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Durability of a natural flavonoid inserted wood against various fungi

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Abstract: Wood has excellent mechanical properties, however outdoor utilization of this renewable resource as an engineering material is limited by unfavorable properties such as low dimensional stability upon moisture changes and a low durability against decaying organisms and insects. However, some wood species are known to produce wood of higher quality by inserting phenolic compounds in the already formed cell walls which is called heartwood formation. In the present study, we transferred the principles of heartwood formation in black locust (*Robinia pseudoacacia*) as a bio-inspiration modification to improve durability of Siberian pine wood by a chemical treatment with commercially available extractable flavonoids. Tosylation treatment was carried out before flavonoid impregnation for hydrophobization of cell walls. The effect of natural flavonoid insertion into wood was studied to improve it's durability by using various fungi. Wood samples including un-treated controls were exposed to brown rot fungi, *Coniophora puteana* and *Postia placenta* attack according to the EN 113 standard, and mold fungi, *Penicillium chrysogenum* and A*spergillus niger* according to the ASTM D4445-10 standard. Flavonoid inserted samples showed higher decay resistance of 50-84% against brown rot fungi but lower resistance against mold fungi than controls. The weight loss results clearly demonstrated modified samples did not have sufficient protection against *P. placenta* according to EN 113 standard since the modified samples had more than 3% weight loss in decaying test but they were found efficacious in suppressing *C. puteana* attack. Mold growth in modified samples was not inhibited by flavonoid insertion. Especially, growth of *A. niger* was very aggressive on both modified and control samples in petri dishes from the first week to the end of the incubation period. Consequently, it is suggested that more successful biomimetic modifications may lead to alternative cell wall modification procedures and improve durability of wood as an engineering material.
Keywords: Brown rot fungi, Decay resistance, Durability, Heartwood, Natural flavonoid, Mold fungi

1. Introduction

The low durability of wood limits its utilization especially in outdoor fields (Rowell, 2000; Hill, 2006). It is known that the wood cell wall determines the main structural properties of wood. Cell walls are composed of bio-synthesized cellulose fibrils embedded in hemicellulose and lignin matrix (Fengel et al, 1984; Salmen et al, 2009). Due to specific chemical structure of wood, its unprotected application in the presence of high moisture results in biodegradation of cell walls by fungi and insects.

Producing durable wood timber by chemical treatment and cell wall modification has been a common application to protect wood from bio-organisms. Anhydrides, carboxylic acids, acid chlorides, isocyanides, epoxides, aldehydes, silicon-containing compounds, graft polymerization methods etc. have been used so far for wood property improvement (Rowell, 2000; Mai et al, 2004; Hill, 2006; Cabane et al, 2016). These chemical treatments are mainly aiming to make covalent bonding between related chemical and cell wall polymers or making cross-links. Such chemical modifications increase hydrophobicity of wood, so reduce water and humidity uptake.

Another treatment model is filling the nano and micro-voids of cell walls which again limit humidity entrance inside cell walls of wood. Thus possible fungi attack can be limited, because fungi can grow inside wood at certain humidity level (Rowell, 2000). Flavonoids which are polyphenolic chemicals found in heartwood of some wood species, can affect wood quality and characteristics significantly, especially durability and color (Magel, 2000; Taylor et al, 2002; Pallardy et al, 2008).

In this study, we bio-inspired from the heartwood formation in black locust to improve the durability of Siberian pine wood, an economically valuable wood species, which has a low durability. Similar wood modification approaches have been reported with different molecules such as simple or polycyclic phenolic compounds (Sakai et al, 1999; Matsunaga et al, 2000; Obataya et al, 2000; Ermeydan et al, 2012). However, a new method of modification with a natural hydrophobic flavonoid has been used for the first time in the study, and there is no literature found that reports fungal resistance of modified wood with flavonoids. In this study, fungal resistance of modified wood with a natural flavonoid, Chrysin, exposed to brown rot fungi, *Coniophora puteana* and *Postia placenta* according to the EN 113 standard, and mold fungi, *Penicillium chrysogenum* and A*spergillus niger* according to the ASTM D4445-10 standard was evaluated.

2. Materials and methods

2.1. Modification process

Para toluene sulfonyl chloride, pyridine, 5,7-Dihydroxyflavone (Chrysin) were bought from Sigma-Aldrich and used as received. Siberian pine (*Pinus sibirica*) wood samples were cut parallel to grain direction and sawn into blocks of 0.5 × 1.5 × 3.0 cm^3 (tangential x radial x longitudinal). Tosylation reaction of pine wood cell walls was carried out as follows. The Siberian pine sapwood samples were dried at 60 °C for 1 day. 25 replicates were weighed (25,7 g, 0,158 mol, calculated as a glucopyranose (MW:162 g/mol)equivalent). 300 mL of pyridine was added to the samples in the flask for swelling for 1 day. The flask was stored in an ice bath with the reactants. P-toluenesulfonyl chloride (45 g, 0,238 mol) was added to the solution and reacted for 1 day at 5 °C.

