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# Quantitative $^{31}$P NMR analysis of solid wood offers an insight into the acetylation of its components

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ABSTRACT

As a solid substrate, wood and its components are almost invariably examined via spectroscopic or indirect methods of analysis. Unlike earlier approaches, in this effort we dissolve pulverized wood in ionic liquid and then directly derive its functional group contents by quantitative $^{31}$P NMR. As such, this novel analytical methodology is thoroughly examined and an insight into the detailed way acetylation proceeds on solid wood and its components is provided as a function of wood density and within its various anatomical features. As anticipated, the efficiency of acetylation was found to be greater within low density wood than in high density wood. The lignin, the cellulose and the hemicelluloses of the low density wood was found to be acetylated nearly twice as fast with remarkable differences in their quantitative degree of acetylation amongst them. This direct analytical data validates the applied methodology and confirms, for the first time, that the order of acetylation in solid wood is lignin > hemicellulose > cellulose and no reactivity differences exist between early wood and late wood.



## 1. Introduction

Wood as a bio-polymeric composite is composed of cellulose, hemicellulose and lignin. These polymers are responsible for most of the physical, mechanical and chemical properties exhibited by wood. Wood is a preferred building and engineering material due to its low price, ease of processing, renewability, strength and aesthetically pleasing characteristics. Despite the above advantages, however, it has several drawbacks such as biodegradability, flammability, dimensional instability at varying moisture contents, and degradability by UV light, acids and base (Hill, 2009; Rowell, 2007).

The presence of chemicals in nature as well as fungi and bacteria are the main factors for wood degradation. It is possible to eliminate or decrease the rate of degradation by changing the basic chemistry of the wood cell wall polymers through chemical modification (Imamura & Nishimoto, 1987; Larsson, Simonson, Bergman, & Nilsson, 2000). Chemical modification of wood is the reaction of a chemical reagent with the wood structural polymeric constituents resulting in the formation of a covalent bond between the regent and the wood substrate(Fuqua, Huo, & Ulven, 2012). One of the most studied wood chemical modification treatments has been acetylation with anhydrate acetic acid with or without catalysts. The reaction of acetic anhydrite with wood results in esterification of the accessible hydroxyl groups in the wood cell wall, with the formation of by-product acetic acid that must be removed (Ozmen & Cetin, 2012; Rowell, 2006). Acetylation is a single addition reaction that implies that one acetyl group adds to one hydroxyl group with no polymerization:

$$\text{Wood—OH} + (\text{CH}_3\text{—CO})_2 \rightarrow \text{Wood—COOCH}_3 + \text{CH}_3\,\text{COOH}$$

Thus, all of the weight gain due to acetyl can be converted into units of blocked hydroxyl groups. Previous research has shown that at weight percent gain (WPG) above 17%, acetylated wood is resistant to attack by fungi, has very good dimensional stability (Papadopoulos & Hill, 2003), is more dense, has improved mechanical properties, is less gas permeable compared with untreated wood and most significantly is of reduced moisture affinity (Rowell, Esenther, Nicholas, & Nilsson, 1987).

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It is known that the anatomical features of wood including density, earlywood, latewood, mature wood and juvenile wood, normal wood and compression wood would affect the efficiency of a given chemical treatment upon it (Hill & Papadopoulos, 2002; Minato & Ito, 2004; Papadopoulos, 2006; Papadopoulos & Ntalos, 2004; Rowell et al., 1987). The reactivity of isolated cell-wall polymers (cellulose, hemicellulose and lignin) toward acetylation showed that lignin is more reactive and hemicellulose follows with cellulose reactivity being the lowest (Rowell et al., 1994). However, the accessibility, the structure, the degree of polymerization and the reactivity of isolated wood components are different from those present within the native whole wood. Questions of reactivity of individual cell-wall polymers cannot be answered until their reactivity can be determined while present in it (Rowell et al., 1994). So far, FTIR (Jebrane, Pichavant, & Sebe, 2011; Stefke, Windeisen, Schwanninger, & Hinterstoisser, 2008), measurement of bond acetyl and weight percentage gain (WPG) (Minato & Ito, 2004; Rowell et al., 1994) and $^{13}C$ NMR (Ohkoshi et al., 1997; Qu, Kishimoto, Hamada, & Nakajima, 2013; Qu, Kishimoto, Ogita, Hamada, & Nakajima, 2012) are the main methods for the characterization of acetylated wood and its components. In general, the solubility of whole wood in common solvents has severely hampered the development of new methods toward its efficient utilization and of its components. Earlier work has demonstrated that both hardwoods and softwoods are soluble in various imidazolium-based ionic liquids (IL's) under gentle conditions (Kilpelaeinen et al., 2007; Qu et al., 2013). Furthermore, it was also shown that wood dissolved in IL's was able to be chemically modified (Kilpelaeinen et al., 2007; Xie et al., 2009; Xie, King, Kilpelainen, Granstrom, & Argyropoulos, 2007). Furthermore, structural analyses of cellulose, lignin and wood samples dissolved in IL's using quantitative $^{31}P$ NMR were reported (King, Kilpelainen, Heikkinen, Jarvi, & Argyropoulos, 2009a; King et al., 2009b).

Overall, this effort attempts to apply state of the art methods to ask and settle remaining questions in the field and also provide additional verification for the applied quantitative $^{31}P$ NMR methodology. More specifically, the purpose of the research described in this paper is applying a novel analytical technique for the reproducible determination of the percent total hydroxyl groups that are modified within the various wood components and the percentage of hydroxyl groups that remain unconverted. To do this we dissolved pulverized acetylated whole wood samples as well as extracted holocellulose and hemicellulose in IL and then quantitatively determined the amounts of hydroxyl groups using the aforementioned quantitative $^{31}P$ NMR technique. Finally we compared the degree of acetylation between two wood boards (a high and a low density).

