei from both the OR and IR samples of *Euterpe oleracea*. These results were also found by Jin, Jin and Shao (2012) when studying the lignin isolation by the Björkman methodology of *Hibiscus cannabinus* (Kenaf) fibers.

The signals at δ_H 2.0, 2.1 and 2.2 correspond to the absorption of methyl adjacent to double bonds or carboxylic groups and the strong signals at δ_H 3,4 and 3,7 may be attributed to methoxyls (-OCH_3). δ_H 4.1-4.5 signals are compatible with C3 lignin unit H_α, H_β and H_γ. Lapierre, Lallemand and Monties (1982) state that the signal around 4,9 ppm represents H_α in bonds. β-O-4 and signals in the δ_H 4,6-4,7 and 4,0-4,5 region originates from H_β and H_γ from β-O-4' structures, respectively. The small signal at δ_H 7.9 was attributed to aldehyde hydrogens as in cinnamaldehyde and benzaldehyde structures.

Furthermore, the ^{13}C NMR spectra of *Euterpe oleracea* lignin samples (OR and IR) were also analyzed in solution with DEPTQ experiments. The spectra of the samples showed signals at 20-30 ppm were attributed to saturated carbon atoms in aliphatic chains. The C_γ, C_α and C_β signals of β-O-4 ether bonds appear in 62, 74 and 80 ppm, respectively.-

The ^{13}C NMR spectrum of *Euterpe oleracea* lignins (OR and IR) was relatively simple in the aromatic system region showing CH signals in 104 and 153, which can be attributed to C2/6 and C3/5 in syringyl units (S), which have symmetry. Their 2/6 carbons of the aromatic rings showed chemical displacement around 104 ppm and their 3/5 oxygenated quaternary carbons are at 153 ppm. This symmetry separates them from their guaiacyl counterparts (G), in which protonated carbons 2, 5 and 6 vary between 110 to 125 ppm, although there is some overlap with quaternary carbons 3 and 4, which vary from 145 to 147 ppm (JIN; JIN; SHAO, 2012). Spectra also showed a broad signal of 169-170 ppm in the ^{13}C spectra of *Euterpe oleracea* lignins (OR and IR), which suggests the presence of carboxyl carboxyl ester or aromatic carbons.

This analysis confirms the observations of infrared spectroscopy that the lignin of the stem is of guaiacyl-syringyl (GS) type. Besides that, the ^{13}C NMR spectra

