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Liquid chromatography and solid state CP/MAS ^{13}C NMR techniques for chemical compound characterizations of cypress wood *Cupressus glauca* Lam. exposed to brown- and white-rot fungi

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Abstract

The main objective of this research was to determine quantitatively the amount of structural sugar polymers (glucan, mannan, and xylan) and acid lignin, after decaying of a conifer wood *Cupressus glauca* Lam., exposed to brown-rot fungi *Gloeophyllum trabeum* (Persoon ex Fries) Murril. and *Lentinus lepideus* Fr. and white-rot fungi *Trametes versicolor* (Linnaeus ex Fries) Pilat and *Ganoderma applanatum* (Pers. ex Wallr.) Patouillard. Extractive and ash contents were homogeneous and showed low value. Chromatographic and spectroscopic techniques were important tools for detecting changes in the wood chemical composition. White-rot fungi showed two different class resistances with almost unchanged decay wood constituents. Brown-rot fungi removed the polysaccharides but not lignin. *G. trabeum* and *L. lepideus* had similar effects on chemical composition of cypress wood. This cypress wood showed lower mannan contents than xylan.

Keywords: Cypress; Biodegradation; Fungi; Carbohydrate; Ash; Lignin; CP/MAS ^{13}C NMR; HPLC; UV

1. Introduction

Timber of cypress, mostly *Cupressus lusitanica* (syn. *Cupressus glauca* Lam.), occupies the leading position in the Kenyan timber market and is one of the few conifers widely planted outside of its natural habitat. It is used as a source of fuel and lumber in Guatemala, as a source of pulp in Venezuela. Colombia established a program to improve cypress timber quality of end products and in the Highlands of Costa Rica it is the most commonly planted species. In Brazil cypress has shown good adaptation in the southern region, but it is missing genetic improvement and diffusion of the timber potential.

Native of Mexico and Guatemala, *C. lusitanica* is now widely planted at high elevations throughout the tropical world. The texture is fine and uniform, and the grain is usually straight. The density of air-dried wood is 512 kg/m^3. Mexican cypress is used mainly for posts and poles, furniture components, and general construction (Haslett, 1986; Miller, 1999).

The interest in cypress species is related to furnish basic data that still has been scarce. The Forest Products Laboratory, a Brazilian federal institution, has been requested to give information about technological and silvicultural aspects of cypress to support a reforestation investment. To fulfill this gap, the first studies using cypress wood evaluated the physical, mechanical and biological properties of flakeboard and particleboard using acetylated and

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non-acetylated particles (Okino, Souza, Santana, Alves, Sousa, & Teixeira, 2004) and cement-bonded particleboards (Okino, Souza, Santana, Alves, Sousa, & Teixeira, 2005) and reported technological characterization of the wood (Okino et al., 2006). Other researches related to cypress were also reported by Guha et al. (1969), Guha, Singh, Singh, Kumar, and Bist (1971), Harcharick & Vaccarone (1974), Shukla and Sangal (1986), Kothiyal, Negi, Rao, Gogate and Dakshindas (1997,1998) but none covered the post-decaying analysis.

Cellulose (~41%), hemicelluloses (~26%), and lignin (~29%) are the principal components of conifer wood. The two major hemicelluloses are (1) the galactoglucomannan (~16%), which is about 70% mannan, and (2) the arabino-4-*O*-methylglucuronoxylan (~10%), which is about 65% xylan (Timell, 1967).

Polysaccharides play a major role as structural components of plants and provide the main source of energy for microbial processes. Wood-destroying fungi metabolize the wood cell wall constituents and seriously reduce the strength of wood before significant weight loss has occurred. The rotted lignin was approximately one atom rich in oxygen, 1.0 and 0.3 atom poor in hydrogen and 25% and 35% deficient in methoxyl content per C_9-unit ("monomer" unit), when attacked by white and brown fungus, respectively. Data have indicated that fungal degradation is largely oxidative and that demethylation of both phenolic and non-phenolic units is the major degradative reaction (Kirk, 1975; Kirk & Chang, 1974). Chua, Chen, Chang, and Kirk (1982) concluded that after partial degradation by *Phanerochaete chrysosporium*, polymeric spruce lignin contained a structure that points to C_α–C_β side chain cleavage, to C_α-oxidation, to aromatic ring cleavage, and possibly to reductive reactions during the fungal attack.

White- and brown-rot fungi are taxonomically very similar, as both are basidiomycetes, and some genera such as *Poria* and *Merulius* contain representatives of both wood-rotting groups. Although the mechanism in which fungal cellulases break down the cellulose in wood cell walls is not yet clearly understood, it is generally accepted that degradation of cellulose by fungi is attributed to the synergistic activity of two types of enzymes: a "C_1" that acts on higly ordered cellulose to produce linear chains, and "C_x" that breaks down the linear chains (Enoki, Tanaka, & Fuse, 1988).

