Correlation of ^{13}C-NMR Analysis with Fungal Decay Tests of Polymeric Structural Wood Constituents. I. Basidiomycetes

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ABSTRACT: Heat treatment at relatively high temperatures (from 150 to 260°C) is an effective method to improve the durability of wood. This study investigates the reasons for the decay resistance of heat-treated and nontreated wood with respect to the polymeric structural constituents by solid-state cross-polarization/magic-angle spinning (CP–MAS) ^{13}C-NMR analysis before and after exposure to brown rot and white rot fungi. An industrial two-stage heat-treatment method under relatively mild conditions (<200°C) has been used to treat the samples. Brown rot fungi attack polymeric carbohydrates of nontreated Scots pine sapwood at C4, resulting in cleavage and eventually depolymerization of cellulose and hemicelluloses. The attack at the carbohydrate C6, which has never been observed before, is remarkable because the C6 —CH_2OH group has no covalent structural function but acts in fixing the three-dimensional carbohydrate configuration just by secondary forces. The —CH_2OH group carries —OH, which forms some of the strongest hydrogen bonds in the structure of the crystalline native cellulose. It is suggested that the fungus tries to cleave this group to open the cellulose crystalline structure into an amorphous structure to decrease its water repellency to facilitate enzymatic cellulose degradation. Considerable degradation of the hemicelluloses occurs during brown rot fungal exposure, whereas in general the attack on lignin is rather limited, being mainly demethoxylation. However, *Gloeophyllum trabeum* is an active brown rot fungus in the (partial) degradation of lignin because there is some indication of ring opening of the aromatic ring of lignin during fungal exposure. Aromatic ring opening has also been observed after exposure to *Coriolus versicolor*, a white rot fungus. The demethoxylation of lignin and some attack on wood carbohydrates are also characteristic of the attack of this white rot fungus. The CP–MAS ^{13}C-NMR spectra of heat-treated Norway spruce reveal similarities but also clear differences after fungal exposure in comparison with nontreated Scots pine sapwood. Brown rot fungi seem to have a preference to attack the carbohydrates of heat-treated wood at C4 and especially C1, cleaving the skeleton of cellulose and glucomannans. In untreated Scots pine sapwood, this attack mainly occurs at C4, the nonreducing end of the glucose unit. An attack on the out-of-the-ring alcoholic group —CH_2OH of the carbohydrates of heat-treated Norway spruce is less obvious than that in untreated Scots pine. The attack on C3/C5 of the carbohydrates is remarkable, indicating ring opening of the glucose units, which has not been observed in nontreated Scots pine sapwood. Lignin degradation is limited to demethoxylation, and low or no aromatic ring opening is observed, even after *C. versicolor* exposure. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 2639–2649, 2006

Key words: NMR; wood modification; heat resistant; decay

INTRODUCTION

Heat treatment at relatively high temperatures (from 150 to 260°C) is an effective method to improve the durability of wood.[1–7] Several authors have tried to explain the molecular reasons for this durability improvement.[1–3,5,6,8–10]

According to Stamm,[2] there is a link between the improvement of the dimensional stability and the durability after heat treatment. This dimensional stability improvement is thought to be caused by hemicellulose degradation into furfural polymers that are less hygroscopic toward water.

Baechler[1] suggested that chemical transformations of minor wood constituents (e.g., minerals and vitamins) that are essential to the metabolism of fungi are involved in the decay resistance of heat-treated wood. Baechler also suggested that changes in wood components, such as low-molecular-weight carbohydrates, which may be necessary for the initial attack by the fungal organisms, are involved in the decay resistance. Highley[3] suggested that the modification of carbohydrates and the formation of toxic compounds are involved.

The results of a ^{13}C-NMR study[8] indicate that an increase in crosslinking within the lignin–carbohy-

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drate complex occurs with a consequent improvement of the hygroscopicity (a lower fiber saturation point). This appears to play a role in a higher resistance against biological deterioration under certain conditions. The modification of the polymeric structural wood components is also believed to contribute to fungal decay resistance, though to a lower extent. Hemicelluloses, the most hydrophilic and easily digested wood component, have been shown to be transformed selectively into a hydrophobic network.[8]

Some decay resistance against lignin-degrading fungi has been found.[5] According to Weiland and Guyonnet,[5] no degradation of cellulose was observed after the addition of cellulase to heat-treated wood. Cellulase is a multienzymatic system consisting of different enzymatic components that are able to convert (isolated) cellulose to glucose.[11] However, because of their molecular weights (30–182 kD), cellulase enzymes cannot penetrate the cell wall in the initial stages of decay[12] and therefore cannot cause the degradation of cellulose after addition to heat-treated wood. The metabolic mechanism of fungal decay in wood is much more complex, including the occurrence of different enzymatic and nonenzymatic systems.

They furthermore claimed that the decay resistance of heat-treated wood is caused by

- A decrease in wood substrates and especially hemicelluloses.
- A chemical modification of the lignin, which cannot be degraded by fungal enzymatic systems.
- A reduction of the hygroscopic wood properties.

Durability experiments with heat-treated maritime pine have shown that despite a strong hemicellulose degradation by heat treatment, fungal attack still occurs.[6] The effect of an initial colonization inhibition due to the thermal degradation of pentosanes seems less than expected. Lignin modifications and the formation of new ether linkages have therefore been suggested as possible causes of decay resistance. The fungal enzymatic systems are thought to be obsolete and not (or less) capable of degrading the modified wood components.