## 2. Materials and methods

### 2.1. Wood samples

Two Southern pine boards, one of high density (0.82 g/cm$^3$) and one of low density (0.54 g/cm$^3$) wood were used in this study. Samples were produced from typical lumber stock, primarily radius edged deck boards by Eastman Chemical Company. Early wood and latewood sections from each board were also carefully isolated.

### 2.2. Acetylation and sample preparation

Samples were selected from acetylated samples produced in the pilot reactor of Eastman Chemical Company. One section from each board was retained (raw). The other section was kiln dried to low moisture conditions, acetylated, and subsequently dried to reduce the residual acetic acid after acetylation. % Bound acetyl was 14.5% for the high density wood and 22.5% for the low density. Acetylated and non-acetylated high and low density boards were cut and then ground to 40 mesh sawdust size particles using the Wiley Mill. Extractives-free samples prepared using acetone and hot water following a standard method (TAPPI T264 CM97, Oct. 2007).

### 2.3. Pulverization of samples

The extractive-free samples (1.0 g) were thoroughly dried in a vacuum oven at 40 °C overnight, followed by planetary ball-milling (Microwolf-VFD-A, Torrey Hill Technology, LLC, USA). This was carried out in a zirconium (zirconium dioxide, 95%) grinding bowls, (5 cm diameter, 4 cm height) in the presence of 8 zirconium balls (10 mm diameter). The milling process was conducted at room temperature at 350 rpm for 40 min with 20 min cooling intervals. The total milling time was 72 h for wood and 10 h for holocellulose and hemicellulose samples. All samples were stored in a desiccator prior to use.

### 2.4. FTIR

The infrared absorption spectra of the acetylated and unmodified samples were obtained using the ATR technique (Perkin Elmer FTIR spectrometer Frontier) at a resolution of 4 cm$^{-1}$ (64 scans).

### 2.5. Isolation of holocellulose

Earlier reported methods for the isolation of holocellulose were used with modifications (Rowell et al., 1994). Low and high density samples (1 g) were weighed in a 100 mL flask, and water (30 mL), 0.2 mL acetic acid, and 0.4 g $NaClO_2$ were added. The contents of the flask were stirred with a magnetic stir bar, covered, and placed in a water bath set at 70 °C. After 30 minutes, another 0.2 mL acetic acid and 0.4 g $NaClO_2$ were added, and the sample was stirred. Same amounts (as used previously) of acetic acid and sodium chlorite were added again after 30 min and then at l-h intervals over a period of 6 h. This resulted in a total of nine additions (total of 1.8 mL acetic acid and 3.6 g $NaClO_2$). The holocellulose was filtered over a fine glass filter, washed with distilled water, air-dried overnight, and then vacuum oven-dried at 40 °C overnight. The yield of holocellulose was 70.1% and 66.3% in the low and high density woods, respectively. The measurements were repeated 3 times and the average is reported.

### 2.6. Isolation of total hemicellulose with NaOH solution (Rowell et al., 1994)

Alkali solution extraction was used to isolate and calculate the total amount of hemicelluloses present in the original wood samples and isolated holocelluose. For the case of hollocellulose one gram of it was stirred in 50 mL of 12% NaOH solution, and nitrogen gas was bubbled through it for 1 min. The flask was then sealed and stirred for 24 h at 25 °C. The sample was then filtered by a coarse glass filter. The residue was extracted with 50 mL of 7.1% NaOH and then filtered using a glass filter. Two filtrates were mixed, and the solution was neutralized with dilute hydrogen chloride (0.2 N) and evaporated by rotary evaporation. The extract was then dissolved in 100 mL of distilled water. The solution was adjusted to pH 4 with acetic acid and the hemicellulose was precipitated with ethanol and the materials were collected by centrifugation. Finally the sample was dried in a vacuum oven at 40 °C.

### 2.7. Isolation of original and acetylated hemicellulose using DMSO as a solvent

Due to the hydrolytic instability of acetyl groups under alkaline conditions our investigation of the degree of acetylation occurring on hemicelluloses was carried out on hemicelluloses extracted by an organic solvent extraction method. Using the same method we also extracted hemicelluloses from the original wood so as to compare the data. To do this we initially isolated the holocellulose from each sample and then we used this to isolate the hemicellulose from it. Literature methods with some modifications were used for these purposes (Goncalves, Evtuguin, & Domingues, 2008; Teleman, Nordstrom, Tenkanen, Jacobs, & Dahlman, 2003). More specifically, the holocellulose sample was first pulverized for 10 h and then the hemicellulose was extracted from it with dimethyl sulphoxide (1 g of holocellulose mixed with 50 mL of DMSO at 45 °C over 24 h). The hemicellulose was finally precipitated by adding an excess of 7:2:1 EtOH—MeOH— water acidified with acetic acid to pH = 5. Complete precipitation of hemicellulose was accomplished by allowing the sample to settle at 4 °C over a period of 24 h. Centrifugation followed by washing (×3) with anhydrous MeOH and driying under vacuum at room temperature, afforded the hemicellulose in the reported yields. The yields of extracted hemicelluloses for acetylated high and low density samples were 48.3 and 70.1%, respectively. The yields of DMSO soluble hemicelluloses were 36.3% and 56.6% for the original high and low density wood samples, respectively. These data is basd on the total hemicellulose content for the original wood as determined by alkali extraction. All measurements were repeated three times and the average is reported.

