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Wood Technology/Products

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Effect of pressurized hot water extraction on the resistance of Scots pine sapwood against mould fungi

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Abstract: The effects of pressurized hot water extraction (HWE) treatment on the mould resistance of wood have not been extensively investigated yet. The activity of the mould fungi is dependent on the availability of nutrients. Therefore, the soluble degradation products produced during HWE treatment could affect the wood's susceptibility to mould growth. Scots pine (*Pinus sylvestris* L.) sapwood specimens were treated with HWE at 140 °C for 1–5 h. Afterwards, the degradation products were either removed via leaching or the wood was dried without applying the leaching procedure. The surface layer (1.5 mm) was removed from half of the leached and non-leached specimens. The resistance of the specimens against mould growth was tested in an incubation chamber. HWE treated wood showed a higher susceptibility to mould growth when it was neither leached nor subjected to surface removal. The susceptibility of wood to mould fungi depended on the availability of hemicellulose-based degradation products produced during HWE treatment. These degradation products were removable via a leaching procedure, but also by removing the outermost layer of the wood. The results show the relevance of removing HWE degradation products located on the wood surface in improving resistance against mould growth.

Keywords: carbohydrate migration; discolouration; hemicellulose-based derivatives; wood composition.

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1 Introduction

Wood is susceptible to deterioration by varying biological organisms, including different fungal species. Wood decaying fungi cause degradation and loss of strength, but mould fungi are also a threat to wood in the built environment. Besides aesthetic damage by discolouration, mould can cause allergic reactions (Curtis et al. 2004; Dales et al. 1991). The optimal environmental conditions vary between species, but mould fungi are able to grow at 0–50 °C and relative humidity (RH) above 75% (Johansson et al. 2012; Viitanen 1997). The growth and activity of mould fungi are also dependent on the availability of accessible nutrients (Imken et al. 2020; Theander et al. 1993).

Previous studies confirm the relevance of low molecular weight nutrients, like carbohydrates and nitrogenous compounds, for the presence of mould growth on the wood surface. The accumulation of soluble nutrients into the outermost layer increases wood's susceptibility to mould growth (Sehlstedt-Persson et al. 2011; Terziev and Boutelje 1998; Theander et al. 1993). In solid timber products, nutrients accumulate during the drying process, as dissolved compounds migrate about 0–3 mm below the wood surface, where water molecules evaporate (Möttönen and Kärki 2010; Terziev and Boutelje 1998).

During pressurized hot water extraction (HWE) wood is treated with high-temperature water (100–240 °C) that is kept in the liquid phase with high pressure (Nitsos et al. 2016; Wikberg et al. 2015). At sufficiently high temperatures water obtains acidic properties and catalyses the degradation of wood components that are more susceptible to hydrolysis. During HWE treatment, hemicelluloses degrade into

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low-molecular-weight carbohydrates and dissolve into the liquid phase, while the more resistant structural compounds, cellulose and lignin, mostly remain in the solid phase (Garrote et al. 1999; Nitsos et al. 2016; Wikberg et al. 2015). Depending on the type of wood and the applied treatment conditions, the extraction liquid contains different oligo- and monomeric carbohydrates, acid-soluble lignin, acids, furan derivatives and inorganic compounds (Amidon and Liu 2009; Garrote et al. 1999; Kyyrö et al. 2020; Nitsos et al. 2013). The liquid and solid phases are simple to separate after HWE treatment, but a leaching procedure is required for the removal of degradation products that are located inside the wood (Altgen et al. 2016; Hill et al. 2021). HWE of wood has the benefit of simultaneous modification of the solid phase and extraction of valuable hemicellulose-based compounds. These compounds can be utilized in the production of biofuel, cosmetics, food additives, pharmaceutical products and other materials (Amidon and Liu 2009; Wikberg et al. 2015). Some previous studies have investigated the potential of HWE in larger wood products (Sattler et al. 2008; Pelaez-Samaniego et al. 2014). Compared to thermal treatment in dry conditions or with steam, HWE treatment mitigates the negative effects on the brittleness and strength of the solid residue (Altgen et al. 2018a). HWE treatment could be a promising method for new material applications if the changes in wood were fully understood. Resistance against biological damage is an important attribute for wood products that withstand temperature and humidity fluctuations. While the results by Altgen et al. (2020) and Kyyrö et al. (2022) show that the HWE treatment has only limited effects on durability against wood-decaying fungi, the mould resistance of HWE treated wood has not been extensively investigated. Since carbohydrates are viable nutrients for microorganisms, the removal of hemicellulose-based soluble carbohydrates in the HWE treated wood via leaching or by removing the outermost surface layer after drying would hypothetically affect the resistance against mould growth.