The presence of traces of polynuclear aromatic hydrocarbons has also been detected in heat-treated wood.[9] It has been suggested that the presence of such compounds might contribute to the resistance of wood to fungal attack. However, the removal of such compounds by extraction (with water, acetone, and chloroform) did not result in any significant change in the weight loss after fungal attack.[10] This indicates that the contribution of these compounds to decay resistance is rather limited.

This study was performed to investigate the reasons for the decay resistance of heat-treated wood with respect to untreated wood in more detail with cross-polarization/magic-angle spinning (CP–MAS) ^{13}C-NMR analysis before and after fungal exposure. A well-established industrial process in current use,[11] based on a two-stage heat-treatment method under relatively mild conditions (<200°C), was used to treat the wood samples. The results of ^{13}C-NMR, Fourier transform infrared, and chemical analysis of similarly heat-treated wood have already been reported and discussed.[8,13,14]

EXPERIMENTAL

Materials

Norway spruce (*Picea abies* Karst) specimens were used for heat treatment and chemical analysis. Untreated Scots pine sapwood (*Pinus sylvestris* L.) specimens were used as references. Norway spruce boards with standard cross-section sizes (32 mm thick and 150 mm wide) and a length of at least 3.0 m were used for heat treatment. The moisture content before the treatment was 16–20% (shipping dry).

Heat treatment

The heat treatment was performed in two separate stages. In the first stage, the wood samples were treated in an aqueous environment at superatmospheric pressure (6–8 bar) with saturated steam as the heating medium to increase the temperature of the boards. This so-called steam hydrothermolysis treatment was performed in an industrial plant at an effective treatment temperature of 165°C. Cooling was accomplished by the flashing of the reactor (a quick but controlled release of the pressure) to atmospheric conditions followed by cold water circulation at the wall of the reactor. The wood samples were then dried with a conventional drying process at 50–60°C. After drying, the wood samples were heat-treated again in a special curing kiln (second stage) under dry and atmospheric conditions for a so-called curing treatment (at 170–180°C). During this stage, superheated steam was used as a sheltering gas to exclude oxygen (reducing fire risks and preventing nondesired oxidation reactions).

Fungal testing

Treated Norway spruce and untreated Scots pine sapwood reference specimens (15 × 25 × 50 mm) were prepared and exposed to brown rot fungi (*Coniophora puteana*, *Gloeophyllum trabeum*, and *Poria placenta*) and white rot fungi (*Coriolus versicolor*) for 16 weeks according to the EN 113 agar test.[15] After exposure, the specimens were oven-dried and weighed to determine the weight loss and moisture content. The Norway

spruce boards were heat-treated with the steam hydrothermolysis treatment.

Solid-state CP–MAS ^{13}C-NMR analysis

Fungus-treated Norway spruce EN 113 specimens with a relative low weight loss (1–4%) and a high weight loss (20–23%) were selected for analysis. The specimens were ground before analysis. Untreated Scots pine sapwood references were also used for analysis. Treated EN 113 specimens with low-to-high weight losses were selected to determine the equilibrium moisture content (EMC) at 90 and 95% relative humidity (RH).

The treated and untreated timber specimens were analyzed by solid-state CP–MAS ^{13}C-NMR. Spectra were obtained on a Bruker MSL 300 FT-NMR spectrometer (Germany) at a frequency of 75.47 MHz and at a sample spin of 4.0 kHz. The impulse duration at 90° was 4.2 ms, the contact time was 1 ms, the number of transients was about 1000, and the decoupling field was 59.5 kHz. Chemical shifts were determined with respect to tetramethylsilane as a control. The spectra were accurate to 1 ppm. The spectra were run with suppression of spinning sidebands.

Hygroscopicity testing

After fungal testing, treated Norway spruce and untreated Scots pine sapwood reference specimens (15 × 25 × 50 mm) were oven-dried (at 105°C for 16 h). The oven-dried specimens were stored in a box (including a ventilator) for 3 days above a saturated potassium nitrate solution (20°C and 95% RH). Before and after the test, the specimens were weighed to determine the EMC. Treated Norway spruce and untreated pine sapwood reference specimens (without fungal testing) were also tested.

TABLE I
Weight Loss and Moisture Content of Heat-Treated Norway Spruce and Nontreated Scots Pine Specimens After Fungal Exposure to Brown and White Rot Basidiomycetes (EN 113)

EN 113 specimen	Weight loss (%)	Moisture content (%)
1. *C. puteana* (brown rot basidiomycete)		
Treated Norway spruce, low weight loss	0.87	36
Treated Norway spruce, high weight loss	23.08	29
Scots pine sapwood reference	45.35	38
2. *G. trabeum* (brown rot basidiomycete)		
Treated Norway spruce, low weight loss	0.17	21
Treated Norway spruce, high weight loss	20.46	27
Scots pine sapwood reference	31.4	29
3. *P. placenta* (brown rot basidiomycete)		
Treated Norway spruce, low weight loss	3.95	36
Treated Norway spruce, high weight loss	21.10	32
Scots pine sapwood reference	38.12	33
4. *C. versicolor* (white rot basidiomycete)		
Treated Norway spruce, low weight loss	3.97	21
Treated Norway spruce, high weight loss	19.98	32
Scots pine sapwood reference	27.84	41

RESULTS AND DISCUSSION

Characterization of the wood after fungal exposure

The weight loss and moisture content of the EN 113 specimens that were used for NMR analysis are shown in Table I. In Figures 1–4 are shown the CP–MAS ^{13}C-NMR spectra of heat-treated Norway spruce and the untreated Scots pine sapwood reference after fungal exposure to brown rot (*C. puteana*, *G. trabeum*, and *P. placenta*) and white rot fungi (*C. versicolor*). The major changes due to fungal degradation are described next.

