Resistance against fungal decay of Scots pine sapwood modified with phenol-formaldehyde resins with substitution of phenol by lignin pyrolysis products

Abstract: Phenol-formaldehyde (PF) resins can be impregnated and cured in situ to improve the wood’s dimensional stability and decay resistance. In search of renewable alternatives, the substitution of phenol by lignin cleavage products (LCP) has been discussed. However, the different chemical nature may affect the performance of the resin against fungal decay, formaldehyde emission, and equilibrium moisture content. In this study, 30 % (w/w) of the phenol in PF resins were substituted by LCP obtained from microwave-assisted pyrolysis. Scots pine sapwood was modified with the resin. The decay resistance against Rhytisma acerinum, Gloeophyllum trabeum, and Trametes versicolor was determined. Additionally, effects of specimen organisation within the Petri dish, different substrates, length of leaching, and type of inoculum were studied. Further, the materials water vapor sorption properties and formaldehyde emission were determined. All modifications effectively reduced fungal decay. With 10 % weight percent gain (WPG), initial decay was detected, while 20 % WPG and 30 % WPG provided efficient protection. The substitution of phenol increases the formaldehyde emission. While further reduction in formaldehyde in the resin admixture or formaldehyde scavengers may be required, the method described herein can be used to partly replace fossil-based phenol, while maintaining good fungal resistance.

Keywords: basidiomycetes; bio-oil; durability; phenol-formaldehyde resin; wood

1 Introduction

Wood is prone to biological degradation, for example by bacteria, fungi, insects, or marine borers. Fungal attack on wood can be divided into aesthetic disfiguration (mould and staining fungi) and decay fungi. Mould and staining fungi cause damage to the surface of wood. While they can stain the wood and increase the water absorptivity, the strength of the wood is usually not altered. Decay fungi can be separated into brown rot fungi, white rot fungi and soft rot fungi. Brown rot fungi utilize a two-step oxidative-enzymatic mechanism (Goodell et al. 2017; Zhang and Schilling 2017) for depolymerisation of hemicellulose and cellulose and leave behind a brown modified lignin (Filley et al. 2002; Yelle et al. 2011), hence the name. The decay mechanism leads to a fast decrease of the strength properties of wood even at low mass loss (Wilcox 1978; Winandy and Morrell 1993). For white rot both simultaneous and selective decay pathways can be found; selective white rot leave behind a cellulose enriched material while simultaneous white rot fungi degrade cellulose, lignin, and hemicelluloses at the same rate (Eriksson et al. 1990). The strength properties decrease gradually along with increase of decay. While brown- and white rot is caused by basidiomycetes, soft rot is caused by ascomycetes and fungi imperfecti. Soft rot fungi usually occur in environments that exclude basidiomycetes, e.g., excessively wet, dry, hot (>50 °C) or oxygen limited environments (Blanchette et al. 1990; Duncan and Eslyn 1966). They preferably degrade hemicelluloses and cellulose over lignin and perform greater decay of deciduous than conifer wood (Nelson et al. 1995).

It was estimated that 10 % of the timber cut annually in the United States is used to replace decayed wood, and immense costs are connected to it (Zabel and Morrell 2020).
Decay probability and decay rate depend on many factors, amongst them the wood species used, climate conditions, and construction details. In studies from Northern Europe and the USA brown rot fungi account for 73–85 % of fungal decay damages in wood structures (Alfredsen et al. 2005; Duncan and Lombard 1965; Schmidt 2007; Viitanen and Ritschko 1991). A reason for this is the extensive use of coniferous timber, which is more susceptible to brown rot decay. A comprehensive study on the associated fungi in wood decay in the US indicated that prevalent fungi species on conifers include Neolentinus lepideus, Gloeophyllum trabeum, Gloeophyllum sepiarium, and Rhodonia placenta. Prevalent species on hardwood include G. trabeum, Trametes versicolor, and Antrodia oleracea (Duncan and Lombard 1965; Zabel and Morrell 2020). Many of these species are included in the European and American standards for durability testing of wood (e.g., EN113-1 2020, AWPA E10-22 2022).

To overcome the challenge of wood decay many different wood protection systems have been developed. Biocidal wood preservatives are deployed into the wood or onto the wood surface with different treatments. Historically, common preservatives included oil borne chemicals such as cresote and pentachlorophenol. Alternatively, waterborne chemicals, e.g., chromated copper arsenate, boron compounds or alkaline copper compounds have been applied. The treatment with those and other preservatives has been the major method for wood decay protection. However, the biocidal and often toxic nature of preservatives, combined with leaching from the wood, has led to major concerns, and many wood preservatives are nowadays restricted in use by government regulations. In addition, some of the preservative treated wood requires specific disposals, which increases costs and waste treatment (Ibach 2005; Hill et al. 2022; Zabel and Morrell 2020). Hence, recently different wood modification systems have emerged on the marked as alternatives, e.g., furfurylation, acetylation and thermal modification (Jones and Sandberg 2020).