Before impregnation of flavonoid in the cell walls, 3 g of Chrysin was dissolved in 340 ml acetone (7,5:1) ethanol mixture. Tosylated pine wood samples were washed with pyridine for 3h to leach out the unreacted tosylates before Chrysin process. Chrysin solution was poured onto the swollen and tosylated samples. Acetone was evaporated harshly by heating the flask to 80°C under vacuum to remove acetone. After evaporation of acetone, samples were washed with distilled water by stirring to leach out pyridine and flavonoid molecules that had not been impregnated in the cell walls but precipitated in the lumen.

2.2. Decay-resistance test

Decay performance was determined using the mini-block agar plate test according to principles EN 113 standard with some modifications. Samples were sterilized in an autoclave by steaming, and then placed into petri dishes inoculated with the brown-rot fungi, *Coniophora puteana* (Schumach.) P. Karst. (Mad-515) and *Postia placenta* (Fr.) Lars. & Lombard (Mad-698-R). After 8-week-exposure period at 20°C and 65% RH, weight loss that had occurred in the samples during fungal exposure was calculated.

2.3. Mold-resistance test

Modified and control samples were evaluated for resistance to mold fungi according to the ASTM D4445-10 standard method with slight modifications. Two mold fungi, *Aspergillus niger* ATTC 16434 and *Penicillium chrysogenum* ATTC 101016 were grown on 4% malt agar at 25°C and 80% RH. Spore suspension was prepared by adding 10mL to petri dishes and rubbing the surface of culture. Suspensions were diluted to yield $1.42x10^7$ spores for *A. niger* and $1.52x10^7$ spores for *P. chrysogenum*. Spore suspensions of 0.25 mL were leaved along the length of one flat side of each sample in the petri dishes. All samples were then incubated at 25°C and 80% RH for four weeks. Following incubation, specimens were visually rated every week on a scale of 0-5 with 0 indicating the specimen is completely free of mold growth and 5 indicating the specimen was completely covered (0: no growth, 1: 20%, 2: 40%, 3: 60%, 4: 80%, 5: 100% coverage with mold fungi). At the end of the test weight loss of samples was also calculated.

3. Results and discussion

3.1. Modification process and physical changes

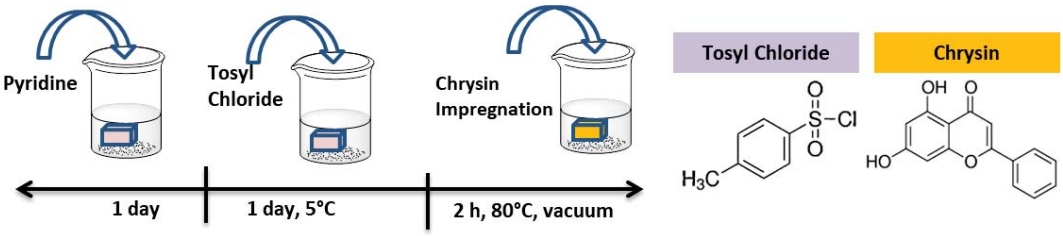


Figure 1. Left: Schematic representation of modification process. Right: Chemical structure of tosyl chloride and chrysin molecules.

Siberian pine samples were first activated by tosylation treatment to increase hydrophobicity of wood cell wall (Ermeydan et al, 2012). Then wood was impregnated with Chrysin molecules (see methods part and Figure 1). Success of modification was observed by simple weight percentage gain (%) and volume change (%) calculations (Table 1). P-toluen sulfonyl chloride (tosyl chloride) is a reactive reagent and can establish covalent bonds with hydroxyl groups of cell wall polymers. Thus, due to the chemistry of tosyl chloride and blocking of hydroxyl groups onto the cell wall polymers, wood cell walls can be hydrophobized (See Figure 1). The aim of hydrophobization of wood cell walls is to be able to insert hydrophobic Chrysin molecule to increase durability of wood.