Untreated Scots pine reference

Fungal exposure to *C. puteana* results in a strong attack on the carbohydrates (cellulose and hemicelluloses) of untreated Scots pine [Fig. 1(a)]. The crystalline peak (89 ppm) and especially the amorphous peak (82 ppm) of the carbohydrate C4 decrease after fungal exposure. The high peak at 105 ppm shows a clear decrease, and the shoulder on the right side of this peak has almost disappeared. This peak and its shoulder belong to C1 of the carbohydrates. Fungal attack at C1 and C4 is believed to cause cleavage of hemicelluloses and cellulose, reducing the degree of polymerization. The decrease after the fungal exposure of the crystalline (65 ppm) and amorphous (62 ppm) peaks of the carbohydrate C6 band is remarkable. This attack at C6, the out-of-the-ring alcoholic function —CH_2OH, is very interesting, especially because this group has no covalent structural function. One reason is likely that the carbohydrate C6 is a pendant external group that is easier to attack. However, as enzymes work by evolutionary adaptation, a very different reason could be the cause. The —CH_2OH group carries the —OH, which forms the strongest and more complete hydrogen bonds in the structure of crystalline native cellulose.[16] The fungus tries to cleave this group to open the cellulose crystalline structure, step by step, to an amorphous structure both to facilitate cellulose degradation and to decrease the water repellence of the (crystalline) structure. The C2 peak at 75 ppm and C3

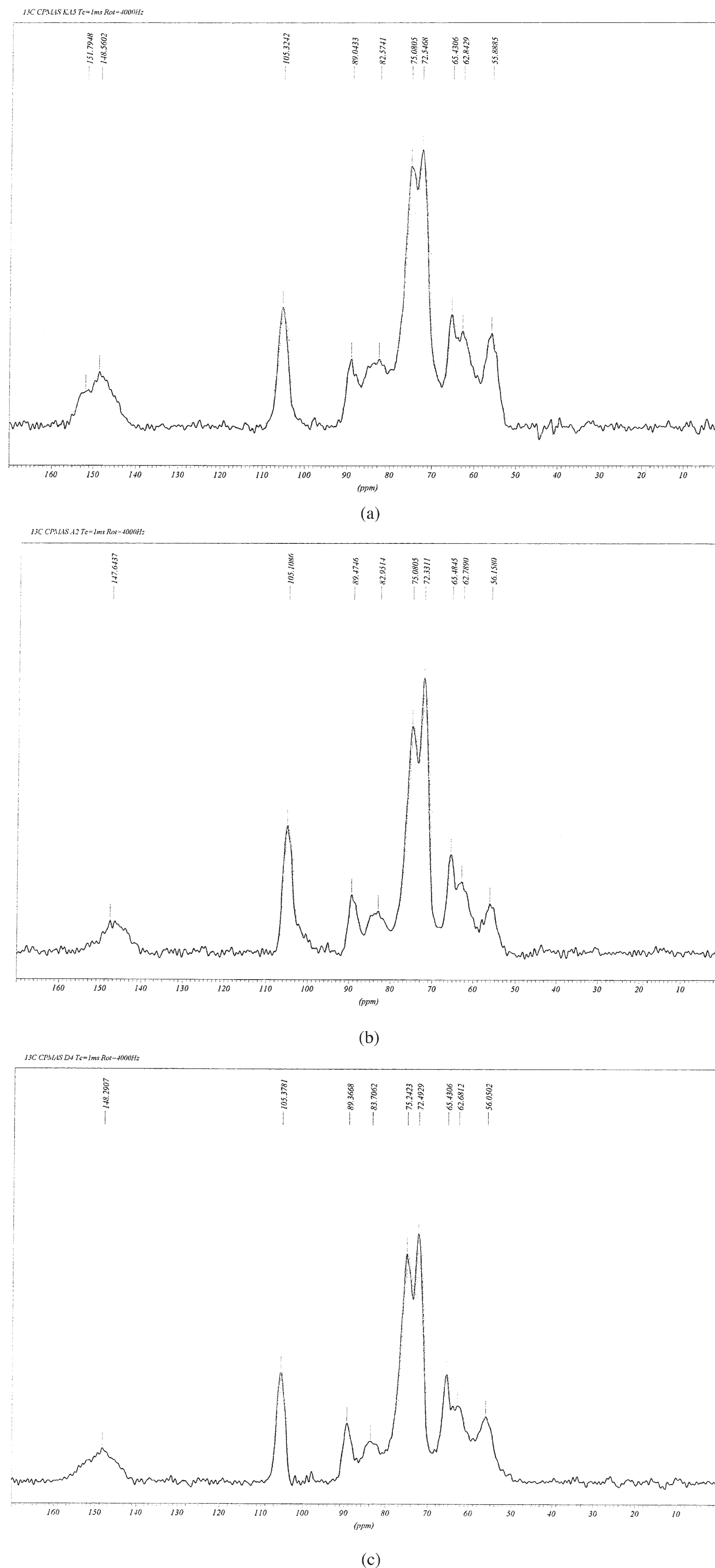

Figure 1 CP–MAS ^{13}C-NMR spectra after *C. puteana* exposure (EN 113) of (a) untreated Scots pine sapwood, (b) heat-treated Norway spruce at low weight loss, and (c) heat-treated Norway spruce at high weight loss.