There are two factors that affect the cell wall degradation rate, the lignin concentration and the monomer composition. Most conifer lignin is mainly composed of guaiacyl propyl (G), while hardwood also contains syringyl propyl (S/G). The lignin of Ceibo (*Erythrina crista-galli* L.), Ulin wood (*Eusideroxylon zwageri*), Bangkirai (*Shorea laevis*), Koki (*Hopea pierrei*), Malas (*Homalium foetida*), are primarily constituted of guaiacyl units, notwithstanding the fact the woods are typical hardwoods. The major structural differences between guaiacyl and syringyl/guaiacyl lignins are probably associated with the degree of condensation – involving C–C and C–O–C linkages, primarily at C_5 (Faix, Mozuch, & Kirk, 1985).

The mode of degradation of most natural polymers by microorganisms is hydrolytic. Linkages between recurring units are hydrolyzed through catalysis by extracellular enzymes to yield soluble monomers or oligomers which are taken into microbial cells and further metabolized (Kirk & Chang, 1974).

Chemical, micromorphological, and ultrastructural studies demonstrated that two distinct types of decay patterns are found in white-rotted wood (i) a simultaneous removal of all cell wall components and (ii) a selective removal of lignin. The selective removal of cell wall components by white-rot fungi may be changed by nutritional factors that influence fungal physiology (Blanchette, 1984).

Detailed quantitative analysis of the various monosaccharide units in wood is useful for the study of wood decay by various fungal or bacterial strains. Solid state CP/MAS ^{13}C NMR, can be used to observe all organic polymers in intact wood samples. Early studies using this technique were reported by Atalla, Gast, Sindorf, Bartuska, and Maciel (1980) and Earl and VanderHart (1980).

The main objective of this research was to determine the changes in the chemical constituents of the cypress wood (*C. glauca* Lam.) exposed to white-rot and brown-rot fungi, using analytical and spectroscopic techniques such as HPLC, UV and CP/MAS ^{13}C NMR.

2. Materials and methods

The alcohol–toluene extractives (1:2, v/v), insoluble lignin and ashes were gravimetrically determined. Simple sugars (glucose, xylose, mannose, galactose and arabinose), carbohydrates degradation products, and byproducts of interest from acidic hydrolysis of the extractive-free cypress sample were analyzed by high performance liquid chromatography and soluble lignin was determined in spectrophotometer UV. Sound cypress wood was used as the reference material.

2.1. Materials

Samples of 17-year-old cypress wood (*C. glauca* Lam.) were exposed to accelerate laboratory test according to ASTM D 2017-05 (2005), using two brown-rot fungi *Gloeophyllum trabeum* (Persoon ex Fries) Murril. and *Lentinus lepideus* Fr. and two white-rot fungi *Trametes versicolor* (Linnaeus ex Fries) Pilat and *Ganoderma applanatum* (Pers. ex Wallr.) Patouillard. Twelve samples were used per fungus in the bioassay test. They were conditioned, weighed, sterilized, incubated for 12 weeks in an incubation room at (26.7 ± 1) °C and (70 ± 4)% relative humidity. Differences between initial and final weights resulted in the resistance classification according to the decay resistance of the wood species (Okino et al., 2006).

2.2. General methods

Wood flakes for chemical analysis were ground to a fine particle size. This material was classified and the fraction between 40 and 60 mesh was used.

Sample preparation, extractives removal and moisture content determinations were according to TAPPI T 264 om-88 (1996a) specification.

Extractive-free wood was obtained when the classified material was extracted subsequently with toluene–ethanol (2:1, v/v), ethanol, and hot water, according specification.

The percentage of ethanol–toluene was determined according to TAPPI T 204 om-88 (1996b). The mixture of solvents extracts waxes, fats, some resins, and possibly some portions of wood gums.

The percentage of ash extractive-free wood was analyzed according to TAPPI T 211 om-93 (1996c) and ASTM D 1102-56 (1994). Results were expressed as the percentage of residue remaining after dry oxidation of the wood.

2.3. Sugar analyses

The polysaccharides present in wood samples were hydrolyzed to their sugar monomers by sulfuric acid in a two-stage hydrolysis process. The hydrolysis of the extractive-free comminuted sound and decayed samples were performed, in duplicate, and quantified by HPLC based on the methods proposed by Ruiz and Ehrman (1996a) and Kaar, Cool, Merriman, and Brink (1991), with modifications.

