Resistance of Scots Pine (*Pinus sylvestris* L.) after Treatment with Caffeine and Thermal Modification against *Aspergillus niger*

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Various natural products are potentially suitable for wood protection, particularly against fungi attack. Caffeine is a well-known compound of natural origin, commercially and economically available, which could facilitate its future use for wood protection. This work investigated the antifungal resistance of the sapwood of Scots pine (*Pinus sylvestris* L.) treated with a water solution of caffeine and then thermally modified. The samples after impregnation were thermally modified at different temperatures selected on the basis of thermogravimetric analysis (TGA) of caffeine. The paper presents preliminary results of resistance of treated pinewood towards *Aspergillus niger* van Tieghem (ATCC 6275). The treated samples were subjected to both a leaching procedure and to the artificial aging procedure imitating natural weather conditions. Thermal modification of wood treated with caffeine improved the fungistatic properties of samples after artificial aging. It occurred through partial elimination of caffeine leaching from the wood structure.

Keywords: Caffeine; Wood preservation; Moulds; Thermal modification; Scots pine

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INTRODUCTION

The increasing range of wood applications and growing demands on its service life have prompted the search for new wood preservatives that are more effective and environmentally friendly. Because of the latter, there is much interest in substances of natural origin having fungicidal properties. Various natural products are potentially suitable for wood protection, particularly against wood decaying fungi (*Basidiomycetes*) and moulds attack. In addition to plant extracts, essential oils, and other natural products, caffeine has been indicated as an attractive alternative (Raut *et al.* 2013; Barbero-López *et al*. 2018). In the meantime, the research on the use of caffeine in wood preservatives is being conducted (Kwaśniewska-Sip *et al.* 2018). This well-known alkaloid exhibits a wide range of biological activities including fungistatic properties against decay fungi and moulds (Lekounougou *et al.* 2007). Due to the fact that water-soluble caffeine is easily leached from wood structure, it is important to explore improvements while keeping them environmentally friendly. This problem also concerns the use of caffeine solutions for the protection of wood against moulds after aging tests (Kwaśniewska-Sip *et al.* 2016).

One effective method of wood treatment is thermal modification (Tjeerdsma *et al.* 1998). Thermal modification reduces the sorption of water and changes the nature of wood material from hydrophilic to hydrophobic through degradation of polysaccharides, mainly thermally labile hemicellulose, in the wood cell wall (Kamdem *et al.* 2002; Lee *et al.* 2018). In addition, heat modification changes the chemical composition of wood material, making it less accessible for fungal attack, especially by wood-degrading fungi (Kotilainen 2000; Sivonen *et al.* 2003). Unfortunately, thermal modification has not increased the wood resistance towards *Aspergillus* spp. or other mould species. Works by Ahola *et al.* (2002) and Chen *et al*. (2016) revealed that there is no difference in fungal growth between thermally modified wood and untreated wood tested. Further studies by Gobakken and Lebow (2010) showed that thermally modified Scots pine acts similarly to non-modified Scots pine sapwood against fungal attack. The similar results by Yilgör and Kartal (2010) were described. According to them, the thermal modification did not increase the resistance of wood specimens to *A. niger*; however slight improvements were seen towards *R. javanicus* and *G. virens*. A possible reason is that upon the thermal modification the basic structural components of wood are degraded to sugar monomers. The degradation of wood components such as lignin and cellulose occurs mainly above 200 °C. During thermal treatment between 160 °C to 200 °C, hemicelluloses degrade, and wood specimens are more susceptible to attack by moulds (Percin *et al.* 2016). The combination of thermal modification with caffeine impregnation of wood may broaden the practical uses of modified wood characterized by insufficient resistance to mould fungi. Secondly, thermal modification could eliminate the leaching of caffeine from the wood structure.

The aim of this study was to investigate the resistance of thermally treated pinewood previously impregnated with a caffeine solution towards the mould fungus *Aspergillus niger*. The additional objective was to improve the fungistatic properties of samples subjected to a simulated aging procedure by finding methods to prevent the leaching of caffeine from the wood structure.