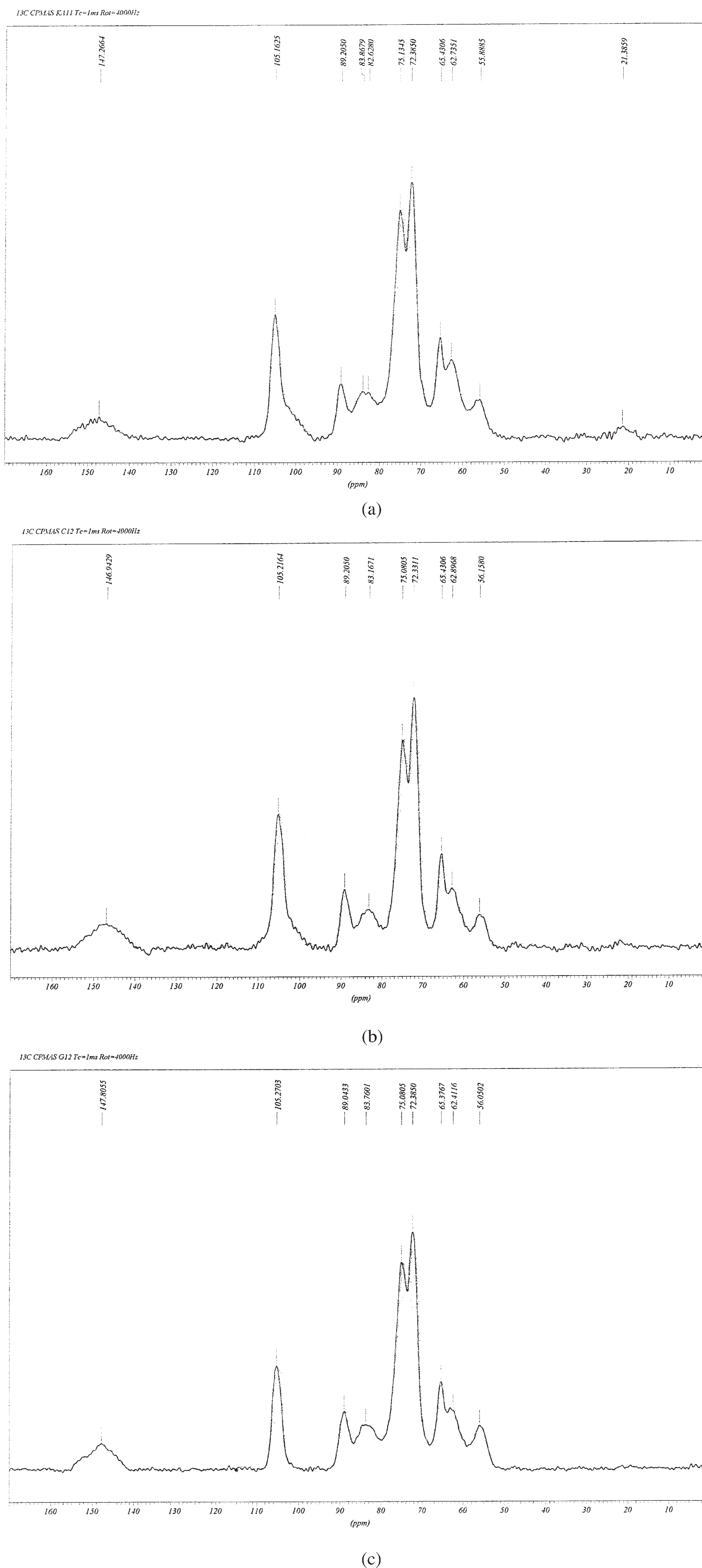

Figure 2 CP–MAS ^{13}C-NMR spectra after *G. trabeum* exposure (EN 113) of (a) untreated Scots pine sapwood, (b) heat-treated Norway spruce at low weight loss, and (c) heat-treated Norway spruce at high weight loss.