A series of sugar calibration standard solutions was prepared in deionized water containing five sugars in approximately the same proportions found in wood, in order to create a calibration curve for each sugar of interest and determine the response factors. The calibration curve was a set of multi-component standards containing glucose, xylose, galactose, arabinose and mannose in the range of 0.01–3.0 mg/mL. The chromatographic system was controlled by a Varian® workstation. The hydrolysate was adjusted to pH ~ 5.3 using $CaCO_3$, then centrifuged and applied to a mixed-bed ion-exchange column. The neutralized hydrolysate samples were analyzed using Bio-Rad Aminex column HPX-87P, 7.8 × 300 mm. A Micro-Guard Carbo-P column, 30 × 4.6 mm, cartridge was used before the main column. The sample was done in duplicate and each hydrolysate was injected two times. Integrated peak areas from standard sugar solutions were used to compute the response factors.

The following instrumental conditions were used for the carbohydrate column:

Sample volume: 50 μL.
Eluante: 0.20 μm filtered and degassed, deionized water (18 MΩ).
Flow rate: 0.60 mL/min.
Column temperature: 85 °C.
Detector: Refractive index.
Run time: 25 min data collection plus a 10 min post-run.

The mobile phase was degassed to prevent the absorption of CO_2 giving carbonate, which acts as a display and shortens the retention time (Martens & Frankenberger, 1991).

The determination of acid insoluble lignin was determined according to Templeton and Ehrman (1995) and acid soluble lignin was conducted according to Ehrman (1996), using a UV spectrophotometer (Femto® Model 700 plus).

The determinations of acetyl groups and other byproducts were analyzed using the methodology described by Ruiz and Ehrman (1996b) and Ehrman and Ruiz (1998). A set of multi-component standards was prepared in deionized water, except furfural that was prepared in alcohol. A calibration curve for each component of interest was determined. The concentrations of acetic acid and levulinic acid were in the range of 0.02–0.5 mg/mL, and HMF and furfural in the range of 0.02–5.0 mg/mL. Both the calibration standards and the samples were analyzed sequentially by HPLC system, consisted of 300 × 7.8 mm Bio-Rad Aminex column HPX-87H (H^+) cation-exchange resin, with a Micro-Guard Cation-H, 30 × 4.6 mm, cartridge. The following instrumental conditions were used:

Sample volume: 50 μL.
Mobile phase: 0.20 μm filtered and degassed, deionized H_2SO_4. 0.01 M.
Column temperature: 35 °C.
Run time: 40 min data collection plus a 10 min post-run.

During the hydrolysis reaction the glycosidic linkages of the wood polymers are cleaved with the addition of water, producing monomeric compounds such as glucose, xylose, galactose, mannose and arabinose. These monomers were then determined quantitatively by HPLC. In order to ascertain its true contribution to the original wood, the weight of each constituent determined quantitatively in the hydrolysate must be calculated stoichiometrically as the weight of that moiety as it occurs in the wood. Thus, each monosaccharide could be reported as its pure theoretical homopolymer, such as glucan, mannan, and galactan for the three hexoses and xylan and arabinan for the two pentoses, and also the degradation products (Kaar and Brink, 1991). The stoichiometric factors used to calculate the weight of the moieties occurring in the wood based upon the weight of the constituents in the hydrolysates are listed in Table 1. The general factor is given by the molecular weight ratio: (compound − water)/compound.

The determination of uronic anhydride content was based on the method proposed by Scott (1979) using a spectrophotometer UV–vis. A colorimetric method was developed by using 3,5-dimethylphenol, very specific for hexuronic acid in the presence of neutral sugars, particularly glucose, mannose, and xylose.

Table 1
Stoichiometric factors used in the homopolymer determinations

Compound	Reported as	Factor used
Glucose, galactose, mannose	Glucan, galactan, mannan	162/180
Xylose, arabinose	Xylan, arabinan	132/150
Levulinic acid	Glucan	162/116
Uronic acids	Uronic anhydride	190/208
2-Furaldehyde (2-F)	Xylan	132/96
5-(Hydroxymethyl)-2-furaldehyde(HMF)	Glucan	162/126
Acetic acid	Acetyl groups	43/60

2.4. Solid state CP/MAS ^{13}C NMR

The solid state nuclear magnetic resonance employing the technique of cross-polarization magic angle spinning (CP/MAS) gave information about the nature of wood polymer constituents as well as its semi-quantitative and specific molecular interactions.

CP/MAS ^{13}C NMR experiments were performed on a Varian® Mercury plus 300 spectrometer (7.05 T), at 76.46 MHz. The spectrometer was equipped with a 7 mm CP/MAS probe and the samples were packed in silicium nitrate rotors and spun at the magic angle at 6 kHz. The spectra were acquired using a spectral width of 100 kHz, a single pulse length of 4.8 μs, a recycled delay of 1 s, acquisition time of 0.05 s, contact time of 1.0 ms, 2000 scans were accumulated and externally referenced to hexamethylbenzene (17.3 ppm). A line broadening of 50 Hz and a Gaussian functions of 0.003 s were used for processing the spectra.