EXPERIMENTAL

Materials

Test specimens (40 ± 0.5) × (40 ± 0.5) × (5 ± 0.5) mm (longitudinal × radial × tangential) with nine to thirteen annual growth rings per 10 mm and the average density of 540 kg×m^{-3} were prepared from Scots pine (*Pinus sylvestris* L.) sapwood from the area of Wielkopolska Province. The samples were without knots and free of visible evidence of resins. Pine is a good material for thermal modification. Pine is usually used in outdoor construction, and that is why it is also treated quite hard (Syrjänen 2001). The wood samples were oven-dried (24 h, 103 ºC) to get their initial mass before impregnation (m_1) and after (m_2). The impregnation of the wood lasted for 15 min under vacuum conditions 0.08 MPa, and then the samples were soaked for 2 h under atmospheric pressure in a caffeine solution of 20 mg/mL (in H_2O) at room temperature (Kwaśniewska-Sip *et al.* 2018).

Caffeine as a white powder ($C_8H_{10}N_4O_2$) was purchased from Sigma-Aldrich, Steinheim, Germany.

Thermal Analysis of Caffeine

Thermal analysis (TA) of pure caffeine was performed using LabsysTM thermobalance (Setaram, Caluire, France) at the following conditions: final temperature, 400 °C; rate of temperature increase, 3 °C / min; and atmosphere, helium flowing at the rate of about 2 dm^3/h. The decomposition temperature was determined using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). TGA determines the melting point of caffeine as well as permits the choice of parameters describing the changes in caffeine structure upon thermal treatment.

Thermal Modification

The modification of wood preliminary treated with the caffeine solution was conducted in a thermal treatment chamber under a nitrogen atmosphere. The heating temperatures were selected near the technological drying regimes. The target temperatures were 100 °C, 120 °C, 140 °C, 160 °C, 180 °C, and 200 °C. The process included four stages: drying samples (24 h) at temperature 103 °C, gradual heating of wood samples to a maximum temperature (1h), proper thermal modification of wood (12 h), and cooling (5 h). The samples were heated at the rate of about 2 to 3 °C /min. Control samples were also subjected to thermal modification. After thermal modification, samples were dried at 103 °C to the constant mass (m_3) in order to calculate their mass loss (ML) using Eq. 1. Thereafter, treated and untreated samples were conditioned at 20 ± 2 °C and 65 % (±5) of relative humidity in a climate chamber for 2 weeks to reach the equilibrium mass (m_e) and to calculate the equilibrium moisture content (EMC) using Eq. 2.

$$\text{ML (\%)} = ((m_2 - m_3) / m_1) \times 100 \quad (1)$$

$$\text{EMC (\%)} = ((m_e - m_3) / m_3) \times 100 \quad (2)$$

Aging Procedure

The samples assigned to the aging procedure were divided into two groups. The samples from the first group were subjected to the leaching procedure for outdoor applications according to EN-84 (1997). The samples were soaked in deionized water and impregnated with deionized water under vacuum. The water was exchanged 10 times for the duration of 2 weeks. The second set of samples was aged according to a modified method recommended by ISO 11341-1 (2005) in a Solarbox 1500e chamber (TECNOSERVICE Equipment, Bucharest, Romania). The wood samples were aged for over 10 cycles. One cycle included 102 min in the following conditions: wood surface temperature, 65 °C; relative moisture content of 65%; visible radiation wavelengths of 550 W/m^2; and 18 min of soaking in distilled water.

Mould Resistance

Wood samples were subjected to mycological tests with *Aspergillus niger* van Tieghem BAM 4 (ATCC 6275). The treated samples (5 replicates) were evaluated for resistance towards *A. niger* in the procedure based on EN ISO 846 (1997) part B. The spore suspension of fungus was prepared by washing the surface culture with deionized water and diluted to approximately 1×10^6 spores/mL examined in Thoma's chamber. Each wood sample was placed in a potato dextrose agar medium (Sigma Aldrich) in Petri dishes and then inoculated. The plates were incubated for 4 weeks at 28 ± 1 °C and relative humidity above 95%. The growth of mycelium on the surface of the samples was visually observed every week and individually rated using a five-degree scale (Table 1), with 0 indicating no growth of mould and 5 indicating heavy mould growth (EN ISO 846 1997).

Table 1. Mould Resistance Rating Scale

0	no visible growth under the microscope
1	growth invisible to the naked eye, but clearly visible under the microscope
2	covering up to 25% of the sample
3	covering more than 50%,
4	intensive growth
5	covering the entire surface of the tested sample.

Statistical Analysis

Statistical analysis was conducted using Statistica 10.0 software (StatSoft, Cracow, Poland) with a Kruskal-Wallis ANOVA. The multiple comparisons were performed using Dunn's test at a significance level of 0.05.