### 2.8. Milled wood lignin extraction (MWL)

Milled wood lignin was determined using the method of Bjorkman (Lin & Dence, 1992). The 72 h milled wood sample was dispersed in dioxane: water (96:4, v/v) and mechanically stirred (1 g of milled wood in 50 mL of dioxane: water). After 24 h, the suspension was centrifuged, and the residue was re-dispersed in fresh dioxane:water and stirred for an additional day. The extracts were combined and then freeze-dried. The non-acetylated crude lignins were purified by dissolution in 90% acetic acid, followed by dropwise precipitation in cold water. For acetylated materials, the crude lignin was purified by dissolving them in pyridine-acetic acid-water (9:1:4, v/v/v) and further extracted with chloroform. These samples were then used for FTIR and quantitative $^{31}$P NMR analyses. The yield of MWL in unmodified (original) materials was found to be 21% of the determined klason lignin. For the acetylated samples, the yield of isolated MWL was increased up to 38% and 28% in the low and the high density wood, respectively. The measurements were repeated three times and the average is reported.

### 2.9. Quantitative $^{31}$P NMR analyses

Such analyses were carried out as per the reported methods (King et al., 2009a,b). Initially a sample of pure ionic liquid [(AMIM) $^{+}$ $CL^{-}$] was prepared to dissolve the pulverized wood samples. The sample (50.0 mg) was vortexed (Dynalon-mx-s) in [AMM] Cl (~0.75 mL, 750 mg) and then heated for 18 h at 80 °C in a 10 mL screw-top glass sample bottle. Pyridine (300 μL) was then added in one portion to dissolved sample in the ionic liquid and the sample was again vortexed for 1 min until it became visibly homogeneous. The sample was allowed to cool to room temperature, whereby 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxophospholane; 2-Cl-TMDP (~300 μL, 1.89 mmol) were added in one portion and vortexed again until visibly homogeneous (1 min.) appearing as a creamy paste. 200 μL of e-HNDI solution (9.27 mg/mL of 3/2 pyridine/$CDCl_3$) as internal standard was then added in one portion and the solution was vortexed again (~30 s). A prepared stock solution of Cr (acac)$_3$/$CDCl_3$ (1 mg/mL, 3.4 mL) was added in three portions with vortexing (~30 s) between each addition. NMR measurements were acquired using a Bruker 300 MHz spectrometer equipped with a Quad probe dedicated to $^{31}$P, $^{13}$C, $^{19}$F, and $^{1}$H acquisition. Quantitative $^{31}$P NMR spectra were recorded with 1000 μL of prepared dissolved samples, using a 5 mm NMR tube. $CDCl_3$ was used as locking solvent, and standard transients of 2000 were collected by 5 s delay time.

**Table 1**
Chemical composition of low and high density wood samples based on extractives-free materials (triplicate analyses).

| Wood components | Amounts in wood (%) (w/w) | |
|---|---|---|
| | Low density sample | High density sample |
| Acetone extractives* | 2.1 | 5.8 |
| Holocellulose | 70.1 | 66.3 |
| Cellulose | 43.2 | 42.2 |
| Total hemicellulose** | 26.4 | 23.8 |
| Extracted hemicelluloses with DMSO*** | 19.9 | 13.85 |
| Klason lignin | 28.2 | 33.3 |
| Acetylated MWL | 10.7 | 9.45 |

* Based on non extracted wood sample.
** Extracted with NaOH.
*** Acetylated samples.

## 3. Results and discussion

The general chemical composition of the high density and low density samples is presented in Table 1. The high density wood sample showed higher acetone soluble extractives (5.8%) and lignin (33.3%) than the low density one (2.1% and 28.2%, respectively). Quantitative $^{31}$P NMR analyses of the acetone extractives showed the presence of 3.85 and 2.3 mmol total OH groups per gram of extractives in high density and low density samples, respectively. This revealed that pre-extraction of wood extractives may assist in obtaining better resolved (and thus more accurate) quantitative $^{31}$P NMR spectra that made the quantitative estimations more reliable. Furthermore the data showed that the yield of extracted milled wood lignin and hemicellulose were improved in the acetylated samples. The yields of milled wood lignin in the unmodified samples were 19% and 18.15% of the determined Klason lignin for low and high density woods, respectively. However, for the acetylated low and high density woods, the yields were increased to 38% and 28%, respectively. The yields of hemicellulose (DMSO extraction) for non-acetylated samples from milled holocellulose were 27% and 25.2% (based on of the total hemicellulose extracted by NaOH) for low and high density samples, respectively. After acetylation, the hemicellulose extraction yields were increased to 75% and 55% in low and high density samples, respectively. Apparently, acetylation of samples facilitates the lignin and holocellulose extraction from wood.

The complete dissolution of dried milled wood powder in ionic liquids ([amim]Cl and [bmim]Cl) has been reported earlier (Kilpelaeinen et al., 2007; King et al., 2009a,b; Swatloski, Spear, Holbrey, & Rogers, 2002; Zhang, Wu, Zhang, & He, 2005; Zhu et al., 2006) (80 °C for 18 h). Furthermore, the dissolution of cellulose in various ionic liquids has also been discussed in detail. Furthermore, there have been various accounts where the complete dissolution of the wood in ionic liquids allowed the use of a wide range of chemical reactions to modify these materials (Kilpelaeinen et al., 2007; Qu et al., 2013, 2012; Xie et al., 2007). In one instance, however, these advances were taken by our team to an analytical level where it was demonstrated that ionic liquid such as [Amim]Cl can be used as a medium for the phosphitylation reaction (for both wood &