The present study investigated the effect of HWE treatment on the resistance of Scots pine sapwood (*Pinus sylvestris* L.) against mould growth. Different levels of degradation were obtained and studied by varying the HWE treatment time, while the effects of the degradation products were analysed by applying leaching and surface removal procedure to the HWE treated specimens. The chemical composition changes in the solid residue and the extraction liquid were also investigated.

2 Materials and methods

2.1 Materials

Specimens with approximate dimensions of $10 \times 75 \times 100$ mm^3 (R × T × L) were cut from kiln-dried Scots pine (*P. sylvestris* L.) sapwood in accordance with BS 3900:G6 (BS 1989). The number of late wood rings in the transverse plane was 4–6. A hole with a ca. 2 mm radius was drilled 9 mm from the midpoint of the transverse plane through the tangential plane. The specimens did not contain heartwood, knots or other visible defects.

All specimens were oven-dried at 105 °C for 24 h to determine their initial dry mass with 0.001 g accuracy and initial dimensions with 0.01 mm accuracy. The average oven-dry density of the specimens was 450 ± 50 kg/m^3. The dried specimens were treated according to the procedure summarized in Figure 1. They were evenly distributed between 14 different groups, with treatment duration (0, 1, 3 and 5 h), water-leaching (yes/no), and surface removal (yes/no) as the grouping variables. Each group contained seven replicate specimens.

2.2 Hot water extraction (HWE)

Two of the specimen groups were untreated reference groups and not HWE treated. Twelve specimen groups were saturated with deionized water via vacuum-impregnation for 12 h at 50 mbar before HWE treatment. The saturated specimens were HWE treated in a rotating air-bath reactor (Haato Oy, Model 16140-538, Vantaa, Finland) with space for up to six treatment vessels that had a volume of 2.5 l. The saturated specimens and deionized water to result in a liquid-to-solid ratio (l/s) of 10 g/g were placed inside tightly sealed treatment vessels. The water temperature within the vessel was gradually increased to 140 °C within` 115 min. The peak temperature was held for 1 h, 3 h or 5 h, and four different specimen groups were treated per HWE treatment duration. After HWE treatment vessels were removed from the reactor and cooled with water.

2.3 Leaching

After the HWE treatment specimens and extraction liquid were separated with 50 µm filtering cloths. Any remaining droplets on the wood surface were carefully removed with a paper tissue. A small amount of liquid (~10 mL) was collected for carbohydrate and soluble lignin analysis according to standards NREL/TP-510-42623 (Sluiter et al. 2008) and NREL/TP-510-42618 (Sluiter et al. 2012), respectively, as previously described by Kyyrö et al. (2020). Half of the specimens, two specimen groups per HWE treatment duration and one untreated reference group, were leached according to standard EN 84:2020 (CEN 2020). Approximately five volumes of water were added per volume of wood and leaching was performed for a duration of 14 days. The water was exchanged at the end of the first and second leaching day and afterwards every 3 days. The water from the first and second leaching days was collected and used to evaluate the amount of leached carbohydrates according to standard NREL/TP-510-42623 (Sluiter et al. 2008),