The reaction process of tosylation step was successful as shown in Table 1. WPG% of wood after tosylation is around 15%, which means there is a certain amount of tosyl groups entered and boned in wood structure. However, after impregnation of chrysin into wood, WPG% of the initial material reduced to 7,7% which means during chrysin impregnation,

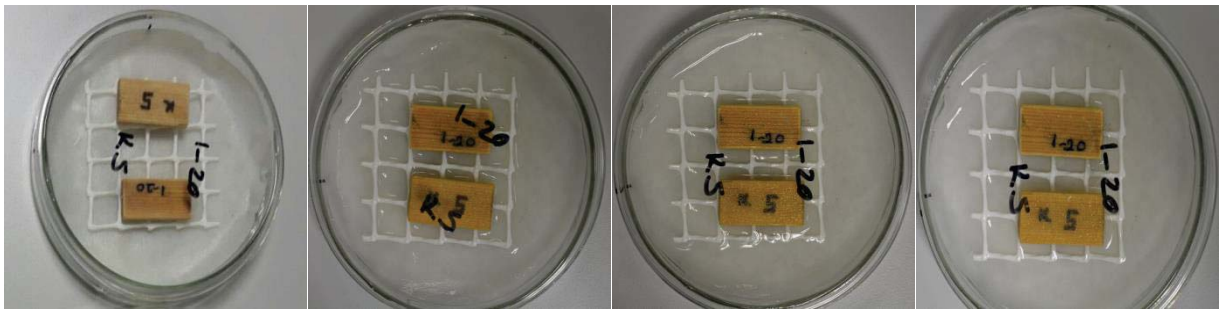
wood lost weight about 7%. This is probably due to the removal of tosyl groups and wood constituents, because a harsh process carried out during the acetone leaching after impregnation (80°C and vacuum). Volume change is another important parameter that shows chrysin or tosyl groups still inside cell walls, and swell them to make a volume increase about 4%. Another observation to prove existence of chrysin in wood is color change of wood after chrysin impregnation. Color of wood become yellowish after chrysin impregnation as can be seen in Figures 2 and 3.

Table 1. Weight Percentage Gain (WPG%) and Volume Change% of wood after tosylation treatment and chrysin modification

	WPG%	Volume Change%
tosylation only	15±1,4	N/A
after chrysin mod.	7,7±1,7	4,3±1,5

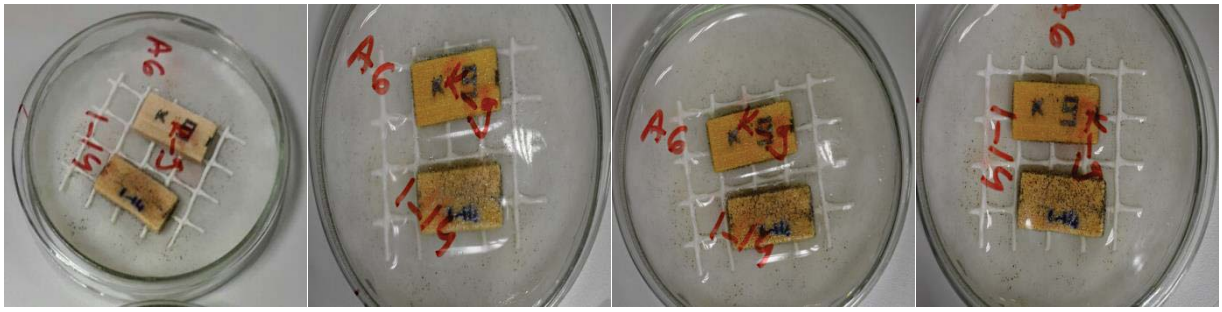
3.2. Decay resistance

Growth and average rating of mold fungi on the wood surfaces is shown in Figures 2-5. In mold-resistance tests, average rating of mold fungi was found to be lower in controls than in chrysin modified samples. Chrysin modification did not enhance mold resistance of wood samples. These results may suggest that concentration level of chrysin is not enough to protect wood against mold growth, and higher concentration levels of chrysin are needed for better performance. Among the mold fungi, growth of *A. niger* was very aggressive on the wood from the first week to 4 weeks. Growth of *A. niger* on the surface increased as the incubation period increased. The molds and soft rot fungi tolerate high levels of some toxicants. These include *Penicillium (cyclopium) aurantiogriseum* on mercury compounds, *Scopulariopsis brevicaulis* on arsenic compounds, *Hormoconis (Cladosporium) resinae* on creosote, and *Trichoderma* sp. on sodium fluoride. The roles of these fungi and the significance of their detoxification abilities on treated wood in the natural environment remains unknown, but under ideal conditions, they could detoxify preservative treatments, permitting decay fungi to colonize the treated wood (Zabel and Morell 1992). *Aspergillus niger*, *Cephalosporium* sp., *Penicillium frequentens* and *Cladosporium elatum* was resistant to high copper levels (Sharp 1975). Some studies also showed nano-CuO, nano-ZnO, nano-B_2O_3, nano-Ag (Kartal et al. 2009), nano-CeO_2, nano-TiO_2 and nano-SnO_2 (Terzi et al. 2016) known as effective chemicals against basidiomycetes failed to provide sufficient protection against mold growth (*Antrodia* sp., *Aspergillus niger*, *Trichoderma harzianum* and *Penicillium pinophilum*) on the wood surfaces.