3. Results and discussion

Table 2 lists some chemical analysis of the sound and decayed cypress wood (*C. glauca* Lam.) exposed to decay fungi in accelerated laboratory test. Cypress wood showed a broad decay resistance, where the increase susceptibility to fungi attacks was in the following sequence: *G. applanatum* < *T. versicolor* < *L. lepideus* < *G. trabeum*. Gravimetric analysis showed an increase in their amounts after decaying when compared to the non-attacked sample.

The limits found to alcohol–toluene extractive were 2.5% and 3.5% for decayed sample, slightly higher when compared to sound samples. The percentage of ethanol–toluene extractives of sound cypress wood was lower than that reported by Costa, Colodette, and Foelkel (1998) in the interval 5–8%, and Guha, Singh, Singh, Kumar, and Bist (1971) that reported 1.8%. The increment on the extractive contents might be explained by the fact that the brown-rotted material became very fragile, porous and friable causing a better wettability or penetration of the solvent, making the removal of the extractable material easier. Also, the brown-rot fungi causes a rapid depolymerization, and some of the non-metabolized low molecular weight carbohydrate fragments became soluble and were leached out by the solvents.

Except the decayed sample by *G. applanatum,* all ash contents showed an increase of 0.08%. The mean values of ash contents are in accordance with the intervals of 0.2–0.9% cited by Browning (1963) for softwoods. The ash content for sound wood was quite similar to 0.47% found by Foelkel and Zvinakevicius (1978) and Guha et al. (1971) who reported 0.4%. In general, there was an increment of the ash contents after decaying due to the concentration of the inorganic elements of the wood.

The amount of extractive and ash contents were low to studied cypress. The first property is a positive and important characteristic for pulp production, finishing and the second to wood processing.

The moisture content range was 7.05% up to 9.50%, a little higher than the sound sample value. Both samples decayed by brown-rot showed higher moisture content than those decayed by white-rot. From these differences in moisture content it can be concluded that fungi attack corroborated the changes in the wood polymers structure. Otherwise, the long exposure of the samples to high moisture content room incubation may interfere with the water of constitution of the samples becoming hard to eliminate under mild conditions.

Table 2
Weight losses, moisture content, ethanol–toluene extractive and ash contents of cypress wood (*C. glauca* Lam.) after wood decay fungi exposure in accelerate laboratory test

Treatment	Percentage			
	Weight loss[a]	Moisture content[b]	Extractive[c]	Ash[b]
T. versicolor	27.79 (3.10)	7.05 (0.04)	3.47 (0.54)	0.48 (0.01)
G. applanatum	10.69 (2.17)	7.30 (0.05)	2.49 (0.54)	0.40 (0.01)
G. trabeum	51.80 (2.41)	8.10 (0.06)	2.99 (0.56)	0.48 (0.07)
L. lepideus	33.60 (2.82)	9.50 (0.03)	2.45 (0.42)	0.48 (0.00)
Sound cypress	–	7.30 (0.02)	2.33 (0.64)	0.44 (0.01)

Values in parentheses are standard deviations.
[a] Data from Okino et al. (2006), mean values of 12 repetitions.
[b] Mean values of two repetitions.
[c] Mean values of three repetitions.

Both, alcohol/toluene and ash contents reported are normal values for conifers. In general, the extractives and ash contents increased after accelerated laboratory decay test. As reported by Kirk and Highley (1973), it reflects the failure of the organisms to attack inorganic compounds, some extractives, and also the formation of additional acid soluble degraded lignin.

Sugars obtained from hydrolysis of cypress wood and converted to their corresponding homopolymers is listed in Table 3; acid soluble and insoluble contents are listed as well.

The arabinose sugar peak was not detected by the HPX-87P column, probably due to the low concentration of this sugar in the cypress wood. Thus, its corresponding homopolymer was not listed in Table 3.