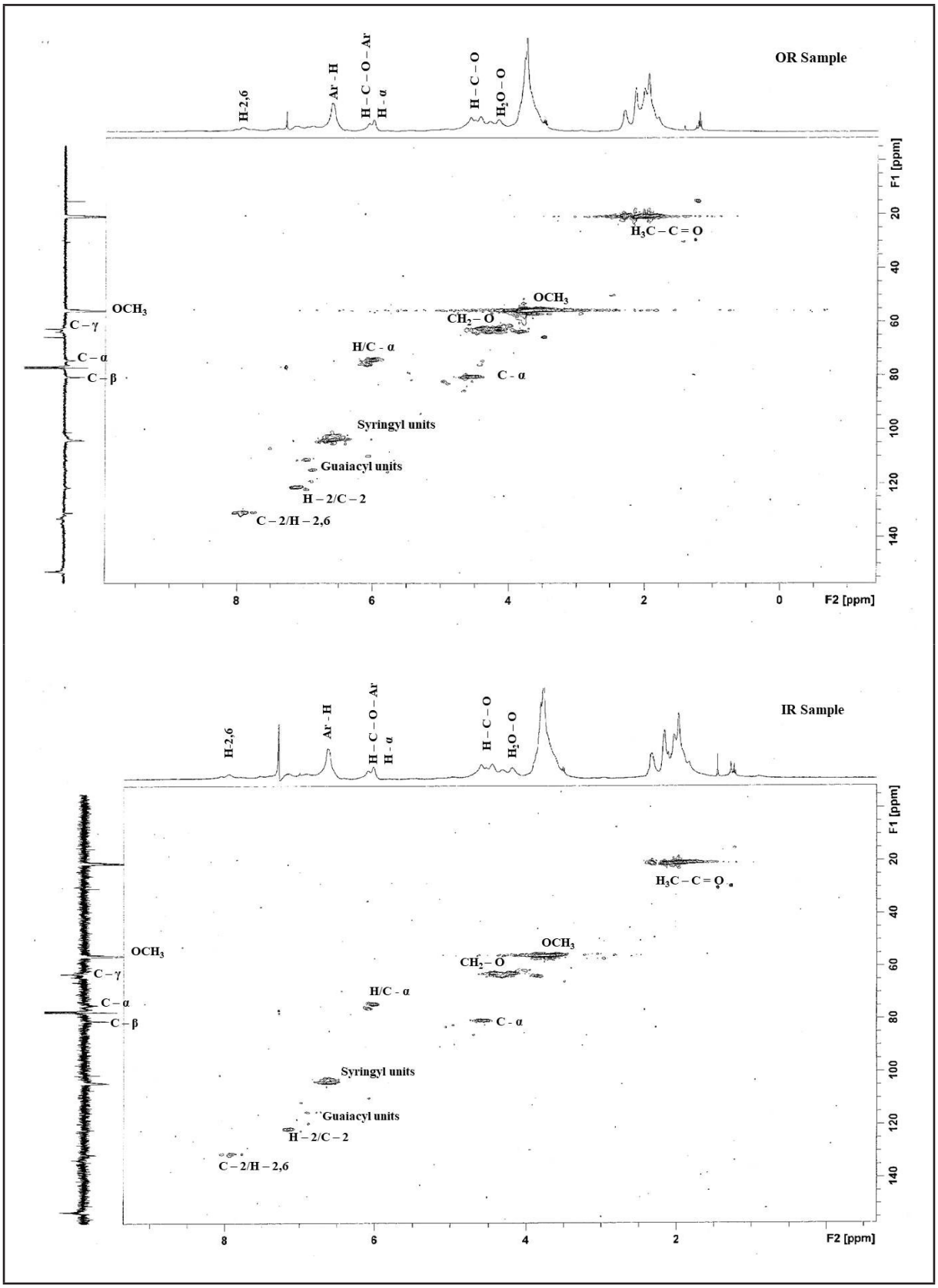
of OR and IR samples also showed signals in 111, 113 and 123 ppm, in which can be attributed the presence of the structure of benzoic acid derivatives in the cell wall of *Euterpe oleracea*. Lu *et al*. (2015) state that these structures are used as "monomers" in the lignification process in palm trees (as well as in willows, poplars and poplars) and that this process occurs through transferases that are responsible for the production of conjugates of *p*-hydroxybenzoates monolignols.

2D NMR experiments ($^{1}Hx^{13}C$-COSY) were also carried out, which was very useful to elucidate the chemical structure of the lignin analyzed. This technique has been used to identify new lignin structures and/or interpolymers crosslinking, as, for example, in the case of the so-called lignin-carbohydrate complexes that result from the lignin-polysaccharide coupling (MANSFIELD *et al*., 2012).

The HSQC spectra showed more correlations ^{1}H-^{13}C ($^{1}J_{HC}$) between the respective nuclei of lateral, oxygenated and non-oxygenated aliphatic chains, and aromatic rings (Figure 2). In addition to, the HSQC spectra of OR and IR showed interaction signals at a binding H2,6 and C2,6 (δ_H 6,8 with δc 104) (Figure 2) and the HMBC spectra of hydrogens 2,6 (δ_H 6,8) with C-3,5 (δ_H 153); C-1 (δ_C 134) and C-α (δ_{CH} 74).

The main $^{1}J_{H\text{-}C}$ interaction signals in the nuclei absorption region of aromatic systems of the HSQC spectra correspond to the benzene rings of the different lignin units, for example, S units, which presented a signal for $H_{2,6}/C_{2,6}$ correlation in δ_H/δ_C 6,6/104 ppm and G units in 7,2/120 for H_6- C_6 correlation, 6,9/113 of H-2/C-2 and 6,8/112 of H-5/C-5. Signal strength 7,6/104 reveals the large amount of S units in the lignin structure. Another significant sign in the absorption region of aromatic system nuclei was observed in correlations $H_{2.6}$-$C_{2.6}$ in 7.9/131 that was attributed to the substructure of sinapyl *p*-hydroxybenzoate (PB) (Figure 2).

Figure 2 – HSQC spectra (400/100 MHz, $CDCl_3$) of the fraction of acetylated lignin from the outer region (OR) and the inner region (IR) of *Euterpe oleracea*



Source: Authors (2017)

The analyses of the signals in the region of aromatic ring nuclei of the spectra revealed that the lignin fraction of the OR and IR samples of *Euterpe oleracea* can be considered as a GS-type lignin containing *p*-hydroxybenzoic and protocatechuic acid units, in which their presence was confirmed by chromatographic analysis. Other substructures and their respective assignments of the main correlations of the samples of the acetylated lignins OR and IR are listed in Table 2.