Another approach for increasing the decay resistance of wood, which might overcome the above-mentioned challenges with wood preservatives is the modification of wood with monomeric resins by curing inside the cell walls. These treatments increase the dimensional stability of wood, while at the same time decreasing the susceptibility of wood towards fungal decay, because of the blocking or removal of susceptible hydroxy groups and the reduction of moisture content (MC) in the cell walls (Stamm and Baechler 1960). Well-known resin types for wood modification are amongst others phenol-formaldehyde (PF), urea-based-, melamine-based resins, or mixtures thereof (Jones and Sandberg 2020).

PF resins are amongst the most used resins for wood modification. Reasons are, amongst others, the easy synthesis, excellent mechanical properties, and weathering resistance (Fleckenstein et al. 2018). Additionally, improved fungal resistance of wood modified with PF resins has been confirmed in many studies (Bicke 2019; Bicke et al. 2012; Biziks et al. 2020; Fleckenstein 2018; Sharapov et al. 2022). According to Biziks et al. (2020) the threshold of PF resin modified beech was a weight percent gain (WPG) of 6–8 % to prevent brown rot fungi, but a higher WPG was needed to provide the same protection against white rot fungi. Larger resin oligomer sizes negatively influence the increase in fungal resistance (Takahashi and Imamura 1990; Biziks et al. 2020).

A major disadvantage of PF resins is that phenol is usually obtained from non-renewable sources. Thus, to improve the environmental impact and potentially the cost, finding renewable replacements for phenol has been a research goal (Sarika et al. 2020). For wood modification, potential substitutes for phenol are lignin cleavage products (LCP). Lignin, the second most abundant biomass on earth, is chemically cleaved from cellulose and hemicelluloses during the pulping process for paper production. Annually, approximately 100 million tons of lignin are produced, and currently mainly burned to regain energy and inorganic pulp chemicals (Olgun and Ateş 2023). The most common pulping process, and thus the most produced lignin type is lignin obtained from the kraft process, referred to as kraft lignin (Rinaldi et al. 2016).

In search of higher value applications of lignin and to replace non-renewable phenol, studies on phenol replacement in PF resins for wood modification by LCP were carried out by Fleckenstein (2018). Up to 40 % of phenol were successfully replaced by LCP, and a wood WPG of 40 % was achieved. The mass loss after 16 weeks of exposure to the brown rot Coniophora puteana and the white rot T. versicolor was measured. For C. puteana a mass loss of 2 % for pure PF resin and 4 % of PF resin with substitution were detected, for T. versicolor the mass losses were 2 % and 3 % respectively (Fleckenstein et al. 2018). While these results indicate that after substitution of phenol the modification leads to good fungal resistance, there are several questions remaining. Firstly, the chemical composition of the LCP was not analysed, resulting in difficulties in comparing the results with other lignin products. Secondly, the LCP were pretreated by distillation. Without distillation, additional compounds with different influences may remain in the cleavage products. Finally, the WPG of 40 % is a high value; good dimensional stabilities can be achieved with significantly lower resin load. However, the influence of a reduction of the WPG on fungal decay should be determined.
In a recent study, up to 45% of phenol in PF resins were substituted by LCP. The LCP were obtained by vacuum low-temperature microwave-assisted pyrolysis of softwood kraft lignin, and its main components are 4-methylguaiacol (13.8 ± 0.8 %), guaiacol (8.1 ± 0.9 %) and 4-ethylguaiacol (6.8 ± 0.5 %). In sapwood of Pinus sylvestris, the substitution of 30 % was achieved without a significant decrease in measured anti-swelling efficiency (Karthäuser et al. 2023).

The aim of this study was to investigate the influence of phenol substitution by the LCP as in Karthäuser et al. (2023) on the fungal resistance of the modified wood. For this, P. sylvestris sapwood was modified with an LCP-PF resin with 30 % phenol substitution by LCP from vacuum low-temperature microwave-assisted pyrolysis of softwood kraft lignin. Different WPGs of 10, 20, and 30 % were achieved to determine if lower concentrations of resin lead to increased fungal decay. The specimens were subjected to determine if lower concentrations of resin lead to increased quantifi-
cation. The specimens were subjected to DVS to determine the vapor sorption properties, and P. sylvestris sapwood was modi-
ed by DVS to determine the vapor sorption properties, and the formaldehyde emissions of the wood specimens were quantified.