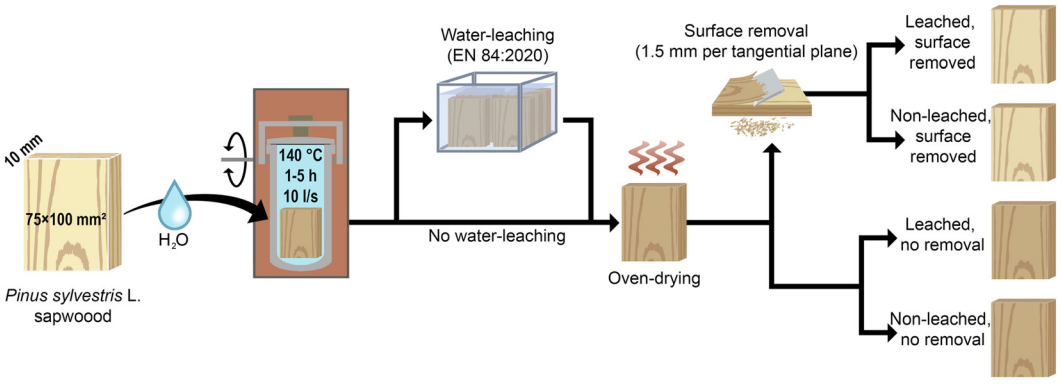

Figure 1: Treatment procedure for wood specimens. The prepared specimens were HWE treated in a rotating air-bath reactor at 140 °C for 1–5 h at a liquid-to-solid ratio (l/s) of 10. Afterwards, selected specimens were subjected to leaching and/or surface removal.

as previously described by Kyyrö et al. (2020). Afterwards, the leached specimens were stored at 20 °C and 65% RH for two days. The other half of the HWE treated specimens and one untreated reference group were stored for 16 days at 20 °C and 65% RH with no leaching procedure. Next, all specimens were gradually dried in an oven using a temperature sequence of 40, 60, 80 and 103 °C, with each temperature being held for 24 h.

The oven-dry mass of each specimen was recorded. The mass loss (ML) caused by HWE treatment and, for half of the specimens, subsequent leaching was calculated using Equation (1):

$$\mathrm{ML} = (m_0 - m)/m_0 \times 100\ \%, \tag{1}$$

where m_0 is the initial dry mass before HWE treatment (g), and m is the dry mass after HWE treatment and potential water-leaching (g).

2.4 Surface removal

The outermost surfaces of the tangential planes were removed from half of the HWE treated specimens. One specimen group per combination of HWE treatment duration and leaching procedure was chosen for the removal of the surface. An outer surface layer with a thickness of approximately 1.5 mm was removed from both tangential planes (altogether 3 mm) of the wood specimens with a planer (Proxxon, Model DH-40, Germany). The specimens with surface removal were oven-dried for 24 h at 103 °C and the masses were determined.

2.5 Chemical composition analysis

One specimen per sample group was chosen for chemical composition analysis. Each specimen was ground in a Wiley mill to pass through a 1.0 mm mesh sieve. Moisture content was measured from the wood powder according to standard SFS-EN 1318-1 (CEN 2002) and extractives were removed with acetone in a Soxhlet apparatus according to standard TAPPI T 204 cm-97 (TAPPI 2007). The extractive-free wood powder was analysed for structural carbohydrates and lignin according to NREL/TP-510-42618 (Sluiter et al. 2012). The hydrolysate was analysed for arabinose, rhamnose, galactose, glucose, xylose and mannose with High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD), as previously described by Lillqvist et al. (2019). Based on the data on the monosaccharide contents, the amounts of cellulose and hemicellulose in wood were calculated according to Janson (1970). Chemical composition measurements were taken in triplicates.

Based on the results, the amount of each chemical component in the wood specimens (C) was calculated according to Equation (2):

$$C = m_c/m \times 100\ \%, \tag{2}$$

where m is the dry mass of the specimen after HWE treatment and potential leaching and/or surface removal (g), and m_c is the dry mass of cellulose, hemicellulose, lignin or acetone in wood (g).

2.6 Mould resistance test

Before the mould resistance assessment, the specimens were stored at 20 °C and 65% RH for at least two weeks. One specimen from each group was stored as reference without inoculation. Other conditioned specimens were inoculated with a suspension of spores from the following mould species: *Aspergillus niger* (BAM 661), *Aspergillus versicolor* (IMI 45554), *Aureobasidium pullulans* (IMI 45533), *Chaetomium globosum* (IMI 45550), *Cladosporium cladosporioides* (IMI 178517), *Humicola grisea* (IMI 75664), *Penicillium minioluteum* (IMI 89377), *Petriella setifera* (IMI 181301), *Phoma violacea* (IMI 049948ii), *Phialophora mutabilis* (DSM 10716), *Rhodotorula mucilaginosa* (DSM 13621), *Stachybotrys chartarum* (IMI 82021), *Sydowia polyspora* (IMI 269217), *Trichurus spiralis* (MG 31) and *Ulocladium atrum* (IMI 79805). The mould species were maintained at 25 °C on potato carrot agar until inoculation. The spore suspension was prepared according to standard BS 3900:G6 (1989). A tangential plane surface of each specimen was evenly sprayed with a spray bottle with 1 ml of spore suspension.