Table 2 – Assignments of 2D-HSQC NMR spectra signals from *Euterpe oleracea* acetylated lignin (OR and IR)

Label	$\delta_H/\delta/_C$ (ppm)	Assignments
OCH_3	3,7/56	C-H in methoxyls
A_γ	3,9/61 e 3,7/59	H_γ-C_γ in β-O-4 substructures
A'_γ	4,1 e 4,4/64	H_γ-C_γ in $_\gamma$-acetylated β-O-4′ substructures
$S_{2,6}$	6,6/104	H_2-C_2 and H_6-C_6 in S units
G_6	7,2/120	H_6-C_6 in G units
$PB_{2,6}$	7,9/131	H_2-C_2 and H_6-C_6 in *p*-hydroxybenzoate

Source: Authors (2017)

These results can provide important information regarding the recalcitrance of this macromolecule, since the knowledge of the structure of lignin. Such knowledge could establish new possibilities or a better usage of this raw material. An example of this are species with higher levels of guaiacyl units, because they have a carbon in the C-5 position available to make a very strong carbon-carbon bond, contributing to a higher degree of condensation of the lignin and consequently making it difficult to remove. More units of the syringyl monomers, or high S/G ratios provide high lignin reactivity, facilitating its removal in processes that have aimed for that objective.

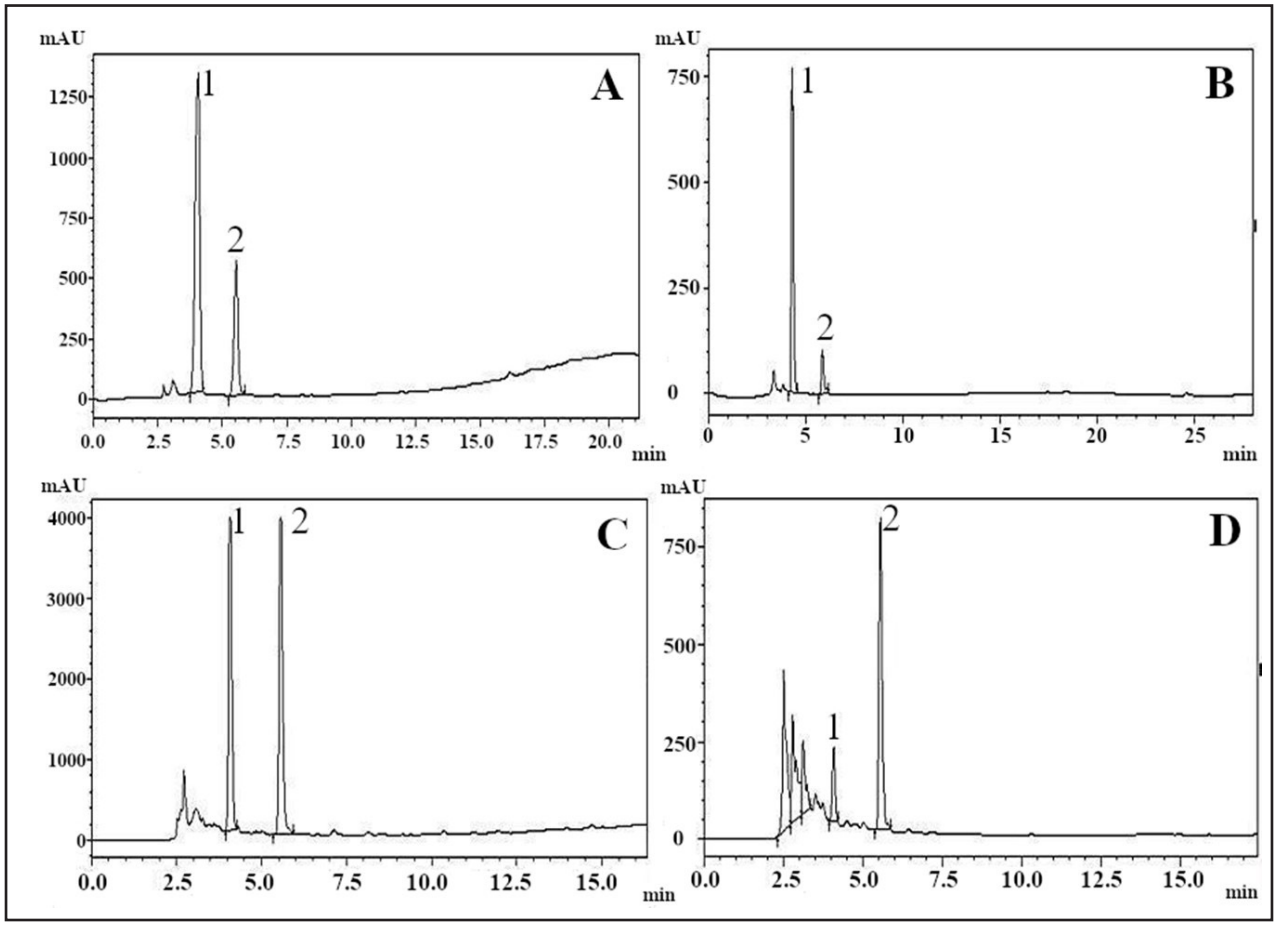
3.3 Chromatographic analyses of phenolic acids of extracts and stem of *Euterpe oleracea*