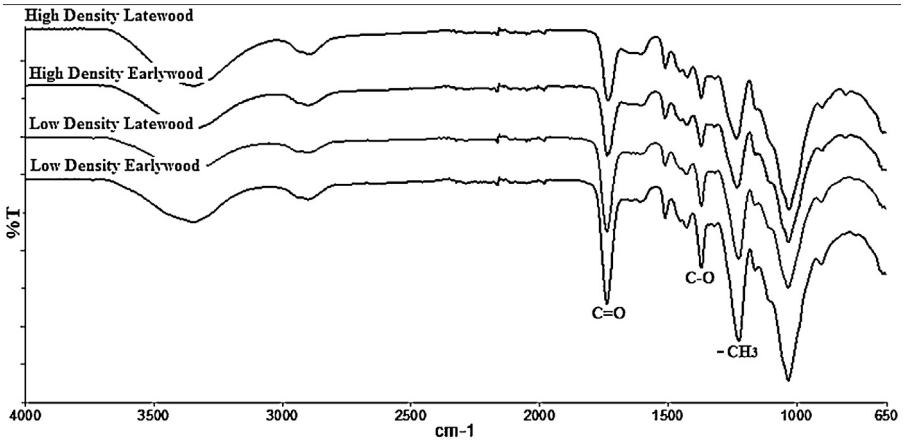


**Fig. 1.** ATR spectra of high density and low density acetylated early and latewood samples.

cellulose) followed by quantitative $^{31}$P NMR (King et al., 2009b). Our earlier work showed that, phosphitylation of dissolved wood and cellulose with 2-Cl-TMDP yielded a phase separated mixture, not suitable for quantitative $^{31}$P NMR analyses. Gradient dilution, however, of a phosphitylated samples with $CDCL_3$, increased the hydrophobicity of the mixture and finally produced a homogeneous mixture suitable for quantitative $^{31}$P NMR analyses. As such we used this method in the present work to determine the precise amounts of hydroxyl groups in the wood and its components before and after acetylation. To address the effect of whole wood acetylation within each component in the wood structure, we isolated lignin, holocellulose and hemicellulose using the previously mentioned methods. All of the components were then examined with FTIR and the described quantitative $^{31}$P NMR technique.

In this work, [Amim] Cl was used for the dissolution of ball-milled unmodified and acetylated whole wood, holocellulose and hemicellulose samples. All wood samples were ball milled for 72 h to ensure complete dissolution (King et al., 2009a) and 10 h for hemicellulose and holocellulose samples. 50 mg of each sample were mixed in 0.75 mL ionic liquid and dissolved at 80 °C for 18 h with stirring. Lignin samples were dissolved directly in our $^{31}$P NMR solvent (Pyridine: $CDCl_3$, 1.6:1 v/v).

### 3.1. Infrared (IR) spectral analyses

Infrared (IR) spectra were obtained on whole untreated and acetylated whole wood, holocellulose, hemicellulose and milled wood lignin samples. All wood samples had been thoroughly extracted with acetone and water to remove any extractives and possible traces of acetic acid entrapped and remaining from the acetylation. There are three major changes that are observed in the IR spectra of wood or its components upon acetylation(Cetin, Ozmen, & Birinci, 2011; Jebrane et al., 2011): (1) an increase in the carbonyl (C=O) stretch region (1735–l765 $cm^{-1}$); (2) increases in various particular frequencies in the carbon-oxygen (C—O) stretch region (1000–1245 $cm^{-1}$) and (3) an increase around 1370 $cm^{-1}$, due to the carbon–hydrogen (C—H) bond of the O—(C=O)—$CH_3$ group (Figs. 1 and 2). The normalized transmission of the carbonyl band stretch frequency (1735, 1765 $cm^{-1}$) for all samples including the ratio of the intensity between of the low density to high density wood samples were calculated.

The obtained data showed that the low density whole wood sample (Fig. 1) shows a higher IR transmission than its high density counterpart. In both low and high density boards the differences between IR transmission for earlywood and latewood was very low. In the low density board the ratio of the IR transmission for earlywood and latewood is 1.08. For the high density board this ratio was 1.00. Overall the data shows that in both no significant differences were apparent on the degree of acetylation for earlywood or latewood.

For the acetylated low and high density holocellulose samples (Fig. 2), this transmission in the low density wood sample was found to be about 1.52 times higher than the high density sample (latewood sample). As anticipated, but never demonstrated, this unequivocally (and supported by additional data in latter parts of this paper) shows that better holocellulose acetylation occurs within a low density board than a high density one.

Usually alkaline media are used to extract hemicelloses from wood. However, in this effort we used an organic solvent (DMSO) to extract hemicellulose from holocellulose so as to prevent acetyl groups from hydrolyzing under alkaline extraction conditions. The yield of hemicellulose extraction from holocellulose was found to be 75% for the low density wood sample and 58% of total hemicellulose for its high density counterpart. The IR transmission for the C=O bond in the hemicellulose of the low density wood was greater than in the high density one (1.55 times greater) (Fig. 2).

Furthermore, our data for the acetylated isolated lignin samples also showed large differences between the low density and the high density wood samples. The ratio for these C=O band transmissions was 1.82.