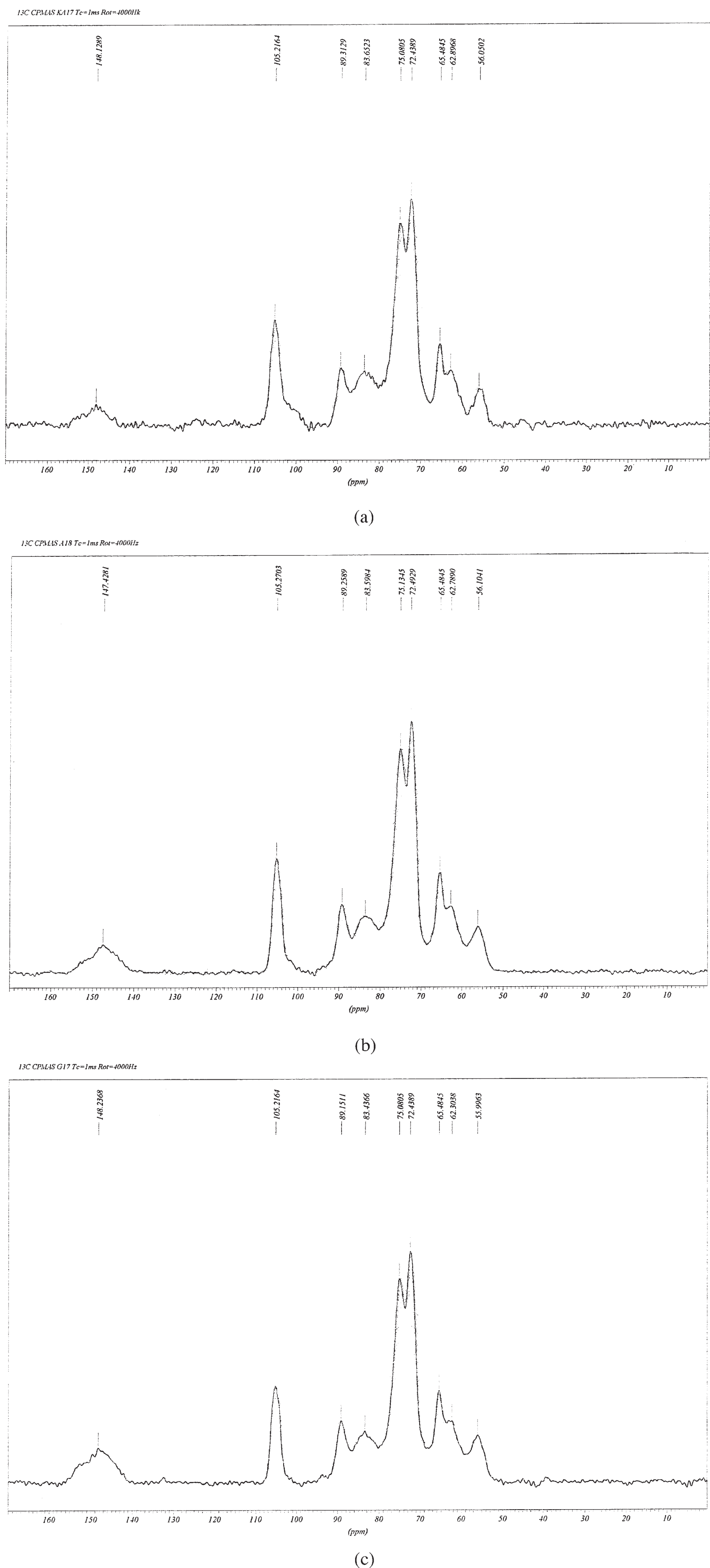

Figure 3 CP–MAS ^{13}C-NMR spectra after *P. placenta* exposure (EN 113)of (a) untreated Scots pine sapwood, (b) heat-treated Norway spruce at low weight loss, and (c) heat-treated Norway spruce at high weight loss.