After inoculation, the specimens were placed in glass containers serving as incubation chambers. A chamber was prepared in advance by first attaching a heater to the bottom of a chamber with dimensions of 40 × 25 × 25 cm^3. Next, about 5 L of distilled water was filled into the chamber and metallic supports were horizontally attached close to the lid of the chamber. The inoculated specimens were hung up with metal hooks from the supports in random order. The space between specimens was at a minimum of 25 mm and the inoculated surfaces were faced in the same direction. The incubation chamber and the specimens were kept at 23 °C. To keep the relative humidity level in the chamber sufficiently high for mould growth, the water was heated every 10 h for 2 h. Temperature and relative humidity were regularly checked: every

day throughout the first week of incubation and afterwards twice a week.

The specimens were incubated for a total of 56 days. The surface of each specimen was visually assessed after 14, 28, 42, 49 and 56 days. The assessment was supported by surface analysis with a VHX-5000 microscope (Keyence, Osaka, Japan). Each specimen was given a grade between 0 and 5 based on the area of surface covered by mould in accordance with the standard BS 3900:G6 (1989): 0 for no visible growth, 1 for up to 1%, 2 for 1–10%, 3 for 10–30%, 4 for 30–70% and 5 for over 70%. Only integer numbers were assigned. After the incubation period, the specimens were gradually dried in an oven using a temperature sequence of 40, 60, 80 and 103 °C, with each temperature being held for 24 h and the moisture content (in %) of the specimens was determined according to Equation (3):

$$\mathrm{MC} = (m_\mathrm{i} - m_{\mathrm{i},0})/m_\mathrm{i} \times 100\ \%, \tag{3}$$

where m_i is the mass of the wood specimen immediately after incubation and $m_{\mathrm{i},0}$ is the oven-dry mass of the wood specimen after incubation. Representative images of the surface mould in selected regions were taken with Olympus SZX 10 microscope equipped with 1.25× objective and digital camera DP74. The images were taken at 8× magnification.

3 Results and discussion

3.1 Mass loss and chemical composition

The results in Figure 2 show an increasing loss in mass as a function of the HWE treatment time. Figure 2 also shows a notably higher mass loss in samples that were leached after HWE treatment, and this difference increased with increasing treatment time. Previous research highlights the role of the leaching step in the removal of degradation products located in the wood after thermal treatment (Altgen et al. 2018b; Biziks et al. 2015). A small mass loss of ca. 1% was also recorded for untreated specimens, due to a loss of wood extractives by water leaching or by their emission as volatile compounds during oven-drying.

The results in Figure 3 show the chemical composition of untreated and HWE treated specimens. HWE treatment caused a loss of hemicellulose and simultaneously increased the proportion of cellulose and lignin in the wood. This effect is consistent with findings in previous studies (Nitsos et al. 2016; Wikberg et al. 2015).

The removal of a surface layer had a clear effect on the chemical composition of the HWE treated and non-leached specimens (Figure 3A), but surface removal did not change the chemical composition of the leached wood (Figure 3B). Post-HWE leaching reduced the wood's overall hemicellulose and acetone soluble content, which simultaneously increased the proportion of cellulose and lignin. A similar change was also obtained by removing the surface layer of the non-leached wood. The chemical composition of the non-leached specimens without surface removal differed clearly from the other specimen groups, which suggests that the HWE degradation products of the non-leached wood resided in the outermost surface layer. However, whether the degradation products resided in the top layer of the specimens during the HWE treatment or migrated to

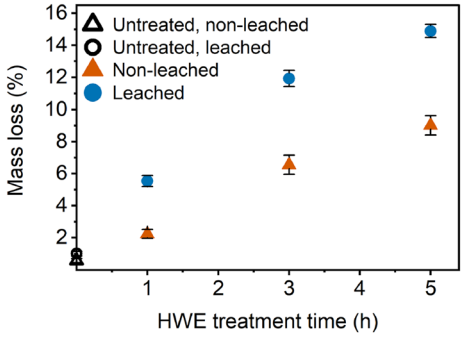

Figure 2: Mass loss (%) of wood as a function of HWE treatment time. The error bars indicate the standard deviation.