Although each fungus showed different percentage of weigh loss, the mean value of glucan, xylan and acid insoluble lignin were approximately 33.6%, 13.0% and 32.0%, respectively, for attacked white-rot sample. The percentage amounts of glucan, xylan and soluble lignin in partial white-rot samples were higher than the amount of the respective compounds in sound cypress wood. From the lignin contents, it seems that both fungi were unchanged in the acid insoluble lignin, but their action was better perceived from the increase of acid soluble lignin in 47% and 30%, to *T. versicolor* and *G. applanatum*, respectively, demonstrating an altered residual lignin. These finding states that white-rot degrades the lignin polymer, leaving behind the cellulose and hemicellulose compounds, so the whiteness of the decayed sample. The contents of glucan, xylan, galactan and mannan in brown-rot samples were lower than the amount of the respective sugar polymer in sound cypress wood. Conversely it was found to acids soluble and insoluble lignin. *G. trabeum* showed an increment of 51% and 66% in the acid insoluble and soluble lignin contents, respectively, when compared to the sound sample. *L. lepideus* also showed an accumulation of 34% and 53% in the acid insoluble and soluble lignin contents, respectively. It looks as if there is a positive correlation between percent weight loss and acid lignin contents.

The rate of sugars degradation by *G. trabeum* was in the following sequence mannan > galactan > xylan > glucan, with maximum of 78.7% to mannan and minimum of 24.9% to glucan. In the case of *L. lepideus*, the rate of degradation was in the same sequence but at a lower rate, where the maximum was 71.0% and the minimum was 5.8%. So the preferential depletion of mannan during brown-rot fungi attack was clear. In relation to white-rot fungi there was a relative increase in some sugar contents. The effects of degradation was observed to mannan content when attacked by *T. versicolor* showing a decrease in 4.4%, while *G. applanatum* showed a decrease in both mannan and galactan contents of 4.1% and 9.1%, respectively, meanwhile the degradation was less during white-rot exposure. Differences in the residual sugar contents have shown the selective metabolism of each fungal attack.

Usually, the mannan content in softwoods is higher than the xylan content. In this study cypress sound wood showed mannan and xylan contents of 3.38% and 12.54%, respectively. Rowell (1984) reported sugar contents of 9.3% xylan and 7.4% mannan for *Pinus resinosa* Ait. and to *Thuja occidentalis* L., with 10.0% xylan and 8.0% mannan. The later specie is a softwood from the Cupressaceae family.

There were differences in the amount of polymers when comparing decayed and sound wood and also brown- and white-rot fungi. This characteristic is related to differences in the biochemistry of the degradation. In brown-rot decay, cellulose and possibly the hemicelluloses undergo an extensive depolymerization before much weight loss has occurred (Kirk & Highley, 1973). Cypress decayed wood showed that glucomannan were removed faster than cellulose. *T. versicolor* presented a slight rate of insoluble lignin degradation showing that this fungus degrades both polysaccharides and lignin simultaneously.

Among the four fungi tested, the lower carbohydrate content was exhibited to brown-rot *G. trabeum* that showed its aggressive attack to the cypress wood *C. glauca*, which is the preferential host for this fungus.

The amount of insoluble lignin in sound cypress wood was 32.1%, quite similar to 33.5%, reported by Foelkel and Zvinakevicius (1978), and the range 26–32% by Sjöström (1981), although in compression wood this interval can vary from 35% to 40%. There was an increment in

Table 3
Lignin and polysaccharide contents in sound and decayed cypress woods (*Cupressus glauca* Lam.), exposed to wood-rot fungi in accelerate laboratory test

Treatment	Polysaccharide content[a] (%)				Lignin[c]	
	Glucan[b]	Xylan[b]	Galactan[b]	Mannan[b]	Insoluble	Soluble
T. versicolor	33.59 (0.32)	13.12 (0.07)	2.35 (0.04)	3.23 (0.06)	31.89 (0.21)	0.69 (0.01)
G. applanatum	33.61 (0.83)	12.64 (0.42)	1.90 (0.16)	3.24 (0.19)	32.21 (0.37)	0.61 (0.04)
G. trabeum	24.61 (0.66)	8.04 (0.41)	0.77 (0.26)	0.72 (0.17)	48.43 (0.76)	0.78 (0.00)
L. lepideus	30.86 (0.79)	10.44 (0.18)	1.35 (0.06)	0.98 (0.64)	43.13 (0.17)	0.72 (0.05)
Sound cypress	32.77 (0.23)	12.54 (0.15)	2.09 (0.07)	3.38 (0.12)	32.06 (0.14)	0.47 (0.01)

Values in parentheses are standard deviations.

[a] Percentage of anhydromonomer on oven dry, extractive-free basis.

[b] Mean values of four repetitions.