RESULTS AND DISCUSSION

Thermal Analysis

The acquired thermogram demonstrated the effect of heat flow on pure caffeine (Fig. 1). The modification temperatures were selected based on the TGA analysis of caffeine. The DSC and DTG curves showed thermal transformations at two temperatures T_1 = 151 °C and T_2 = 181 °C. The caffeine thermogram showed an endothermic peak at 237 °C corresponding to the melting point, indicating that the thermal modification should not be carried out above this temperature.

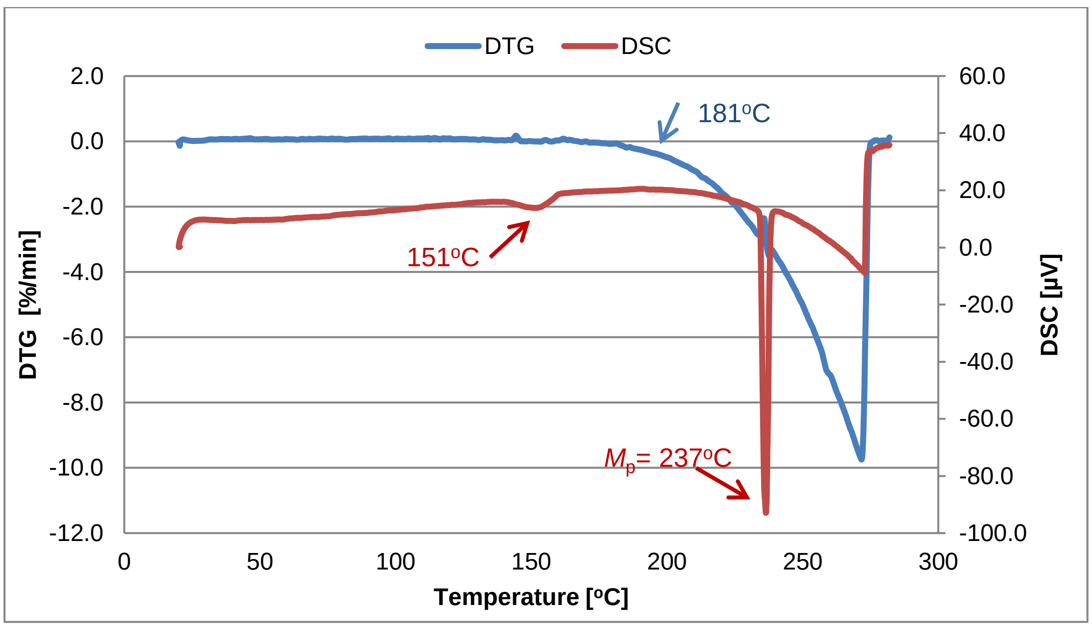

Fig. 1. Thermogram of caffeine

At the same time, the DTG curve showed mass loss. The enthalpy variations associated with the T_1 point could come from transformation to the trigonal phase of α-anhydrous caffeine at 155 °C (Edwards *et al.* 1997). This phase is stabilized by weak CH_3 to CO hydrogen bonds. The rate of mass loss at 181 °C was the most pronounced and can be explained by the sublimation of caffeine. In an open system caffeine is more susceptible to sublimation, while in the TGA analysis the sample is confined, which minimizes sublimation. The parameters of modification were chosen to account for the types of possible bonds between caffeine and wood.

Thermal Modification

The positive changes in the wood properties after thermal modification include darkened colour, increased thermal insulation, lower equilibrium moisture content, reduced water absorption, and decreased wettability of wood (Yilgör and Kartal 2010). The colour of the sample edges became a little darker during the thermal modification at temperatures of 100 °C to 160 °C than in the reference sample. Significant change in colour occurred in the samples treated at higher temperatures 180 °C to 200 °C (Fig. 2a). Slight changes in colour and maturation after were observed the aging of samples (Fig. 2b).