2 Materials and methods

2.1 Resin synthesis

Two resins were synthesized: one resin containing only phenol and formaldehyde as educts (100 PF), and a second resin, in which 30 % of the phenol were substituted by pine kraft lignin-based pyrolysis oil (referred to as LCP; main components 4-methylguaiacol (13.8 ± 0.8 %), guaiacol (8.1 ± 0.9 %), and 4-ethylguaiacol (6.8 ± 0.5 %)) obtained by vacuum low-temperature microwave-assisted pyrolysis (70/30 LF; LF for lowered formaldehyde content). Detailed information on the LCP and the pyrolysis process can be found in Karthäuser et al. (2023). The phenol (99.5 %, Th. Geyer GmbH & Co. KG, Renningen, Germany) and the LCP were weighed into a three-neck flask, where it was melted at 55 °C. NaOH (50 % solution, AppliChem GmbH, Darmstadt, Germany) was added as a catalyst, and nitrogen atmosphere was applied. Finally, formaldehyde (37 % solution, Th. Geyer GmbH & Co. KG, Renningen, Germany) was added, and the temperature was adjusted to 65 °C. For larger resin amounts, the formaldehyde was added in three steps over an hour, to reduce the exothermic heat release. After 4 h the resin was obtained. The molar ratio of phenol, formaldehyde and NaOH was 1:1.5:0.1 in case of the pure PF resin. For the resin, in which 30 % of the phenol were substituted, a ratio of phenol + LCP, formaldehyde, and NaOH of 1:1.38:0.1 was applied to take into account the reactivity of the LCP, which is lower compared to pure phenol.

2.2 Resin analysis

To prepare modified specimens with a precise WPG, resins with a known solid content must be prepared. To do so, the solid content of the resins was determined. For this, about 2 g of the resin were weighed in an alumina cup. A small amount of butanol (1-Butanol, >99.5 %, Appli-
Chem GmbH, Darmstadt, Germany) was added to ensure a flat surface. The resin was cured at 135 °C for 2 h. The final weight was collected, and the solid content was determined. Prior to impregnation, the resins were diluted with demineralized water to solid contents of 6.25, 12.50, and 18.75 % w/v.

2.3 Wood treatment

Scots pine (P. sylvestris L.) sapwood mini-blocks with a size of 30 × 10 × 5 mm³ were sawn, dried at 103 °C for 18 h and weighed. The specimens were submerged into the resins with different solid content. To assure complete impregnation, the specimens were treated with vacuum (80–100 mbar, 1 h) followed by pressure (12 bar, 2 h). The solution uptake was determined, and the specimens were slowly dried at room temperature, 40 °C, 60 °C, and 80 °C. Finally, the specimens were cured at 140 °C in an oven for 16 h. The specimens were weighed, and the WPG was determined. The modified specimens and control specimens were leached according to EN 84 (1997) and dried at 103 °C for 18 h. The WPG after leaching was calculated. Then the specimens were conditioned to stable weight at 65 % RH/20 °C and the equilibrium moisture content (EMC) was calculated.

2.4 Fungal decay experiments

2.4.1 Main experiment: A modified AWPA E10-22 (2022) soil-block test was used. The brown rot fungi were R. placenta (Fr.) Niemelä, K.H. Larss. & Schigel (syn. Postia placenta), strain FPRL 280 (BAM113), and G. trabeum (Pers.) Murrill, strain BAM Ebw. 109, the white rot fungus was T. versicolor (L.) Lloyd, strain CTB 863 A. Initially, the fungi were cultivated on a 4 % (wt/vol) Difco malt agar medium (VWR International, Radnor, PA, USA), and plugs containing actively growing mycelia were transferred to a liquid culture containing 4 % (wt/vol) Difco malt (VWR International, Radnor, PA, USA). After a period of 14 days, the liquid culture was homogenized using a tissue homogenizer (Ultra-Turrax T25; IKA Werke GmbH & Co. KG, Staufen, Germany). Soil containing 2/3 compost soil and 1/3 sandy soil were adjusted to 95 % of their water-holding capacity according to ENV 807 (2001). Wood specimens, soil and plastic mesh were autoclaved at 121 °C. Into each Petri dish (TC dish 100, standard; Sarstedt AG & Co., Nümbrecht, Germany) (Ø = 87 mm, h = 20 mm) 20 g of sterile soil was added. Further, a sterile plastic mesh was used to avoid direct contact between the test specimens and the soil (i.e., to avoid water logging). A 1 mL inoculum of homogenized liquid culture was added on top of each specimen. In addition, modified specimens without any fungal inoculum (termed check test specimens in EN 113-1 2020) were incubated on the same sterile soil substrate and used for correction of the mass loss (i.e., any mass loss of the check test specimens is subtracted from the measured mass loss of treated speci-
mens). This is an approach used in EN 113-1 (2020) as a check of potential mass changes not related to fungal activity of the specimens during incubation (typically leaching from the specimens). Two specimens of the same treatment were added to each plate, and three replicate plates were used (n = 6). Every third week the weight of the plates was
measured, and sterile water was added to the soil when needed to keep the moisture conditions in the plates stable. Specimens were incubated at 22 °C and 70 % RH until harvest. At harvest fungal mycelium was manually removed from the wood surface with delicate task wipes (Kimtech Science, UK) and the specimens were dried at 103 °C for 18 h to provide data for calculation of final mass loss. This test approach was used because it allows the test to run as long as needed (malt agar plates tend to dry out after approximately 12 weeks) and because soil is more similar to in-service conditions than malt agar. Three harvest intervals were included to gain a better understanding of the mode of action and decay rate. In addition, wood moisture content (MC) after intervals were included to gain a better understanding of the mode of action.