[c] Mean values of two repetitions.

decayed soluble lignin amount compared to sound cypress wood. The increase extractability of lignin from decayed wood was noted by Kirk and Chang (1974), who reported that freeing of lignin from decayed wood reflects two changes: (1) release of soluble fragments by degradation of the lignin polymer and (2) disruption of some of the intimate lignin-carbohydrate association. As reported by Blanchette (1984), white-rot fungi degrade both lignin and polysaccharides from wood. A distinction has been made between white-rot fungi that simultaneously remove lignin, cellulose, and hemicellulose, and those that successively decompose lignin and hemicellulose, followed by cellulose removal only at a later stage. According to Scheffer and Cowling (1966), lignification of wood cell walls is by far the most important non-toxic factor that contributes to the natural resistance to microbial deterioration. They also stated that lignin apparently acts as a physical barrier preventing the polysaccharide-splitting enzymes of many organisms from reaching a sufficient number of glycosidic bonds to allow significant hydrolysis. Thus, all organisms that degrade wood cell walls possess the necessary enzymes not only to degrade wood polysaccharides but also to depolymerize lignin, or at least disrupt its association with the polysaccharides.

Results from chemical analysis of white-rotted wood poorly indicated the capacity of mainly *G. applanatum* fungus to cause selective lignin degradation. The amount of delignified portion in decayed wood was concentrated in the surface specimens and had little visually apparent success in colonizing deep into the wood. Results of this study demonstrated only a slight or no preferential to insoluble lignin and mannan degradations by *T. versicolor* and preferential decay to galactan and mannan by *G. applanatum*, after 12 weeks decay period.

According to Almeida, Gomide, and Silva (2000), the carbohydrate degradation of the wood occurs by hydrolysis of the reducing end of the polysaccharides chain (terminal depolymerization reaction or peeling reaction). The aleatory cleavage reactions of the glycosidic β-*O*-4 linkage result in a reduction of the chain length. These cleavages result in new reducing terminal groups, becoming possible the occurrence of secondary terminal depolymerizing reactions and all these processes result in a mixture of compounds that were analyzed.

Mean values of carbohydrate degradation products, such as HMF and levulinic acid, as well as byproducts of interest, such as acetyl groups and uronic anhydrides, are listed in Table 4.

From Table 4, the *T. versicolor* and *G. applanatum* showed a decrease in 5.9% and 4.1% to acetyl group content, respectively, while for HMF these values were 7.5% and 5.0%, respectively. Conversely, *T. versicolor* and *G. applanatum* showed an increase of 4.2% and 6.3% to levulinic acid, respectively, while for uronic anhydride these values were, 23.8% and 27.6%, respectively. The same behavior was found to *G. trabeum* and *L. lepideus* in relation to acetyl group and HMF, but in great extend. Briefly, comparing the sum of carbohydrate degradation products and byproduct between sound and decayed samples, it is possible to state that white-rot fungi increased from 6.7% to 9.4% the amounts of these products, while brown-rot fungi decreased these amount from 10.25% to 24.0%.

As stated by Martens and Frankenberger (1991), isolation of uronic acids from polysaccharides is very difficult because their glycosidic linkages are more stable than that of the neutral saccharides. The total amount of uronic acid units or uronic anhydride in wood is about 2–6% (Santana, 1990). Data found in this study are within this range.

According to Fengel and Wegener (1984), in most softwood xylans investigated the ratio of Xyl:Me-GluU is 5–6:1, sometimes even 3–4:1. In this work cypress wood showed ratio of 3.8–5.3:1.0 in the range previously reported.

Wood and related products are known to give an increased acetyl values due to the formation of additional acetic acid from sugar decomposition when strong mineral acids are employed. Furfural, a degradation product obtained from xylose, was not detected in this study. As reported by Niola, Basora, Chornet, and Vidal (1993), the production of furfural increased by 15% when two acids are used together instead of individually, as conducted in this study. Springer and Zoch (1968) reported that uronic acid side chains stabilize the adjacent main-chain linkages. They also reported that the hydrolysis rate varied inversely with the D-glucuronic acid content of the isolated xylans. Consequently, the more uronic acid side chains, the lower will be the overall rate of polymer degradation. So this might be the cause of the absence of furfural peak observed in this study.

In general, the amount of acetic acid and HMF decreased after decay, conversely, the amount of levulinic acid increased, except for the *L. lepideus* fungus. The

Table 4
Percentages of carbohydrate degradation products and by products of sound and decayed cypress wood (*Cupressus glauca* Lam.) by brown- and white-rot fungi

Treatment	Acetyl group	Levulinic acid	HMF	Uronic anhydride	Total
T. versicolor	2.54 (0.02)	0.50 (0.01)	0.37 (0.01)	2.96 (0.05)	6.37
G. applanatum	2.59 (0.01)	0.51 (0.02)	0.38 (0.00)	3.05 (0.04)	6.53
G. trabeum	1.65 (0.04)	1.32 (0.82)	0.27 (0.01)	2.12 (0.07)	5.36
L. lepideus	1.69 (0.01)	0.48 (0.03)	0.22 (0.15)	2.15 (0.04)	4.54
Sound cypress	2.70 (0.02)	0.48 (0.01)	0.40 (0.00)	2.39 (0.08)	5.97

Mean values of four repetitions. Values in parentheses are standard deviations.

amount of uronic anhydrides were an average of 2.0% and 3.0% to brown- and white-rot fungus, respectively.