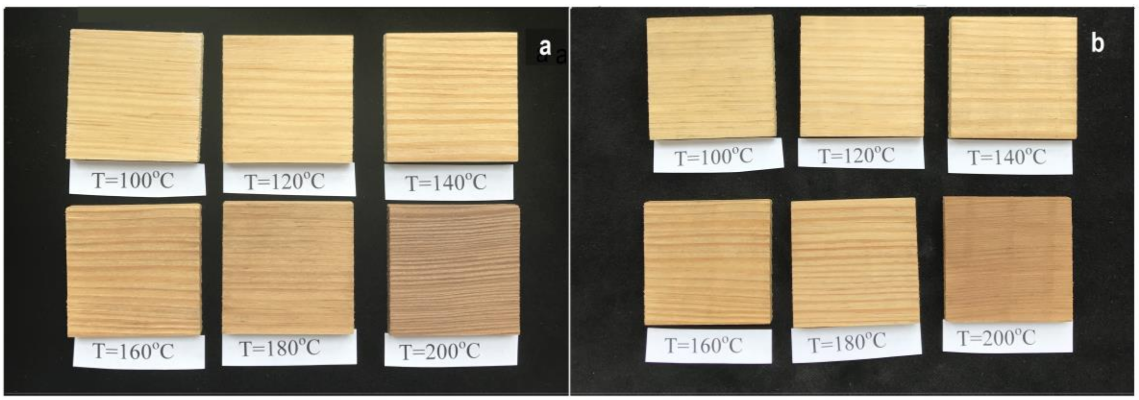

Fig. 2. Samples after caffeine impregnation and thermal modification before aging (a) after aging in Solarbox (b)

The MLs and EMC value of samples thermally modified after impregnation with caffeine (labelled CAF) and without impregnation as control are shown in Fig. 3. Only the results of samples modified at temperature above 140 °C are presented. Below this temperature there were slight changes. The ML of the thermal modified wood after impregnation were 3.0% and 10.6% for 180 °C and 200 °C, respectively. Any noticeable differences between MLs of impregnated and control samples were found. The results demonstrated that pre- impregnation with caffeine did not affect the ML of thermally modified wood.

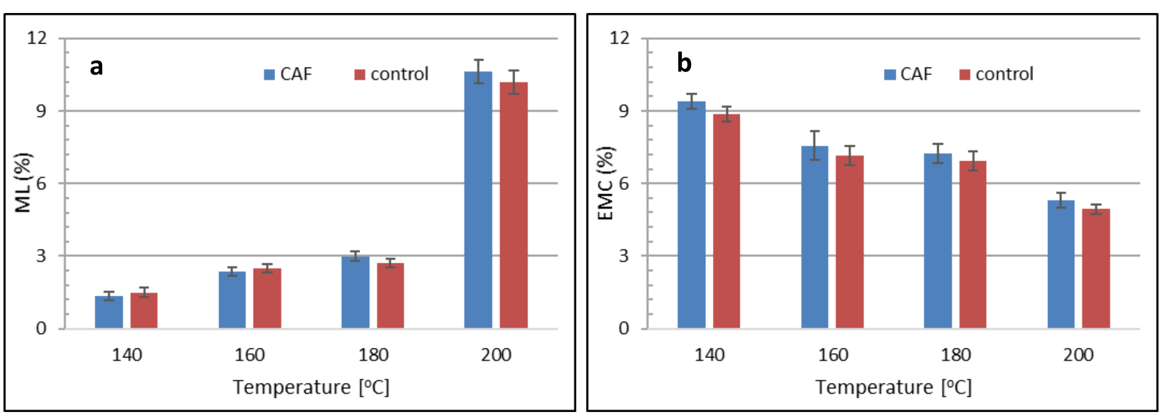

Fig. 3. a) MLs value **b)** EMCs value of wood samples thermally modified

Thermal modification decreased the EMC of Scots pine samples, which correspondences to previous studies (Esteves and Pereira 2009). The EMC for non-impregnated wood was 8.9% and it decreased to 4.95 % in the case of wood modified at 200 °C. A slight increase of EMCs value for samples after impregnation was observed. The reason may be the presence of caffeine in the wood structure, which has hydrophilic properties (Edwards *et al*. 1997).

Mould Resistance

The antifungal effects of caffeine applied together with thermal modification were assessed by visual observation of the growth of mycelium on wood surface. The results determined after 4 weeks are presented in Fig. 4. *A. niger* growth on the thermally modified control specimens was fast, and after 1-week of incubation the surface of the pine samples was completely overgrown (rating scale of 5). Similarly to previous works, control samples after thermal modification were covered with the mycelia of the *A. niger* rapidly. In the work of Chen *et al.* (2016), *A. niger* mycelium covered about 75% of the surface of modified Southern pine sapwood at 180 °C to 200 °C. A similar phenomenon was noticed in the studies by Yilgör and Nami Kartal (2010). In their results both treated and untreated specimens were covered by the fungus in the end of the test.