As a verification of the setup in the main experiment versus other test approaches four different follow-up experiments were conducted. For all of them: 1) only the brown rot fungus *G. trabeum* was used, and 2) all specimens were harvested after 12 weeks. An overview of the fungal decay experiments performed is listed in Table 1.

2.4.2 Addition of an untreated control specimen (follow-up experiment; FE1): FE1 was carried out to determine if the mass loss of PF 100 or 70/30 LF modified specimens is changed by adding an untreated control in each Petri dish (EN113-1 2020) instead of using two similarly treated specimens (main experiment). If the modified wood has any biocidal leachates to the soil or air this would influence the decay rate of the controls versus the virulence specimens (two untreated specimens).

Further, the control included in each plate could potentially increase the decay rate of the modified specimens by translocation of nutrients via hyphae. The wood specimens were the check test specimens from the main experiment. Four replicates from each harvest point (week 8, 12 and 16) were tested. Hence, this test also serves as an additional check of potential change in resistance against fungal depolymerisation of check test specimens during incubation.

2.4.3 Influence of the substrate (FE2): FE2 was conducted to determine if there is a significant effect on mass loss of PF 100 or 70/30 LF modified specimens if malt agar is used as substrate (EN113-1 2020) instead of sterile soil (main experiment). Potentially, a nutrition rich substrate (malt agar) could change the results compared a nutrition poor substrate (sterile soil). The wood specimens were spare unexposed specimens from the main experiment.

2.4.4 Prolonged leaching (FE3): To determine the effect of prolonged leaching, the specimens were treated with two times leaching according to EN 84, instead of one time (main experiment). It has been argued that the standard leaching procedure EN 84 (1997) is not severe enough and that non-fixed chemicals could affect mass loss and durability classification (Emmerich et al. 2021). The prolonged leaching included the EN 84 procedure followed by a drying step and a second EN 84 procedure. Initial dry weight was recorded after the final leaching step. The analysis of the leaching water from the prolonged leaching procedure is reported in Section 2.6 below.

2.4.5 Feeder strips instead of sterile soil or malt agar (FE4): FE4 was conducted to determine if there was a significant effect on mass loss of PF 100 or 70/30 LF modified specimens due to the use of feeder strips (AWPA E10, 2022) instead of sterile soil (main experiment) or malt agar (EN113-1 2020). The same batch of specimens and the same extended leaching procedure as in 2.4.4 was used. Briefly, in AWPA E10 (2022) untreated wood inoculated with fungi is placed on soil and the test specimens are placed on top of the feeder strips.

2.5 Dynamic vapour sorption (DVS)

The wood equilibrium moisture content (EMC) is an important parameter for fungal resistance, as fungi can only attack wood with a specific range of moisture content, typically above 20 % (Meyer et al. 2014; Stienen et al. 2014). Hence, the vapor sorption properties of the wood were determined via dynamic vapor sorption (DVS) to obtain further insights into the resin influence on the material properties and fungal resistance.

Specimens of pure cured resin prepared for solid content determination were collected for DVS analysis. The modified wood material (prepared as described above) and the cured resins were ground to powder and around 100 mg of material was weighed and analysed for its vapor sorption properties. A DVS instrument (VSorpt advanced, ProUmid GmbH & Co. KG, Germany) was used at 25 °C in 5 % humidity steps from 0 % to 95 % and back to 0 % relative humidity (absorption and desorption isotherm). The EMC of the wood and pure polymer powder was defined at < 0.01 % mass change/40 min. An empty crucible was added to determine the potential drift of the balance. The wood specimens were dried at 103 °C before the analysis. Untreated Scots pine wood powder and pure polymer from 100 PF and 70/30 LF was used as a reference. All