### 3.2. Distribution of acetyl content

All southern pine wood samples were initially extracted with acetone and hot water to remove the extractives that could interfere with our measurements. Extractives-free whole wood sawdust samples were milled in a planetary ball mill for 72 h and the holocellulose samples were isolated from acetylated and unmodified wood samples using sodium chlorite by removing the lignin. The sodium chlorite isolation procedure did not hydrolyze the acetyl groups as per previous accounts (Rowell et al., 1994). As already mentioned the hemicelluloses were extracted from the 10 h milled holocellulose samples using DMSO at 40 °C for 24 h, while milled wood lignin (MWL) was extracted using the standard Biorkmman method. The distributions and amounts of hydroxyl groups in unmodified and acetylated whole wood, holocellulose, hemicellulose and milled wood lignin was determined by the quantitative $^{31}$P NMR technique described above.

Figs. 3–5 show actual quantitative $^{31}$P NMR spectra of acetylated and un-acetylated whole wood, hemicellulose and milled wood lignin (MWL) samples for the high and low density wood samples, respectively. Table 2 shows the determined hydroxyl groups (mmol/g sample) as determined by $^{31}$P NMR for all modified and unmodified samples. Furthermore, Table 3 shows the percentage

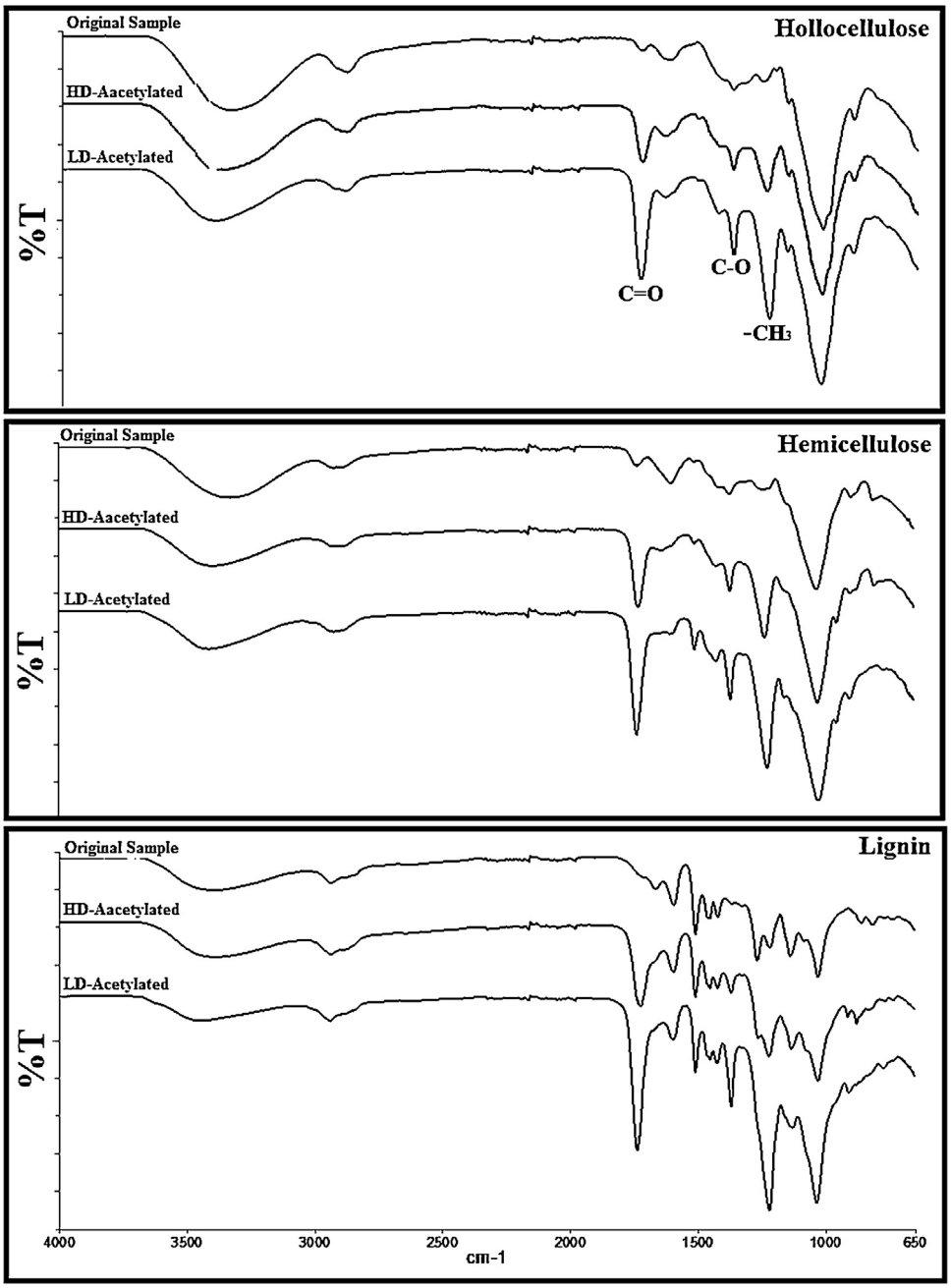


**Fig. 2.** ATR spectra of holocellulose, hemicellulose and lignin samples isolated from high density (HD) and low density (LD) original and acetylated samples.

of OH acetylation in high and low density acetylated wood and wood components. The determined hydroxyl groups present in the unmodified samples were found to be in good agreement with those previously reported (King et al., 2009a).

Our quantitative $^{31}$P NMR analyses of the low and high density whole wood samples showed two main resonances at 146.1 ppm and at 147.4 ppm that were of almost identical integrations. All resonances were seen to overlap due to the presence of high molecular weight wood components and mixtures within them as per previous accounts (King et al., 2009a).