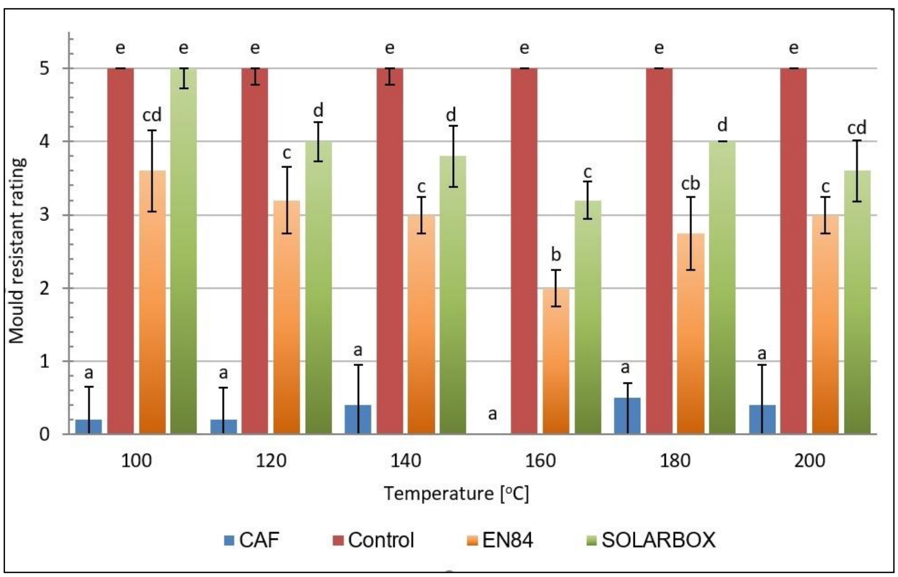

Fig. 4. Average mould resistance rating after 4 weeks. Averages from the samples accompanied by different letters (a, b, c, d, e) are significantly different at $p < 0.05$.

In the present study, thermal modification did not change the effect of caffeine towards *A. niger*. Compared to previous studies, caffeine protects wood against mould attack. However, the impregnated wood was not protected after aging tests (Kwaśniewska-Sip *et al.* 2018). Only the thermally modified samples treated with caffeine after the aging procedure had an increased resistance towards the tested fungus. An increased value of the index of the fungus growth was attributed to the partial leaching of caffeine from the wood structure. Despite the effect of reduced hygroscopicity of wood after thermal modification, the treatment improved the fungistatic properties of the samples impregnated with caffeine solution after quick aging in Solarbox chamber (labelled SOLARBOX) and after leaching procedure (labelled EN84). The best results were obtained for the samples treated at 160 °C. This result may be related to the transformation of the caffeine molecule into the trigonal phase (Edwards *et al.* 1997). Future chemical analysis will be necessary to understand the chemical interactions between caffeine molecules and wood structure. Further research on the use of caffeine to an effective wood protection against microbial attack is also needed. Furthermore, research on caffeine resistance to leaching is being continued by the use of mixtures of caffeine with natural oils, silanes, and natural extracts (Ratajczak *et al.* 2017).

Statistical Analysis

A Kruskal-Wallis test conducted for *A. niger* indicated a dependence between the rating and temperature of thermal modification for samples assigned to the aging procedure (p-value = 0.006). However, these differences were not very significant in visual assessment among all temperature modifications equal at least to 12 °C (Fig. 4).

CONCLUSIONS

1. Thermal modification changes the chemical composition of Scots pine wood lowers equilibrium moisture content and leads to mass loss simultaneously.
2. Thermally modified wood did not prevent mould growth to a significant extent when compared to unmodified wood.
3. The thermal modification of Scots pine sapwood previously impregnated with caffeine solution improved the resistance of the samples subjected to quick aging against the fungi attack. The best results among used thermal treatments were obtained for the samples treated at 160 °C. This may be related to the transformation of the caffeine into the trigonal phase, which is influenced by temperature (155 °C).
4. The combination of thermal modification with caffeine impregnation of wood could partly eliminate the leaching of caffeine from the wood structure. In addition, this could widen the practical uses of modified wood characterized by insufficient resistance to mould fungi.

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