The estimated total hemicelluloses percentage was the sum of xylan, mannan, arabinan, galactan, uronic anhydride and acetyl groups contents. The estimated cellulose percentage was the sum of glucan, HMF and levulinic acid contents.

The totals shown in Table 5 were not 100% and were lower than reported in the literature. This summative analysis is very complex due to the fungi biochemistry route, and because the major samples were exposed sequentially to two types of hydrolysis, one enzymatic and the other mineral. Analysis of glycosidic units comprising polysaccharides presents many problems as suggested by Martens and Frankenberger (1991), where the susceptibility of the glycosidic linkage to acid hydrolysis varies greatly along with the stability of the resulting monosaccharide unit in the acidic solution.

Mean values listed in Table 5 showed that, when comparing sound and decayed wood samples, the carbohydrate amount decreased in 30.4% and 15.1% to *G. trabeum* and *L. lepideus*, respectively, but in the case of total lignin it increased in 51.3% and 34.8% to *G. trabeum* and *L. lepideus*, respectively. Both white-rot fungi showed a non-selective attack of all wood cell wall components due to the slight increase in the total amount of polysaccharide and total lignin.

Kaar and Brink (1991) reported a summative analysis of nine common North American woods, and the total polysaccharides of *Pseudotsuga menziesii* heartwood and *Abies concolor* sapwoods were on average 9% higher than the results of this study. As reported by them, several undetermined factors are thought to contribute to the remaining mass balance. These factors include: (1) methanol from cleaved methoxyl groups in the polysaccharides and lignin, (2) dehydration of the lignin during the concentrated acid hydrolysis, (3) trace amounts of polysaccharides moieties, such as rhamnose and fucose, that were present in quantities below the detection limit of the analytical method, and (4) experimental error associated with the moisture content determination due to bound water in the comminuted wood that was not removed and therefore unaccounted.

Study using CP/MAS ^{13}C NMR technique corroborated results of chemical and chromatographic analyses. The spectra CP/MAS ^{13}C NMR, in solid state, to sound and decayed cypress wood are shown in Fig. 1 where the peaks related to carbohydrates at 46–110 ppm region were consistent with those semi-quantitative mean values listed in Table 6.

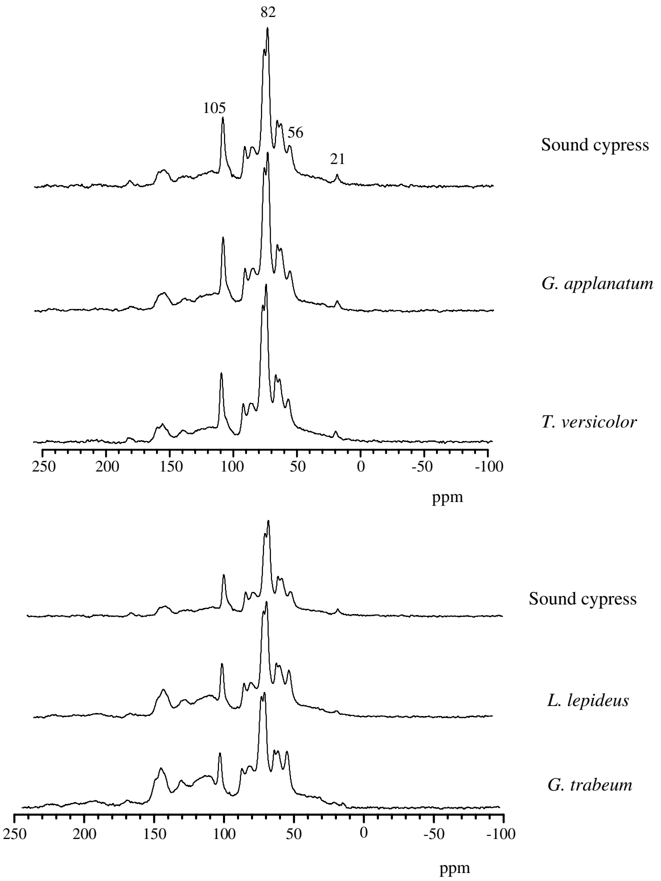

Fig. 1. CP/MAS ^{13}C NMR spectra of sound and decayed samples.