Table 2 shows the amounts of total OH groups present in the original and the acetylated samples. For the low density whole wood sample, we determined that about 6.4 mmol OH/g wood in latewood and 6.6 mmol/g in earlywood were acetylated which is equal to about 41% and 43% of the total OH present in latewood and earlywood, respectively. For the high density board, these amounts were 3.6 mmol/g and 3.5 mmol/g wood for the earlywood and latewood, respectively, which is equal to 28% and 24% of total OH present in earlywood and latewood, respectively. Based on the amounts of OH acetylated in the whole wood samples the weight percent gain (WPG) for the low and the high density samples after acetylation were thus calculated and were found to be around 25% and 17%, respectively. Both of these values are close to previously determined amounts using wet chemical methods (22.5% and 14.5%, respectively). The data for whole wood in the high and the low density boards showed that there are no significant differences between the degree of acetylation in the earlywood and latewood for both samples. Notably, in our effort related to the detailed analyses concerning individual wood component acetylation, we only examined latewood.

Since the acetylation procedure utilized no catalysts, the different responses of the low and high density wood samples to

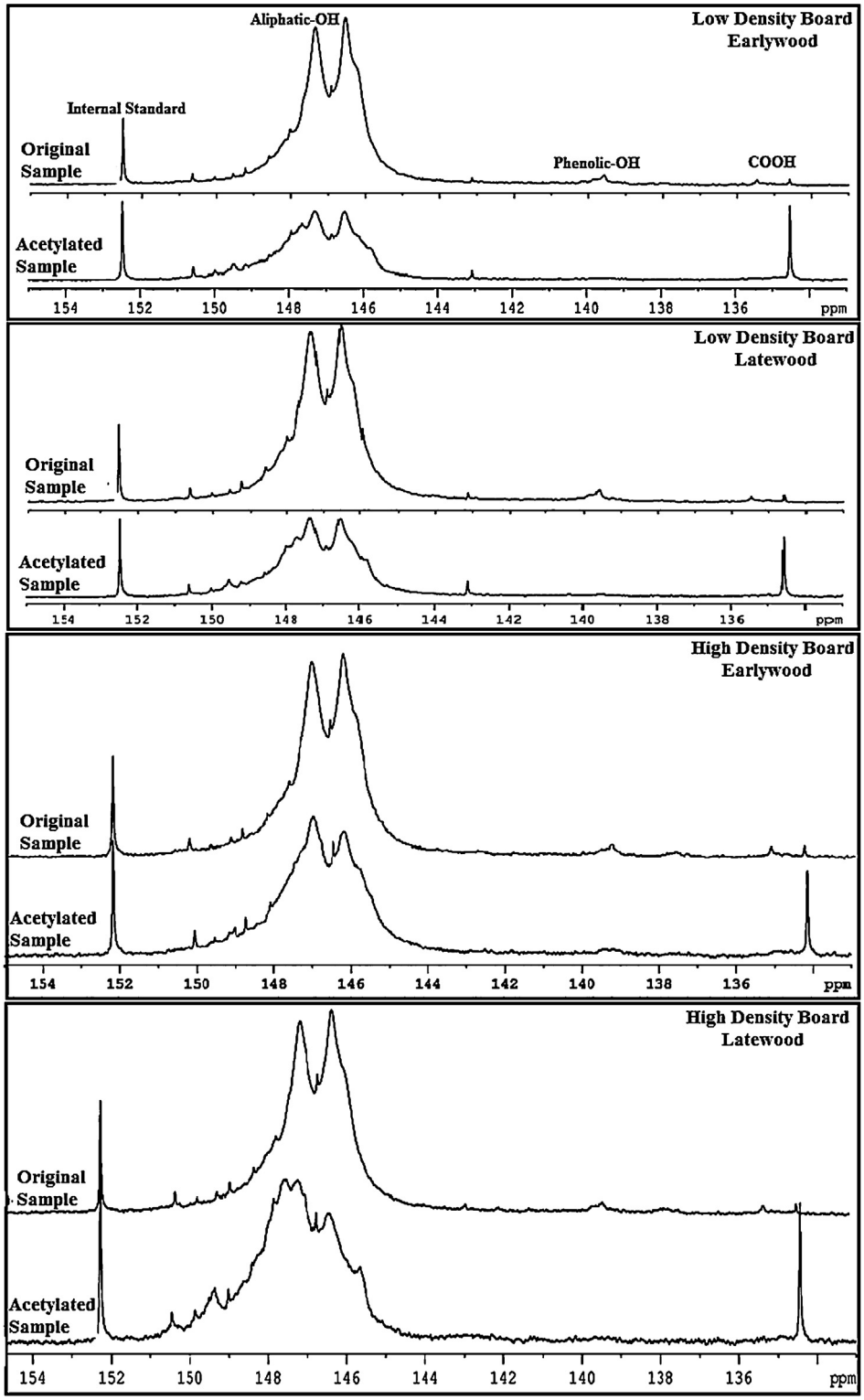


**Fig. 3.** Representative quantitative $^{31}$P NMR spectra of high and low density original and acetylated earlywood and latewood samples.

acetylation can be related to the swelling, accessibility or other chemical effects and not due to catalytic effects (Minato & Ito, 2004). More specifically it is known that during the chemical modification of wood, diffusion effects into the wood structure affect the accessibility of its hydroxyl groups (Papadopoulos & Ntalos, 2004). Table 1 reveals that the low density wood contains 4% more holocellulose and about 5% less lignin than its high density counterpart. Furthermore, Table 2 shows that the total OH in the high and the low density woods were almost same. These data imply that the chemical composition of the low and the high density woods were almost identical and as such cannot explain the magnitude of the difference in the degree of acetylation observed, which is approximately 18% more in the low density material. Therefore, an explanation other than chemical composition may be operational here. For any