| Table 1: Overview of fungal decay experiments: main experiment and FE1-4. |
|-------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Experiment | Leaching | Within plate organisation | Substrate | Fungi | Inoculum | Weeks of exposure | Replicates (n) |
| Main experiment | EN 84 | 2 modified | Sterile soil | *G. trabeum* | Liquid | 0, 8, 12, 16 | 6 |
| FE1 | EN 84 | 1 modified 1 untreated | Sterile soil | *G. trabeum* | Liquid | 12 | 12 |
| FE2 | EN 84 | 1 modified 1 untreated | Malt agar | *G. trabeum* | Agar plug | 12 | 3 |
| FE3 | 2 × EN 84 | 2 modified | Sterile soil | *G. trabeum* | Liquid | 12 | 4 |
| FE4 | 2 × EN 84 | 2 modified | Sterile soil | *G. trabeum* | Feeder strips | 12 | 6 |
23 specimens, including three replicates per treatment and treatment level, three untreated Scots pine sapwood controls and two pure polymer specimens, were analysed in the DVS at the same time. In addition, repeated moistening of the same specimens was performed after the first sorption experiment using 25 °C from 0 % to 90 % and repeated two times. The EMC of the wood- and pure polymer powder was defined at < 0.01 % mass change/60 min, where the water mass in the samples at each moisture equilibrium level was related to the dry mass of the samples, as described in Eq. (1). This was done to study the stability of the polymer in high relative humidity.

\[
\text{EMC} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100 \%
\] (1)

2.6 Formaldehyde analysis of leaching water

The formaldehyde content released during the extended leaching, i.e., EN 84 (1997) with a drying step (103 °C for 20 h) before the second leaching was analysed according to the Japanese Industrial Standard A 1460 (JIS 2001). The only deviation from the standard was: 1) the use of leaching water rather than desiccator water and 2) the use of 6 mL sample and 3 mL acetylacetone-ammonium acetate solution (to improve the detection threshold). The detection limit is 0.03 mg/L.

2.7 Statistics

For comparison of means Tukey HSD was performed using JMP®. Version 16, SAS Institute Inc., Cary, NC, 1989–2023. A probability of 0.05 was used as a statistical type-I error level.

3 Results and discussion

3.1 Resin synthesis and analysis

The 100 PF resin was obtained as a light reddish liquid. The resin containing LCP is a dark brown liquid. The solid content of the 100 PF resin was 51.4 ± 0.2 %, while the solid content of the 70/30 LF was 53.4 ± 0.1 %. The slightly higher value for the resin with substituted phenol was expected because the amount of formaldehyde (37 % solution in water) was decreased.

### Table 2: Mean values for solid content of resin, solution uptake, weight percent gain (WPG) before and after leaching and equilibrium moisture content (EMC).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Solid content (%)</th>
<th>Solution uptake (%)</th>
<th>WPG before leaching (%)</th>
<th>WPG after leaching (%)</th>
<th>EMC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 PF, 10WPG</td>
<td>6.25</td>
<td>168.2 ± 10.5</td>
<td>9.8 ± 0.7</td>
<td>8.6 ± 0.7</td>
<td>8.2 ± 0.2</td>
</tr>
<tr>
<td>100 PF, 20WPG</td>
<td>12.50</td>
<td>172.1 ± 10.8</td>
<td>19.5 ± 1.3</td>
<td>18.6 ± 1.3</td>
<td>7.4 ± 0.4</td>
</tr>
<tr>
<td>100 PF, 30WPG</td>
<td>18.75</td>
<td>180.2 ± 11.1</td>
<td>30.9 ± 2.0</td>
<td>29.9 ± 2.0</td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>70/30 LF, 10WPG</td>
<td>6.25</td>
<td>169.5 ± 11.5</td>
<td>9.8 ± 0.8</td>
<td>8.4 ± 0.9</td>
<td>8.3 ± 0.3</td>
</tr>
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<td>18.2 ± 1.6</td>
<td>7.5 ± 0.5</td>
</tr>
<tr>
<td>70/30 LF, 30WPG</td>
<td>18.75</td>
<td>177.5 ± 12.2</td>
<td>30.4 ± 2.2</td>
<td>29.5 ± 2.3</td>
<td>6.8 ± 0.7</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>10.5 ± 1.0</td>
</tr>
</tbody>
</table>

3.2 Specimen characterisation

After vacuum-pressure-treatment with the different resins, the solution uptake of the mini-block specimens was determined. As described in Table 2, the solution uptake of the resins increases with higher solid content of the resin. Between the 100 PF and the 70/30 LF, only slight differences in the solution uptake were observed. The cured specimens are darker than the original wood, and the specimens containing LCP are darker than the pure PF-resin modified wood. The EMC is reduced with increasing resin content.