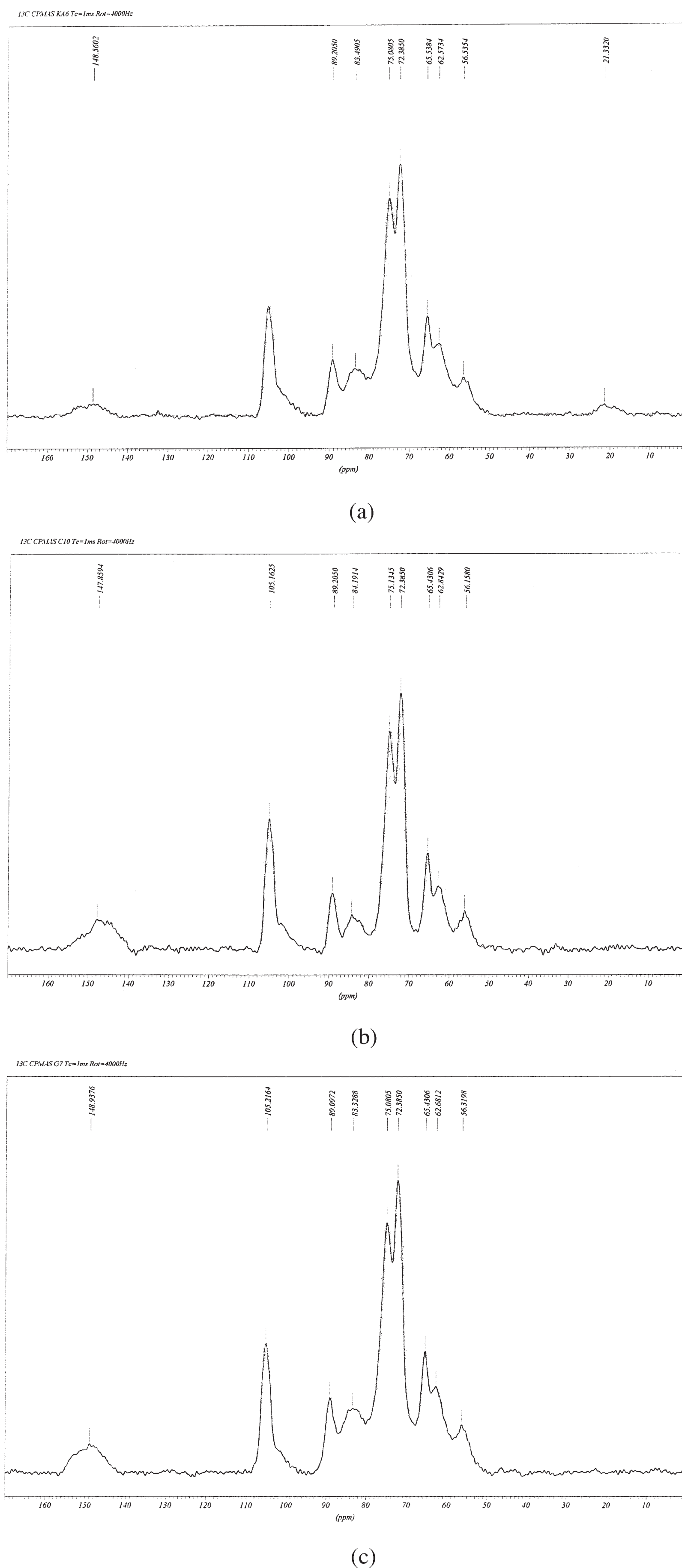

Figure 4 CP–MAS ^{13}C-NMR spectra after *C. versicolor* exposure (EN 113)of (a) untreated Scots pine sapwood, (b) heat-treated Norway spruce at low weight loss, and (c) heat-treated Norway spruce at high weight loss.

and C5 peaks at 72 ppm do not change, and this indicates that no ring opening of the glucose units occurs during fungal exposure. The acetyl group (CH_3COO—) peak of hemicelluloses (at 23 ppm) disappears, and this indicates marked degradation of hemicelluloses.

The attack on lignin of untreated Scots pine after exposure tot *C. puteana* is rather limited. There is a slight decrease of the —OCH_3 peak (56 ppm), which means some, but not too extensive, demethoxylation of lignin. A peak around 148 ppm can be taken as furfural formation superimposed to (Ar)*C*—OH of lignin. The shifts of solid-state NMR are not as well defined as those of liquid-state NMR, and the band (or shoulder, more often) that sometimes appears at 152–153 ppm means that two things can occur. The band at 152–153 ppm is like that of an Ar*C*—OH phenolic group of lignin with no methoxy group as a substituent, whereas the band at 148 ppm is the same type of carbon but with an Ar*C*—OCH_3 group as a substituent. Superimposed on the 148 ppm peak is the band of furfural too. Thus, when the 153 ppm band appears, it likely indicates demethoxylation of lignin and hence a proportionally higher level of phenolic hydroxy groups. Both furfural production and demethoxylation can be due to radical reactions characteristic of fungal enzymatic and nonenzymatic degradation mechanisms.[12,17,18]

All three wood components (cellulose, hemicelluloses, and lignin) are attacked after fungal exposure to *G. trabeum*, *P. placenta*, and *C. versicolor* fungal species. *G. trabeum* shows a clear preference to attack the amorphous carbohydrates at C4 and C6 because the C4 peak (at 82 ppm) and the C6 peak (at 62 ppm) decrease after fungal exposure [Fig. 2(a)]. A slight decrease of the 83 ppm peak (amorphous C4) and a clear decrease of the 62 ppm peak (amorphous C6) are visible after exposure to *P. placenta* [Fig. 3(a)]. Therefore, this fungus shows a preference to attack the amorphous carbohydrates at C6, although some attack at amorphous C4 also occurs. The C1 peak of the carbohydrates (at 105 ppm) does not change, and the shoulder on the right side of this peak is still visible after fungal exposure to *G. trabeum* and *P. placenta*. The depolymerization of the carbohydrates occurs at C4 and probably either later or not at all at C1 when untreated Scots pine sapwood is exposed to these fungi.

The white rot fungus *C. versicolor* shows an attack on the amorphous carbohydrates at C4 and C6 because the amorphous peaks of C4 (82 ppm) and especially C6 (62 ppm) are reduced after fungal exposure. Furthermore, the high C1 peak at 105 ppm clearly decreases, whereas its shoulder is still visible. Thus, this fungus tries to depolymerize the carbohydrates through an attack at C1 and C4.

The CH_3COO— hemicellulose peak (23 ppm) is still visible after exposure to *G. trabeum* [Fig. 2(a)] and *C. versicolor* [Fig. 4(a)], indicating the presence of some hemicelluloses. After exposure to *P. placenta*, the CH_3COO— hemicellulose peak disappears [Fig. 3(a)], and this indicates greater hemicellulose degradation after fungal exposure.

The Ar*C*—OH peak at 148 ppm and the Ar*C*—OCH_3 peak at 145 ppm clearly decrease after *G. trabeum* exposure, and this possibly indicates ring opening of the lignin aromatic structure [Fig. 2(a)]. *G. trabeum* seems to be an active brown rot fungus, partially degrading lignin. In this way, the fungus has better access to the carbohydrates. Neither peak changes after exposure to *P. placenta* [Fig. 3(a)]. Nevertheless, it seems that this fungus also tries to open the aromatic structure of lignin because the Ar*C*—H peaks at 112 and 132 ppm decrease slightly. Exposure to the white rot fungus *C. versicolor* results in a clear attack on lignin [Fig. 4(a)]. A clear decrease of the 145 ppm peak (Ar*C*—OCH_3) and 148 ppm peak (Ar*C*—OH) is visible, indicating ring opening and degradation of the aromatic nuclei. A decrease of the 56 ppm peak (O—CH_3) after exposure to *G. trabeum*, *P. placenta*, and *C. versicolor* indicates demethoxylation of lignin.