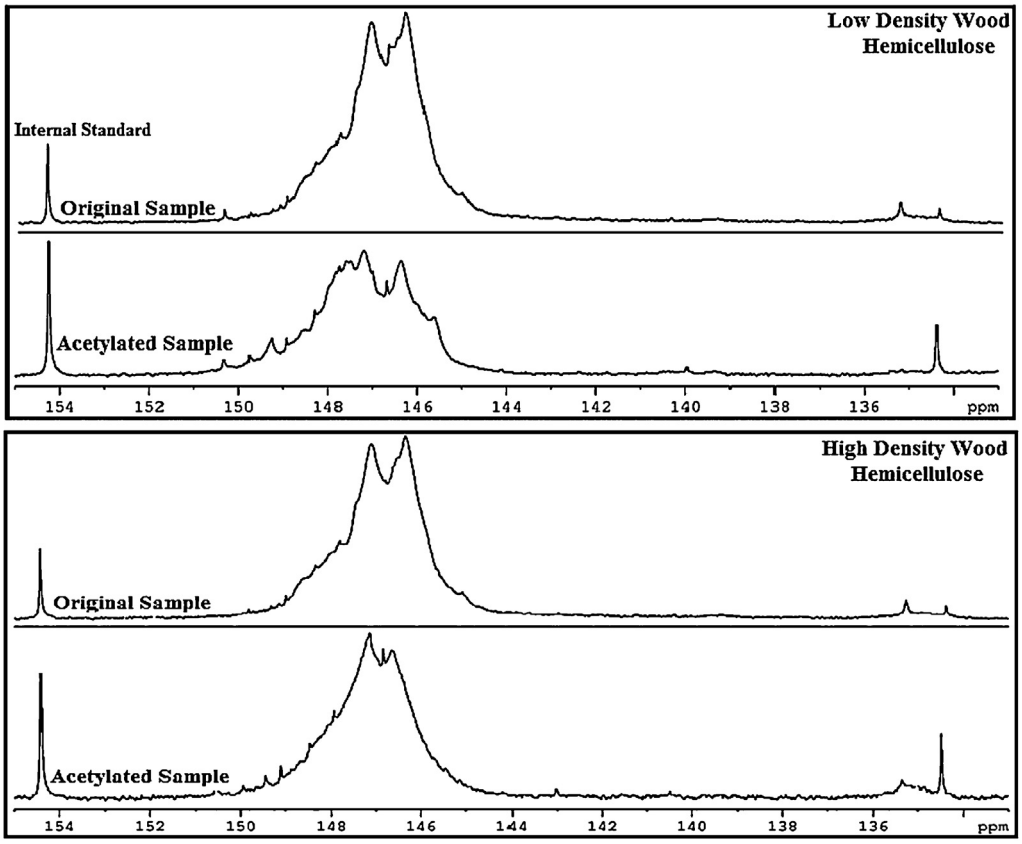


**Fig. 4.** Quantitative $^{31}$P NMR spectra of hemicelluloses originating from high and low density original and acetylated latewood wood samples. Signals between 134–136 ppm are due to COOH groups, while those between 144 and 151 ppm are due to aliphatic OH's.

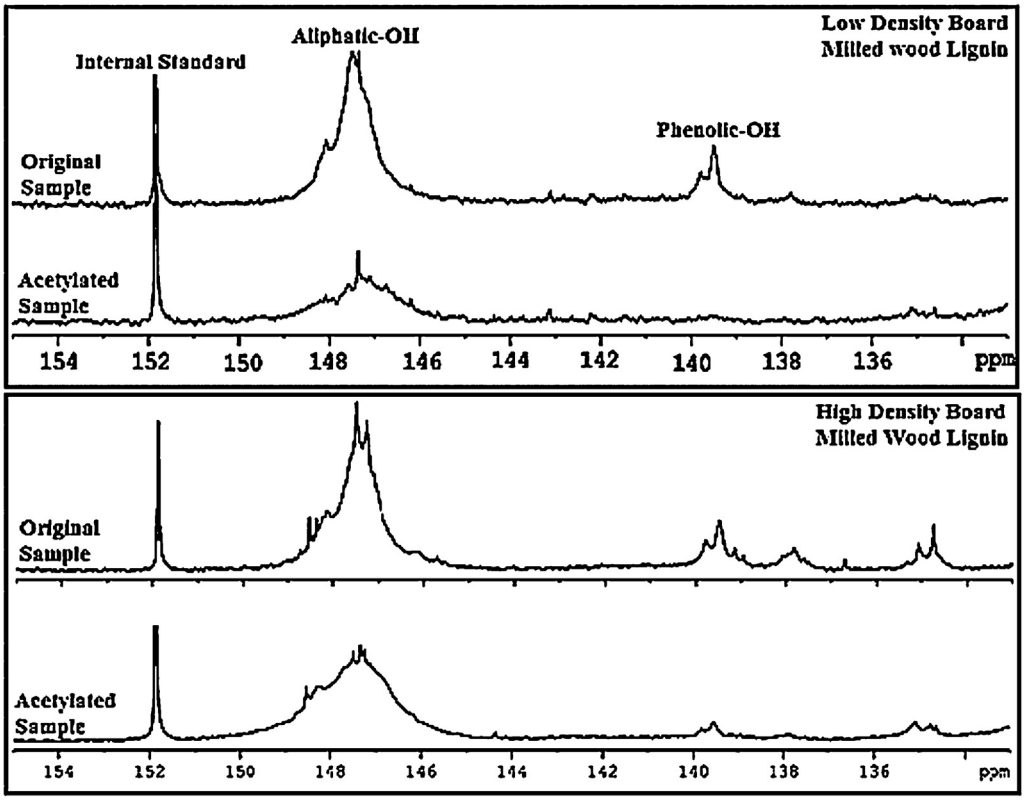


**Fig. 5.** Quantitative $^{31}$P NMR spectra of milled wood lignin originating from high and low density original and acetylated latewood samples. Phenolic OH signals range from 137 to 141 ppm and COOH signals range from 134 to 136 ppm.