The acid identification was performed by comparing retention times (RT) in minutes, together with analyses of the respective UV absorption spectra compared to authentic chromatographic standard. Chromatographic analyses of *Euterpe oleracea* extracts obtained with ethyl acetate showed two peaks, and methanol extracts showed two peaks for the OR sample, while the IR sample presented five peaks, one of which was identified as protocatechuic acid (TR 4.060 min) and another identified as *p*-hydroxyhydrobenzoic acid (TR 5.543 min).

In each sample were observed peaks that allowed identification of the different concentrations of these phenolic acids. The presence of protocatechuic and *p*-hydroxybenzoic acids were observed in all samples, with protocatechuic acid being predominant in extracts obtained with ethyl acetate and *p*-hydroxybenzoic acid being predominant in methanolic extracts (Figure 3).

Phenolic acids may occur in plants in two different ways: a) Either in free forms and extractable from the matrix using organic solvents; b) or bound to the cell wall through ester or ether linkage. Several simple phenolic compounds occur in plants and they participate in plant-herbivore, plant-fungus and plant-plant interactions. Moreover, many of these acids serve as precursors of more complex compounds, while some of them play regulatory and metabolic roles.

Figure 3 – Chromatograms of phenolic acids found in extracts obtained with ethyl acetate and methanol in *Euterpe oleracea* (OR and IR)