Both the solution uptake and the WPG of the mini-block specimens are in the expected range. The leaching procedure did not lead to significant mass loss for any of the treatments, indicating good fixation properties. The standard deviation is low, implying that a uniform wood modification was achieved. The higher solution uptake at higher solid content of resins was expected, both due to higher density and due to higher viscosity of the resins. The similar uptake and WPG between the resins containing only phenol and a mixture of phenol and LCP indicate that the properties of the resins are comparable, and that the LCP do not have a negative impact on the impregnation process or fixation of the resins, which is in line with earlier studies (Karthäuser et al. 2023). As expected, the modified specimens had lower EMC than the control and the EMC of treated specimens decreased with increasing treatment level.

3.3 Fungal decay experiments

3.3.1 Main experiment

Generally, the mass losses in all the modified specimens were low, indicating no decay, or only initiation of decay (Figure 1, Supplementary Table S1). Supplementary Table S1 also provides comparisons of mean mass loss and wood MC values between treatments for each fungus and incubation time. However, since only initial decay was found (i.e., mass
loss generally below 1%) the comparisons should not be overinterpreted.

The mass loss due to fungal depolymerisation decreased with increasing treatment level. This was expected and previously found for PF modified wood (Belt et al. 2023; Biziks et al. 2020) and PF modified LVL (Bicke 2019; Bicke et al. 2012, Fleckenstein 2018; Sharapov et al. 2022). For some specimens an increase in mass was measured. The reason is that it is not possible to remove all the fungal mycelia and “mass loss will not show until the decrease in mass due to degradation becomes larger than the increase in mass due to the fungal colonisation” (Brischke et al. 2008).

For R. placenta no mass loss was found until week 16 where the highest mass loss was measured for 100 PF 10WPG (0.29 %) and 70/30 LF 10WPG (0.10 %).

The virulence specimens exposed to G. trabeum had a significantly higher decay rate than the virulence specimens exposed to R. placenta throughout the incubation period. For G. trabeum initial mass loss was detected in week 8 for 100 PF 10WPG (0.90 %), 20WPG (0.03 %) and 70/30 LF 30WPG (0.19 %). In week 16 a mean mass loss above 1 % was found for 100 PF WPG 10 (3.18 %) and 70/30 LF 10WPG (1.04 %).

For the white rot fungus T. versicolor the virulence specimens had, as expected, significantly lower mass loss than the brown rot fungi throughout the incubation period (white rot fungi tend to prefer deciduous trees). Initial mass loss was detected in week 8 for 100 PF 10WPG (0.05 %), 20WPG (0.30 %) and 70/30 LF 30WPG (0.08 %). However, none of the treatments reached 1 % mean mass loss in week 16. This result contrasts with Biziks et al. (2020) where T. versicolor needed a higher WPG to ensure protection. This deviation between the studies can be due to differences in wood species, formulation, and/or the virulence of the test fungus.

The check test specimens indicated that there was a neglectable change in mass during the incubation period when the specimens were exposed in the Petri dishes without fungi (Supplementary Table S2). This is supporting the results from leaching of good fixation properties (Table 1).

In Figure 2 mean wood MC is presented while statistical comparisons of means between treatments are presented for each fungus and incubation time in Supplement Table 1. As expected, the untreated virulence specimens had higher wood MC than the modified specimens. Specimens exposed to R. placenta had significantly higher wood MC than specimens exposed to G. trabeum and T. versicolor. Wood MC tended to increase with increasing incubation time and, rather surprisingly, with increasing treatment level. The increase in wood moisture content with increasing incubation time is most likely due to fungal colonisation and could be observed in both treated and untreated specimens. When looking at the moisture content during incubation for the check test specimens (Supplementary Table 2) a significant increase in wood MC with increasing WPG was observed. Hence, increasing hygroscopic properties of the modified wood matrix are assumed, which cannot be explained by fungal colonisation and depolymerisation. This would contradict the results obtained from DVS studies (Figure 6). Similar results were reported by Belt et al. (2023), where the treatment of Scots pine sapwood with PF resin decreased the EMC, however, after fungal decay an increase in MC with increasing WPG was detected. While a definite reason for this was not determined, Belt et al. suggest that the increased moisture uptake may be caused by resin in the lumen or by capillary effects. Considering this explanation, it makes sense that the results are contradicting to the DVS measurements, because the samples in DVS are ground to powder, so that resin in the lumen and capillary effects do not play a role anymore.

3.3.2 Addition of an untreated control specimen (FE1)

In FE1 the four check test specimens from 8, 12 and 16 weeks were included. No significant changes as a result of incubation time were found except for 70/30 WPG10 where 12

Figure 1: Percent mean mass loss of wood specimens after incubation for 8, 12 and 16 weeks with monocultures of G. trabeum, R. placenta or T. versicolor (n = 6).
and 16 weeks had significantly higher mean mass loss (0.55 % and 0.38 % respectively) than eight weeks (0.11 %). Hence, in the following the specimens for each treatment will be pooled \((n = 12)\).