Heat-treated Norway spruce

Heat treatment causes a modification of the main components of wood (cellulose, hemicelluloses, and lignin) and changes the composition of these components.[8,13,14] The most important changes found so far are

- Degradation of the hemicelluloses.
- An increase in the relative proportion of the crystalline cellulose.
- Further crosslinking of the lignin network due to polycondensation reactions.

In Figures 1(b) and 2(b), ^{13}C-NMR spectra are given of heat-treated Norway spruce after exposure to *C. puteana* and *G. trabeum* at a very low weight loss (0.87 and 0.17%, respectively). At such a low weight loss, the decay of wood components is still rather limited, and changes due to the heat treatment are clearly visible. The acetyl group (CH_3COO—) peak of hemicelluloses (at 23 ppm) has disappeared, and this indicates thermal degradation of hemicelluloses. A clear decrease of the amorphous C4 (83 ppm) and C6 (62 ppm) peaks of the carbohydrates is visible because of the thermal degradation of hemicelluloses and amorphous cellulose.

The ^{13}C-NMR spectra of heat-treated Norway spruce [Fig. 1(c)] reveal a clear attack on the carbohydrates (cellulose and hemicelluloses) after exposure to *C. puteana* at a high weight loss (23.1%). The amorphous (83 ppm) and crystalline (82 ppm) peaks of C4

decrease after fungal exposure. The 105 ppm peak of C1 shows a clear decrease, and the shoulder on the right side of this peak disappears. The fungus attacks the carbohydrates at both C1 and C4 to cleave and eventually depolymerize cellulose and hemicelluloses. Some decrease of the amorphous (62 ppm) and crystalline (65 ppm) peaks of C6 are visible, indicating some attack on the out-of-the-ring alcoholic group —CH_2OH of the carbohydrates. The clear decrease of the 72 ppm peak, which belongs to C3/C5 of the carbohydrates, is remarkable, indicating ring opening of the glucose units. No changes in the O—CH_3 peak at 56 ppm, the Ar*C*—OCH_3 peak at 145 ppm, and the Ar*C*—OH peak at 148 ppm have been observed, and this indicates that this *C. puteana* does not attack or attacks only slightly the aromatic structure of lignin.

G. trabeum and *P. placenta* show a preference to attack the carbohydrates of heat-treated Norway spruce at C1, rather than at C4. The 105 ppm peak of C1 shows a clear decrease, and the shoulder on the right of this peak has disappeared, whereas the amorphous and crystalline peaks of C4 (83 and 89 ppm) and C6 (62 and 65 ppm) do not change after fungal exposure [Figs. 2(c) and 3(c)]. The attack at C1 has already occurred after exposure to *P. placenta* at a low weight loss (3.95%) because the 105 ppm peak of C1 decreases and the shoulder on the right side of this peak almost disappears [Fig. 3(b)]. The fungus probably tries to cleave the carbohydrates at C1 in the initial stages of decay. *C. versicolor* also attacks the carbohydrates at C1 because the 105 ppm peak clearly decreases after fungal exposure. However, the shoulder on the right side of this peak is still visible as in the spectra of nontreated Scots pine sapwood. The crystalline C4 peak at 89 ppm, which decreases after fungal exposure to *C. versicolor*, is remarkable. In some unknown way, the fungus can attack crystalline cellulose. *G. trabeum*, *P. placenta*, and *C. versicolor* do not attack or attack only slightly the carbohydrates at C6 because the amorphous and crystalline C6 signals (at 62 and 65 ppm, respectively) do not change after fungal exposure. Less obvious but still visible is the decrease of the C3/C5 peak at 72 ppm after exposure to *G. trabeum* and *P. placenta*, indicating some ring opening of the glucose units of the carbohydrates. This is not observed after exposure to the white rot fungus *C. versicolor*.

The exposure of heat-treated Norway spruce to *G. trabeum* seems to have little or no effect on lignin, especially in comparison with untreated Scots pine [Fig. 2(a)]. The Ar*C*—OCH_3 peak at 145 ppm and the Ar*C*—OH peak at 148 ppm do not change after fungal exposure [Fig. 2(c)]. However, the decrease of Ar*C*—H at 119 and 123 ppm indicates some attack of the fungus on the aromatic structure of lignin. The increase of the peak at 148 ppm in the spectra of *P. placenta* [Fig. 3(c)] at a high weight loss (21.1%) is remarkable. This must be due to a higher proportion of furfural probably produced from the carbohydrates by fungal attack because the 148 ppm peak is slightly but noticeably higher than that in the spectra of *P. placenta* [Fig. 3(b)] at a low weight loss (3.95%). Heat-treated wood lignin can also be observed as a separate, distinct peak at 153 ppm, and in many cases it exists as a shoulder at a slightly higher point than the 148 ppm peak, a shoulder always smaller than the furfural peak at 148 ppm. It is believed that the depression of the isotherm and the increased water repellency of heat-treated wood are caused by furfural self-polymerization and crosslinking coupled to some crosslinking of lignin.[8] The ring opening of the aromatic structure of lignin probably does not occur because the increase of the 148 ppm peak is rather small and the Ar*C*—OCH_3 peak at 145 ppm does not change after fungal exposure to *P. placenta*. The 148 ppm peak in the heat-treated Norway spruce spectra after *C. versicolor* exposure [Fig. 4(c)] is much higher in comparison with the untreated Scots pine reference [Fig. 4(a)], indicating lower or no opening of some of the lignin aromatic rings and/or the presence of furfural. The 132 ppm peak (Ar*C*—H or Cγ) disappears [Fig. 4(c)], and this indicates a certain proportion of lignin skeleton cleavage and a decrease in the molecular weight. The 119 peak (Ar*C*—H) decreases [Fig. 4(c)], and this means some attack on the aromatic rings of lignin. A clear decrease of the 56 ppm peak (O—CH_3) is visible after exposure to *C. versicolor*, indicating demethoxylation of lignin. Some demethoxylation also occurs after exposure to *G. trabeum* because the O—CH_3 peak at 56 ppm is slightly decreased.