Table 6
Composition of lignocellulosic substrates in sound and decayed cypress wood *(Cupressus glauca* Lam.) exposed to white- and brown-rot fungi as obtained from integration of CP/MAS ^{13}C NMR spectra

Treatment	Carbonyl/acyl 220–160 ppm	Aromatic C 160–110 ppm	*O*-alkyl C 110–45 ppm	Alkyl C 45–0 ppm
T. versicolor	2.1	16.5	76.1	5.3
G. applanatum	0.0	11.3	80.0	8.7
G. trabeum	7.6	23.3	54.0	15.1
L. lepideus	10.1	20.9	58.0	11.0
Sound cypress	6.1	15.6	72.2	6.1

Table 5
Estimated values of chemical constituents of sound and decayed cypress wood *(Cupressus glauca* Lam.) by brown- and white-rot fungi

Treatment	Percentage				
	Cellulose	Hemicellulose	Total lignin	Ash	Total
T. versicolor	34.46	24.20	32.58	0.48	91.72
G. applanatum	34.50	23.42	32.82	0.40	91.14
G. trabeum	26.20	13.30	49.21	0.78	89.49
L. lepideus	31.56	16.61	43.85	0.42	92.44
Sound cypress	33.65	23.11	32.53	0.48	89.77

In the carbohydrate region (45–110 ppm), the ratio of the intensities of the peaks at 89 and 65 ppm and at 62 and 84 ppm related to the crystalline cellulose and amorphous carbohydrate components, respectively, did not present any change between sound and white-rotted cypress wood. A preferential removal of amorphous carbohydrate components for *T. versicolor*, reported by Davis, Schroeder, and Maciel (1994a), may be due to difficulty by the enzymes of the fungi in penetrating into the crystalline regions of the cellulose.

Only for *G. applanatum* the lignin was removed to a small extent as noticed in Table 6. However, this result was opposite to that described by Davis, Schroeder, and Maciel (1994b) when demethylation of lignin was observed to a greater extent after decaying by *T. versicolor*.

The increase of methoxyl carbon at 56 ppm, evidencing the demethylation of the lignin, was observed for both brown-rot fungi *G. trabeum* and *L. lepideus*. As result of degradation process, there was also a decrease in signal intensity at ~21 ppm, related to methyl carbon in acetyl group (CH_3CO), which almost disappeared at the highest weight loss (*G. trabeum*), suggesting a preferential removing of hemicellulose in comparison to cellulose during the degradative process, as observed by Kim and Newman (1995).

The increases shown in aromatic region (110–160 ppm) for the brown-rot decayed wood when compared to anomeric carbon signal at 105 ppm, indicated the preferential removal of the carbohydrate fractions of the wood with respect to the lignin (Davis, Schroeder, & Maciel, 1994c).

The solid state ^{13}C NMR spectra, in solid state, of the white and brown-rots corroborated the existence of the complex lignin-carbohydrate, characterized at 45–110 ppm and 110–160 ppm regions, as a mixture of both natural polymers present in the wood.

4. Conclusions

In the accelerated laboratory test the cypress wood showed a broad spectrum of decay resistance varying from resistant to moderate resistant class. Among all tested fungi, *G. trabeum* was responsible for the highest weight loss and consequently it accounted for significant changes in the chemical structure of *C. glauca* Lam.

The rate of sugars degradation by *G. trabeum* was in the following sequence mannan > galactan > xylan > glucan, with maximum of 78.7% on mannan and minimum of 24.9% on glucan. Brown-rot fungi removed primarily the structural carbohydrates in the cell wall and degraded mannan faster than the other hemicelluloses.

The effect caused by *T. versicolor* and *G. applanatum* in the sample was a reduction in approximately 4% to mannan content, while further fungus also showed a decrease in 9.1% to galactan content. Meanwhile compared to brown-rot degradation, the degradation was less. White-rot fungi depleted slightly the lignin, exposing the cellulose and hemicelluloses which were metabolized in lower proportions.

Cupressus glauca Lam. showed higher xylan content than mannan content, despite being a softwood.

From the sum of carbohydrate degradation products and byproduct among sound and decayed samples, it is possible to conclude that white-rot fungi increased the total amount, while brown-rot fungi decreased this.

The summative analysis did not total 100%, probably due to sequentially samples exposure to two type of hydrolysis, enzymatic degradation throughout accelerated laboratory test and hydrolytic degradation by *in situ* mineral acid attack.

The CP/MAS ^{13}C of cypress wood attacked by *G. trabeum* and *L. lepideus* indicated the depletion of the carbohydrates, and the hemicelluloses (a peak at ~21 ppm) were removed faster than the celluloses. This similar behavior was shown by chemical analyses as well. In the aromatic region, the spectra indicated major changes occurred in decayed lignin structure through oxidative process promoted by demethylation, which correspond to an increase in methoxy carbon peak at 56 ppm.

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