The 100 PF 10WPG specimens had statistically higher mean mass loss than all the other treatments except 70/30 LF 10WPG (Figure 3a). No statistical difference was found between the other treatments. It is important to note that none of the treatments had above 1 % mean mass loss. The mass loss of virulence specimens was comparable to the main experiment (53.0 % and 55.2 % respectfully). The virulence specimens had statistically higher mean mass loss than the control specimens (42.0 %). Since leaching of chemicals is not very likely (ref. leaching Table 1 and check test specimens Supplement Table 2) the specimens in the three experiments are regarded as comparable.

Statistically the results between the modified specimens are comparable between the experiments, i.e., no or initial mass loss except for significantly higher mass loss for 10WPG for both treatments (Figure 4a). When comparing controls (one control in each Petri dish) and virulence specimens (two untreated specimens in each Petri dish) decay rate was higher when sterile soil was used as substrate compared to malt agar.

No significant differences in wood MC were found between treatments (Figure 4b). However, wood MC was significantly higher for modified specimens in this experiment than in the main experiment and FE1 (both on sterile soil) while for virulence and control specimens the wood MC was lower than in the two previous experiments.

### 3.3.3 Influence of the substrate (FE2)

In FE2 malt agar was used instead of sterile soil. In FE1 the check test specimens from the main experiment were used with soil as substrate while in FE2 spare specimens from the main experiment were used with malt as substrate. Since the change in mass for the check test specimens were neglectable (Supplement Table 2) the specimens in the three experiments are regarded as comparable.

The aim was to test durability after prolonged leaching \((2 \times \text{EN 84})\) compared to standard leaching (EN 84) and to compare liquid inoculum (FE3) and feeder strips (FE4). There was no significant difference between treatments when liquid inoculum was used after prolonged leaching (FE3) compared to the main experiment. It was unexpected that prolonged leaching resulted in statistically similar results for all treatments. However, the interpretation of low mass losses should be done with caution.

Interestingly, the mass loss for 100 PF 10WPG and 70/30 LF 10WPG was significantly higher when exposed on feeder strips (FE4) than when inoculated with liquid inoculum. The reason might be that with the feeder strips the mycelia is already actively growing and therefore allowing faster colonisation of the modified wood and that the feeder strips provide more nutrients than the nutrient poor sterile soil.

The mass loss for virulence specimens with liquid inoculum was similar to the previous experiments. However, the
virulence specimens had significantly lower mass loss when using feeder strips (Figure 5a). The reason might be that the feeder strips already are providing nutrients for the fungus. Hence, it is less urgent for the fungus to utilise the nutrition available in the virulence specimens compared to the setup with nutrient poor sterile soil substrate (FE3).

For liquid inoculum (FE3) there was a significantly higher wood MC for 30WPG compared to 10WPG for both

Figure 3: Percent mean mass loss (a) and mean wood moisture content (b) (wet mass after fungal incubation related to leached dry mass before incubation) of wood specimens from FE1 after incubation for 12 weeks with monoculture of *G. trabeum*. Comparisons of means of treatments is given, treatments not connected with the same letter are significantly different (*n* = 12).

Figure 4: Percent mean mass loss (a) and wood moisture content (b) (wet mass after fungal incubation related to leached dry mass before incubation) of wood specimens from FE2 after incubation for 12 weeks with monoculture of *G. trabeum*. Comparisons of means of treatments is given, treatments not connected with the same letter are significantly different (*n* = 3).
treatments. When using feeder strips (FE4) no significant differences were found between the treatments except for a higher wood MC in 70/30 LF 10WPG. The elevated wood MC for this treatment might be assigned to higher fungal colonisation.

3.4 Dynamic vapour sorption (DVS)

The water sorption isotherms (Figure 6) describe the relationship between atmospheric relative humidity and EMC of the wood powder material. The specimens exhibited a sigmoidal water vapor sorption isotherm, which is typical for cellulosic material. The obtained sorption data of untreated wood is in accordance with published data on Scots pine sapwood sorption (Xie et al. 2011).

Hysteresis occurred between the adsorption and desorption branches of the isotherm of the materials (Figure 7). However, the adsorption curves of the pure polymers were almost linear, indicating equivalent sorption sites or monolayer adsorption of water.

Both modifications reduced the vapor sorption of wood. The sorption was slightly dependent on the treatment level for both types of PF modification. Pure PF-polymer had even lower EMC than PF-modified wood. Slightly lower sorption was measured for 100 PF modified wood compared to 70/30 LF-modified wood. These differences are reduced after correcting the EMC (Figure 6).