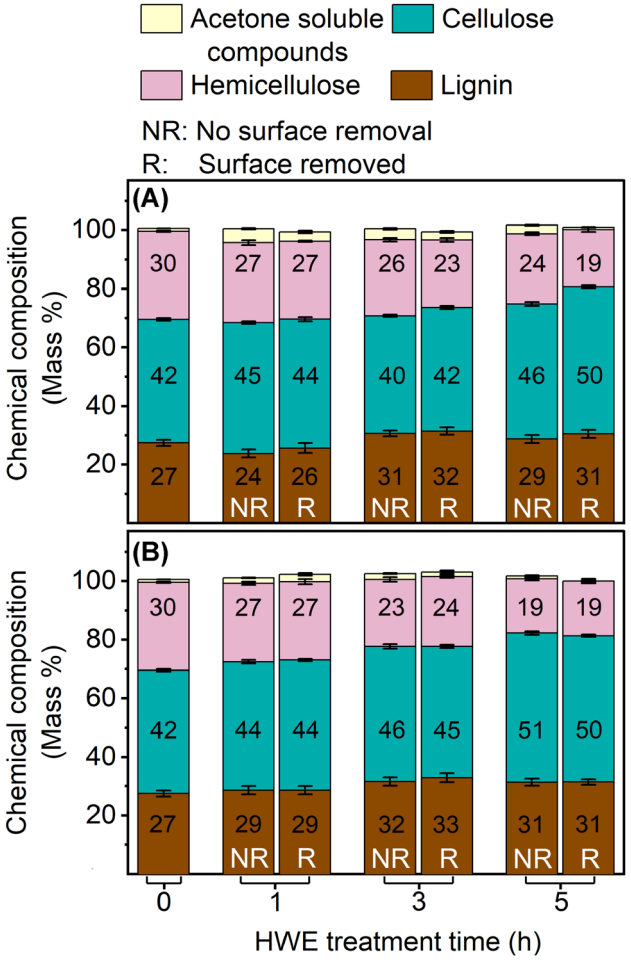

Figure 3: Chemical composition (mass %): (A) non-leached and (B) leached HWE treated specimens. The error bars indicate the standard deviation.

the surface afterwards during drying remained unclear. The latter possibility is supported by previous studies by Möttönen and Kärki (2010) and Terziev et al. (1993) who investigated the migration of water-soluble compounds during wood drying. Möttönen and Kärki (2010) tested a dye solution to observe the migration of liquid in different types of wood specimens and noted that in Scots pine sapwood the liquid migrated during drying in radial, tangential and longitudinal directions towards the surfaces. Aside from the migration of degradation products, the higher content of hemicellulose derivatives in the surface layer could also be due to HWE treatment affecting the outer layer more than the inner parts of the wood, which would explain the gradient in chemical composition. Another potential explanation is the recondensation of degradation products on the wood surface during the cool-down phase of the HWE treatment. While darkening of the outermost layer due to recondensation of degraded lignin is a known phenomenon, other lignin- and carbohydrate-based reactions on the wood surface may also contribute to the formation of gradient in chemical composition (Nistos et al. 2013, 2016; Pelaez-Samaniego et al. 2014).

The decreased amount of hemicellulose in wood due to leaching (Figure 4) was also reflected in the increased amount of obtained carbohydrates. More than half of the wood mass lost due to HWE was obtained as mono- and polycarbohydrates from the extraction liquid and leachates. While leaching increased the overall amount of the obtained carbohydrates, the ratio of mono- to polycarbohydrates remained nearly the same.

3.2 Mould resistance

The standard BS 3900:G6 (BSI 1989) states that the inoculated surface of the control Scots pine sapwood specimens should exhibit visible mould growth within the coverage of 1–30%

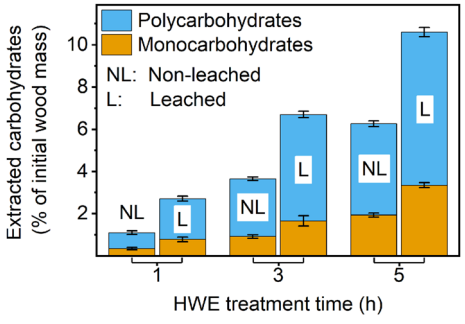

Figure 4: Amount (% of initial wood mass) of mono- and polycarbohydrates extracted from wood as a function of HWE treatment time. The error bars indicate the standard deviation.