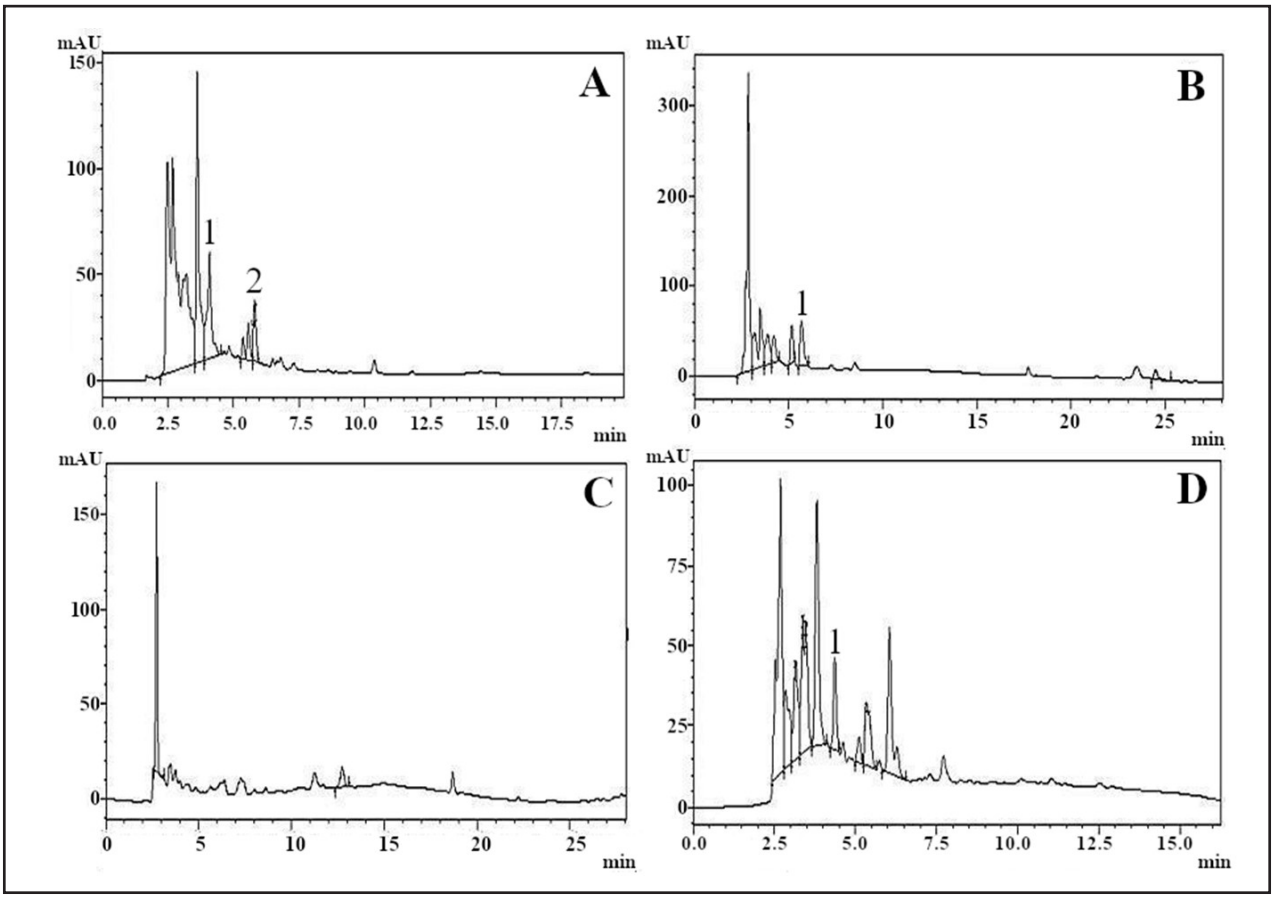


Source: Authors (2017)

In where: Identification of peaks by response time: 1- protocatechuic acid; 2- *p*-hydroxybenzoic acid; A- OR ethyl acetate sample; B- IR ethyl acetate sample; C- OR methanol sample; D- IR methanol sample.

The chromatographic analyses of the stem samples of *Euterpe oleracea* from the outer and inner regions showed five peaks for the extracted OR sample and seven peaks for the extracted IR sample. Protocatechuic acid was identified in both samples, however, only the extracted OR sample evidenced *p*-hydroxybenzoic acid (Figure 4). The presence of these acids in the cell wall corroborates to the spectrometric analysis of NMR.

Figure 4 – Chromatograms of phenolic acids found in the stem of *Euterpe oleracea* (OR and IR)



Source: Authors (2017)

In which: Identification of peaks by response time: 1- protocatechuic acid; 2- *p*-hydroxybenzoic acid; A- OR extracted sample; B - IR extracted sample; C - OR extracted sample + 1% NaOH; D – IR extracted Sample + NaOH 1%.

The derivatives of the cinnamic acid are the most abundant. However, those of benzoic acid and aldehyde origin are restricted to some groups (HARTLEY; KEENE, 1984). Another interesting fact is that only a smaller fraction exists as free acids. Instead, most are linked by glycoside, ester, ether or acetal bonds to plant structural components such as lignin and cellulose, or larger polyphenols such as flavonoids and tannins (WÓJCIAK-KOSIOR; ONISZCZUK, 2008).

After the alkaline treatment, it was possible to observe that the peaks of protocatechuic and *p*-hydroxybenzoic acids of the OR sample disappeared, but this loss was not observed in the IR sample (Figure 4D). A possibility for this loss can be explained by a greater amount of ester-like connections in the outer region than in the inner region, which can be confirmed by the C=O stretch signal ratio in infrared spectra, in which the outer region presented a higher ratio than the inner region.

Alkaline treatments dissolve lignin and release phenolic acids from cell walls by cleavage of ester bonds in the lignin complex. Lignin is known to make polysaccharides resistant to acid hydrolysis or enzyme. Lignin solubilization by alkaline products increases the biodegradability of cell wall polysaccharides (PAN; BOLTON; LEARY, 1998).

4 CONCLUSIONS

The chemical characterization of the cell wall of *Euterpe oleracea* stem samples showed that pre-treatment with 1% NaOH allowed an increase in holocellulose content of the outer and inner regions, and a reduction in lignin content. Pretreatment with 1% NaOH showed a favorable effect to hydrolysis, concluding that part of the material was solubilized.

In the analyses performed, the OR and IR samples treated with 1%NaOH showed a reduction of functional groups linked to lignin and hemicellulose, indicating that the process was favorable to hydrolysis. Lignin from the OR and IR samples of *Euterpe oleracea* was found to be GS-type and incorporated with *p*-hydroxybenzoic and protocatechuic acids. Also, NMR indicated that the main structural units present are β-O-4, β-O-4' Y-acetylated, guaiacyl unit, syringyl unit and *p*-hydroxybenzoate.

In conclusion, this material, which is frequently deemed as residue after consumption of its fruits and leaves, can be converted into products of higher added value and chemicals.

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