An upward curve in the isotherms at higher relative humidities was measured for modified wood powder, indicating a softening of the material, which can be explained by plasticization of wood. The isotherms of the pure polymers suggest no plasticization and were similar to an inert porous material (Metran et al. 2022). This is also reflected by the hysteresis curve for pure polymer with a lower hysteresis effect in higher relative humidities (Figure 7). In contrast, the hysteresis in modified wood is similar to untreated wood.

Repeated moistening of the powder material from the sorption and desorption experiment in high relative humidity indicated no significant differences in material MC between the different cycles (Figure 8). The polymer seemed therefore to be stable and extensive leaching or other changes of the modified wood specimens during the exposure to fungi are not anticipated.

The reduced MC of modified wood compared with untreated wood could explain a significant part of the mode of action in 100 PF- and 70/30 LF modified wood. Reducing the MC limits the fungal decay activity by reducing water accessibility. A similar mode of action can be found for other wood modification treatments leading to improvements in anti-swelling efficiency and decay resistance (Hill 2006).

![Figure 5](image_url)

**Figure 5:** Percent mean mass loss (a) and wood moisture content (b) (wet mass after fungal incubation related to leached dry mass before incubation) of wood specimens from FE3 and FE4 after incubation for 12 weeks with monoculture of *G. trabeum*. Comparisons of means of treatments are given, treatments not connected with the same letter are significantly different. \( n = 4 \) for liquid inoculum and \( n = 6 \) for feeder strips. Letters F and L at the X-axis refer to feeder strips- and liquid inoculation respectively.
3.5 Formaldehyde analysis of leaching water

As mentioned above, the main reason for high fungal decay resistance of wood treated with impregnation resins is the blocking or removal of susceptible hydroxy groups and the reduction of moisture content (MC) in the cell walls (Stamm and Baechler 1960). However, the formaldehyde emission of the specimens may have an additional effect on the fungal resistance of the treated wood. It is known from former...
studies that formaldehyde can inhibit or completely neutralize the growth of mould fungi (Bomar and Bomar 1999; Dennis and Gaunt 1974; Power 1997). To make sure that the high resistance is not due to high formaldehyde emission, the formaldehyde content of the leachates from extended leaching (as suggested by Emmerich et al. 2021) was analysed (Figure 9). The formaldehyde content of the leachates of the untreated reference specimens were comparable to the 100 PF, while the 70/30 LF treatment had slightly higher formaldehyde levels (Figure 9). The peaks are due to accumulations during the weekend (hours 288 and 624) and the drying step between the two leaching procedures (between hour 336 and 337).

Untreated wood releases formaldehyde (Figure 9). However, formaldehyde-based resins may increase the formaldehyde emission. This is mainly due to the free formaldehyde content remaining after resin synthesis. The free formaldehyde can react with hydroxyl groups in cellulose or hemicelluloses to form a hemiacetal. This reaction is reversible, leading to ongoing release of the formaldehyde from the cured specimens (Murata et al. 2013). Substituting phenol by LCP leads to an increase in the free formaldehyde content. Reason for this are reduced numbers of free reactive sites in the bio-oil constituents compared to phenol (Jia et al. 2020; Karthäuser et al. 2023). Because of this, the amount of formaldehyde added to the resin was reduced during the synthesis of the resin. Nevertheless, the free formaldehyde content remains higher than for pure PF resin (Karthäuser et al. 2023). The free formaldehyde content, and thus the formaldehyde emission, could be reduced by further decreasing formaldehyde addition or by adding formaldehyde scavengers to the resin admixture. However, considering that the decay rate of the specimens modified with 70/30 LF resin was not lower than with pure PF resin, the formaldehyde release does not seem to be high enough to significantly affected the fungi added to the specimens.

4 Conclusions

This study set out to investigate if, when used for wood modification, the substitution of phenol in PF resins by organic pyrolysis cleavage products of softwood kraft lignin has an impact on the resistance against fungal decay. The resistance against depolymerisation by basidiomycetes was significantly improved by both modifications. Mass loss above 1% was only measured for the specimens with lowest solid content (10WPG).

The EMC is reduced by the modifications and is believed to be the main reason for the improved decay resistance of the material. No significant differences due to the phenol substitution by the LCP compared with PF were observed.

The formaldehyde emission of specimens modified with resin in which phenol is substituted by lignin cleavage products is slightly higher than for pure PF resin. Additional reduction of formaldehyde or addition of formaldehyde scavengers may be required. However, the mode of action of increase in resistance against fungal decay is suggested to be mainly due to blocking or removal of hydroxy groups, and not formaldehyde emission. Thus, the method presented herein is a possibility to decrease the use of non-renewable phenol, while maintaining the advantageous properties of the modified wood.
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References


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