(grade 2–3) after 14 days of incubation. However, excluding the specimens HWE treated for 5 h, no visible mould growth was seen on the wood surface in the first 14 days of incubation (Figure 5). In most of the investigated specimens, mould growth was observed after 28 days of incubation. A similar phenomenon was also observed in the research by Imken et al. (2020), who tested multiple different specimens, including Scots pine sapwood. The results by Imken et al. (2020) indicated that for Scots pine sapwood and other tested specimens the mould growth was very low in the first 14 days of incubation, when the tests were performed in an incubation chamber.

After 28 days visible mould growth was observed on the part of the inoculated surface that was closest to the lid of the incubation chamber. As the incubation time extended, the mould growth was distributed more evenly throughout the surfaces of more susceptible specimens. Due to the absence of dense growth even after 56 days of incubation, the highest mould grade given was 4. Figure 5 shows the average mould growth grades of different specimen groups as a function of incubation time, while all assigned grades are listed in Supplementary Table S1. Increasing HWE treatment time did not cause notable changes in the average mould growth grading. Apart from non-leached HWE treated specimens with no surface removal, the resistance to mould growth was lower in untreated specimens compared to HWE treated specimens. According to Ahmed et al. (2013) and Sehlstedt-Persson et al. (2011) kiln-dried Scots pine sapwood is particularly susceptible to mould growth as the low-molecular carbohydrates concentrate close to the wood surface, where the moisture migrates during drying before evaporating. However, within this study, the resistance against mould growth did not improve in untreated specimens despite the extensive leaching. Similar results were also obtained by Reinprecht and Grznárik (2015), who also subjected Scots pine sapwood to an EN 84:2020 leaching procedure before the mould resistance test.

Compared to untreated specimens, HWE treatment improved resistance against mould growth, with the consistent exception of the non-leached specimen with no surface removal. Nutrients such as low-molecular-weight carbohydrates increase the grade of mould growth (Theander et al. 1993; Karlsson et al. 2012). Thus, higher mould grades of the HWE treated specimens that were neither leached nor planed were expected due to the presence of hemicellulose derivatives on the wood surface. This theory is supported by the changes in wood composition due to HWE treatment (Figure 3). While this study utilized leaching and surface removal as methods for removing degradation products from the wood, in previous research

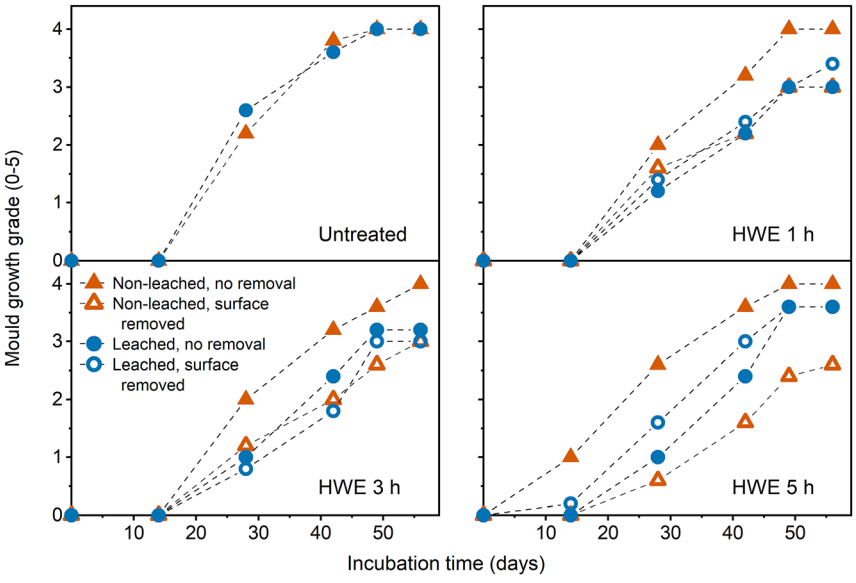

Figure 5: Mould growth grading of untreated and HWE treated (1 h, 3 h and 5 h) wood specimens. Different symbols represent specimen groups subjected to water-leaching (yes/no), and surface removal (yes/no).

by Sehlstedt-Persson et al. (2011) double stacking drying was utilized to direct the migration of water and soluble compounds towards a chosen wood surface. According to Sehlstedt-Persson et al. (2011), the surface with a high amount of monocarbohydrates accumulated in the outward layer (0–1 mm) displayed increased mould growth, while the other surface displayed considerably less visible staining.