Hygroscopicity of the wood after fungal testing

The fungal decay of untreated Scots pine results in a reduction of the EMC due to the degradation of carbohydrates (Table II). Less (amorphous) cellulose and hemicelluloses are available to adsorb moisture. Differences in EMC between the fungi used can be explained by the weight loss of the specimen, with the highest weight loss giving the lowest EMC (fewer carbohydrates available to adsorb moisture). Fungal preference to attack a specific wood component could also be a reason for these differences.

The EMC of treated Norway spruce increases after fungal testing (Table II), despite fungal degradation of some hemicelluloses that are still available after heat treatment and fungal degradation of (amorphous) cellulose. This increase might be due to the transformation of crystalline cellulose in amorphous cellulose and/or specific (oxidation) reactions of wood components during fungal decay making the wood more hygroscopic. It is striking that the EMC of heat-treated Norway spruce after *C. versicolor* exposure is somewhat higher than that after brown rot fungal exposure.

TABLE II
ENC at 95% RH (20°C) of Heat-Treated Norway Spruce and Nontreated Scots Pine Sapwood After Fungal Testing

Weight loss (%)	ENC (%) at 95% RH and 20°C			
	C. puteana	*G. trabeum*	*P. placenta*	*C. versicolor*
Treated Norway spruce				
0	12.5			
9.3	13.8			
23.5	13.4			
0		12.5		
5.93		13.4		
19.1		14.1		
0			12.5	
8.3			12.8	
24.1			14.1	
0				12.5
4.3				12.7
8.5				13.5
19.0				15.2
Nontreated Scots pine sapwood				
0	21.0	21.0	21.0	21.0
58.4	15.4			
36.6		17.7		
40.4			17.3	
29.2				19.5

Tjeerdsma et al.[8] showed an increase in crosslinking within the lignin–carbohydrate complex after heat treatment, and they suggested that this could be the reason for improved hygroscopicity. Lignin degradation during fungal exposure could reduce the crosslinking network around the cellulose fibrils, making wood more hygroscopic, especially when *C. versicolor* acts as a selective white rot fungus and cellulose is not degraded.

CONCLUSIONS

CP–MAS ^{13}C-NMR spectra have revealed similarities but also clear differences in the polymeric structural wood constituents of heat-treated wood and untreated wood after fungal exposure. Brown rot fungi attack the polymeric carbohydrates of untreated Scots pine sapwood at C4, resulting in cleavage and eventually depolymerization of cellulose and hemicelluloses. The attack at the carbohydrate C6, which has never been observed before, is remarkable because the C6 —CH_2OH group has no covalent structural function but acts in fixing the three-dimensional carbohydrate configuration just by secondary forces. The —CH_2OH group carries the —OH, which forms the strongest and more complete hydrogen bonds in the structure of crystalline native cellulose. It is suggested that the fungus tries to cleave this group to open the cellulose crystalline structure to an amorphous structure both to facilitate cellulose degradation and to decrease the water repellence of the (crystalline) structure. Considerable degradation of the hemicelluloses occurs during brown rot fungal exposure, whereas in general the attack on lignin is rather limited, being mainly demethoxylation. However, *G. trabeum* has been found to be an active brown rot fungus in the (partial) degradation of lignin because there is some indication of ring opening of the aromatic ring of lignin during fungal exposure. Aromatic ring opening is also observed after exposure to *C. versicolor*, a white rot fungus. The demethoxylation of lignin and some attack on wood carbohydrates are also characteristic of the attack of this white rot fungus.

Brown rot fungi seem to have a preference to attack the carbohydrates of heat-treated Norway spruce at C4 and especially C1, cleaving the skeleton of cellulose and glucomannans. In nontreated Scots pine sapwood, this attack mainly occurs at C4, the nonreducing end of the glucose unit. The attack on the out-of-the-ring alcoholic group CH_2OH of the carbohydrates of heat-treated Norway spruce is less obvious than that in nontreated Scots pine. The attack on the C3/C5 of the carbohydrates is remarkable, indicating ring opening of the glucose units, which has not been observed in nontreated Scots pine sapwood. Lignin degradation is limited to demethoxylation, and low or no aromatic ring opening is observed, even after *C. versicolor* exposure.

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