**Table 2**
Amounts of hydroxyl groups (mmol/g) determined in whole wood, extracted 461 holocellulose, hemicellulose and MWL for high and low density latewood samples.

| Samples | Whole wood | Holocellulose | Hemicellulose | MWL |
|---|---|---|---|---|
| Original high density wood | 15.51 | 18.25 | 18.08 | 9.08 |
| Acetylated high density wood | 11.87 | 15.21 | 12.62 | 5.05 |
| Original low density wood | 15.61 | 18.12 | 18.16 | 8.45 |
| Acetylated low density wood | 9.22 | 11.9 | 7.28 | 1.77 |

Values in mmol OH/g of sample.

**Table 3**
Percentage of acetylation of total hydroxyl groups in acetylated low and high density latewood wood samples.

| Samples | % of OH acetylation | | | | |
|---|---|---|---|---|---|
| | Whole wood | Holocellulose | Hemicellulose | Cellulose* | MWL |
| High density | 23.4 | 16.6 | 30.2 | 7 | 44.3 |
| Low density | 40.9 | 34.3 | 59.9 | 10 | 79 |

* Cellulose—calculated by difference and based on whole wood composition and by summing up the hemicellulose+ lignin contributions.

reaction to occur with any reactive sites (OH groups in this case) present in the cell wall, accessibility considerations for all reactive groups are of a primary concern. A well swollen state is known to enhance such accessibility considerations since swelling may open up the cell wall micro-voids and promote reaction in otherwise inaccessible sites. This is actually one of the reasons that basic catalysts are used during wood acetylation reactions. However, since no such catalysts were used during this effort, the remaining possibility is that the larger porosity of lower density wood promoted better accessibility of the acetylation reagents within the structure.

### 3.3. Acetylation of hemicelluloses

Around 70% of the total hemicellulose hydroxyls present in the low density wood were isolated, using a DMSO-based extraction method, while only 48% of the hemicellulose present in the high density sample was extracted. It is likely that the higher level of acetylation apparent in the low density sample facilitated the hemicellulose extraction with the organic solvent. This is supported by literature evidence where the main part of the galactoglucomannan present in softwood hemicelluloses was extracted by this method (Goncalves et al., 2008; Teleman et al., 2003). Furthermore, these authors verified that extracting about 50% (or more) of the total hemicelluloses in softwood offer a good representative sample for the whole hemicelluloses present in it.

Fig. 4 shows the quantitative $^{31}$P NMR spectra of the hemicelluloses before and after acetylation for the low and high density samples while the accumulated analytical data is shown in Tables 2 and 3. About 60% of the total OH present in the hemicelluloses of the low density wood was seen to be acetylated while that figure was only 30% for the high density sample. This further demonstrates the higher reactivity of hemicellulose present in low density wood during a non catalyzed acetylation reaction.

### 3.4. Acetylation of lignin & the reactivity of its various groups

Lignin analyses for the non-acetylated and acetylated samples were also carried out after extraction of milled wood lignin from them. Fig. 5 and Tables 2 and 3 show the quantitative $^{31}$P NMR spectra and the accumulated data for the measured OH groups in these lignin samples. About 79% of the total OH present in lignin within the low density sample was acetylated while only 43% of them were acetylated in the lignin of the high density wood sample.

### 3.5. Acetylation of cellulose

Based on the amounts of acetylation determined within the whole wood, hemicellulose and lignin and considering the determined percentage of each component in the wood (Table 1), we calculated the amount of acetylation that occurred in cellulose. Our calculations showed that about 10% of the cellulosic OH groups were acetylated in the low density wood sample and about 7% of them in the high density sample.

## 4. Concluding remarks

Dissolving solid pulverized wood in IL's followed by phosphytilation and quantitative $^{31}$P NMR offers a novel powerfull and previously inaccessible analytical information for it. Low density wood samples showed marked differences toward their propensity to acetylation than higher density in accordance with earlier accounts (Papadopoulos, 2006; Papadopoulos & Ntalos, 2004). In addition our data points to the overall wood microscopic structure influencing the reactivity of its hydroxyl sites toward acetic anhydride in accordance with previous accounts (Ramsden & Blake, 1997). Many researchers suspect that the chemical reactivity of isolated wood components is different to those present in native whole wood due to differences in accessibility, structure and degree of polymerization. Acetylation of isolated wood components showed the reactivity order of lignin > hemicellulose > cellulose (Minato & Ito, 2004; Ramsden & Blake, 1997; Rowell et al., 1994). This study confirmed that the most reactive cell wall component was lignin by examining both the whole wood and its isolated components. Overall, about 80% of the total OH's present in a low density wood sample lignin was acetylated while only 44% of them acetylated in a high density wood sample. With similar methodologies being applied, it was also confirmed that hemicellulose was the next most reactive component in both low and high density wood samples. Hemicellulose hydroxyls were acetylated to a maximum of 60% in the low density wood and about 30% in the high density sample. Finally, only about 10% of the cellulosic OH groups were acetylated in the low density wood sample and about 7% of them in the high density sample. Overall the data show that for both boards no significant differences were apparent on the degree of acetylation for early wood or latewood.

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