By observing magnified images taken from the surfaces of the inoculated specimens, four different types of mould growth were identified (Figure 6): (A) long reddish, partially transparent filaments, (B) small spherical spores that also formed larger clusters, (C) white and grey mycelium and (D) long black mycelium. After 56 days of incubation, all the inoculated specimens had small black spherical spots distributed on the wood surface as well as mycelium growth. The reddish filaments were prevalent primarily in specimens that were untreated or HWE treated for 1 h. While the mould resistance grading was based on the surface

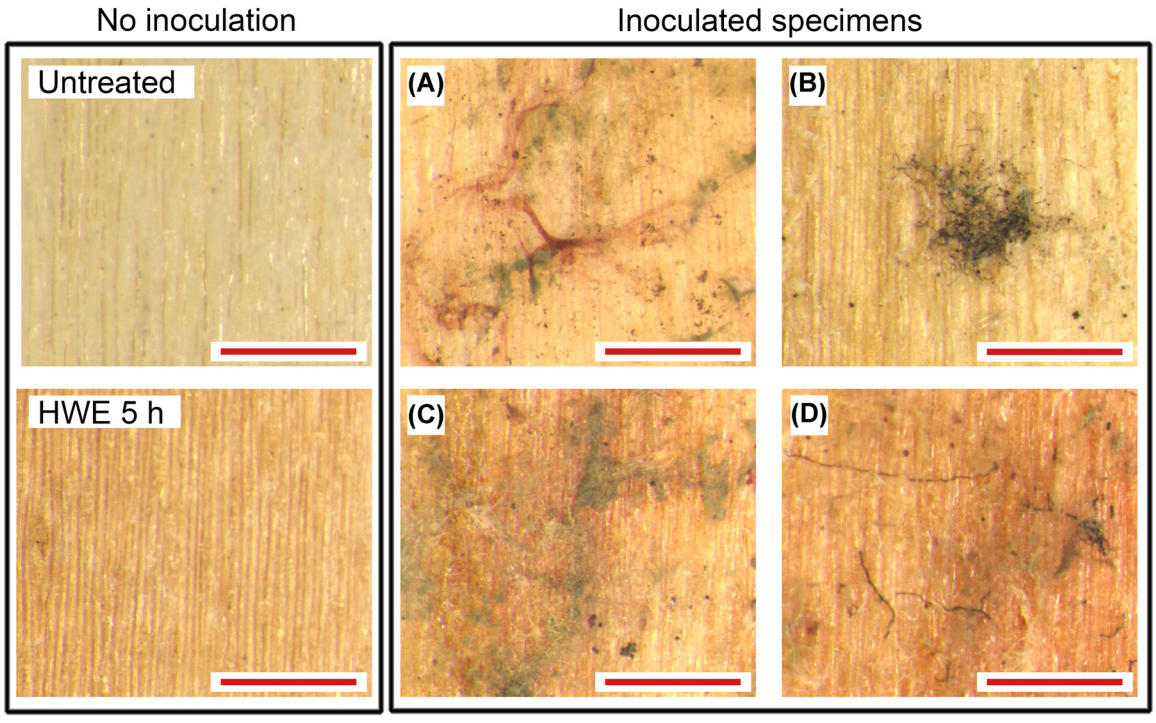

Figure 6: Magnified images taken from the tangential plane of non-inoculated and inoculated wood specimens (includes 500 µm scale bar).

coverage, the variations in fungal flora that grow in differently HWE treated wood could be a topic of interest for future studies.

4 Conclusions

The resistance of HWE treated wood against discolouration by mould fungi was dependent on whether the soluble hemicellulose-based degradation products were removed from the surface after the treatment. By analysing the mass loss, chemical composition and the extraction liquid, it was shown that after HWE a high amount of soluble compounds was located at the wood surface. These degradation products were removable via leaching or planing. The relevance of the HWE degradation products located on the wood surface in promoting discolouration by mould was reflected in studies made in accordance with standard BS 3900:G6 (1989). This research improves the understanding of the mould resistance of HWE treated wood.

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