1 week 2 weeks 3 weeks 4 weeks

Figure 2. Pictures of samples exposed to *P. chrysogenum*.



1 week 2 weeks 3 weeks 4 weeks

Figure 3. Pictures of samples exposed to *A. niger*.

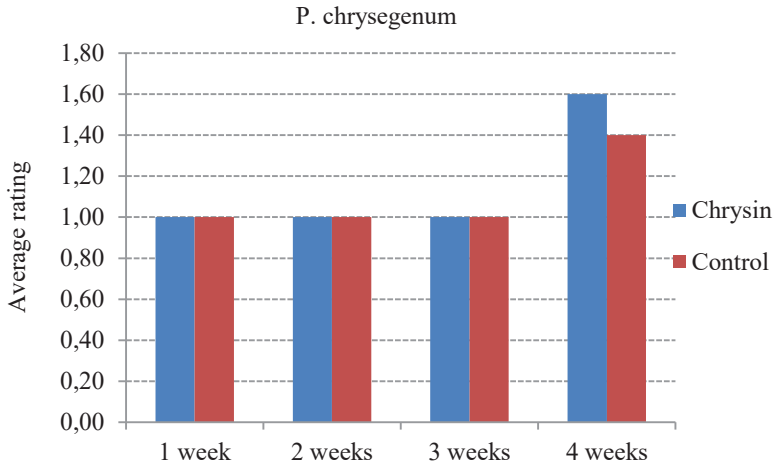


Figure 4. Average rating of *P. chrysogenum* growth on wood samples

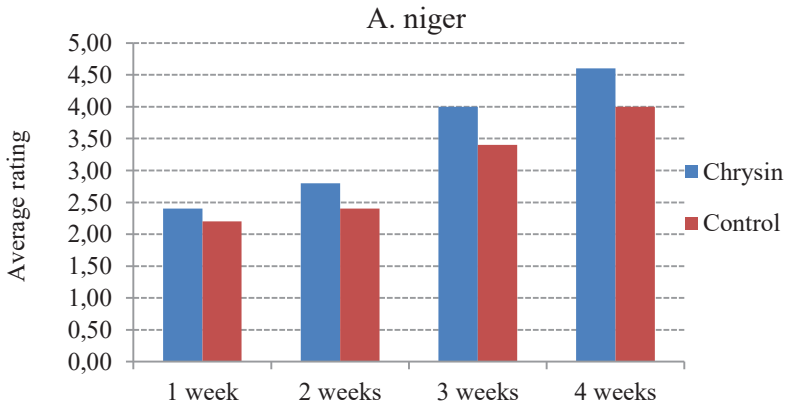


Figure 5. Average rating of *A. niger* growth on wood samples

Figure 6 shows the weight loss of samples by fungi attack. Chrysin modified samples exhibited lower weight loss than controls for both basidiomycetes fungi. Decay resistance in modified samples was found as 50 and 84% for *P. placenta* and *C. puteana* attack, respectively. *P. placenta* was more aggressive than *C. puteana*. This is probably due to the degradation of the cell wood attacked by *C. puteana* occurs firstly on the wall surface, but in the case of *P. placenta* the destruction of cellulose proceeds deep inside the wall even after 1 month of fungal attack (Irbe et al, 2006; Tomak 2014). The weight loss results clearly demonstrated modified samples did not have sufficient protection against *P. placenta* according to EN 113 standard since the modified samples had more than 3% weight loss in decaying test but they were found efficacious in suppressing *C. puteana* attack. Surprisingly, quite high weight loss was observed on the modified samples after mold fungi attack. It has been stated that the mold fungi do not affect the strength of wood; however, in this study mold fungi covered the wood surface and caused weight loss of around 5%. Salem (2016) reported that mold fungi affected significantly the surface elemental composition and showed different degrees of hyphal penetrations. The mold fungi metabolize the carbon-rich constituents of wood and produce large fruiting structures (Salem 2016). Weight loss findings by mold fungi were in accordance with the average rating of fungi growth on surfaces. Control samples exhibited lower weight loss (0.6 and 0.9%) than modified samples (5.16 and 5.4%). Chrysin might support mold growth on the samples, and mold fungi might digest the chemical. Both weight loss of wood components and chrysin due to decay, could be possible reasons for higher weight loss in modified samples than control samples. Wood extractives are commonly degraded by mold decay (Zabel and Morell 1992). *Aspergillus niger* is a producer of many pectinases and hemicellulose degrading enzymes, like xylanases and arabinases (Salem et al, 2016). Hydrolytic enzymes of *Aspergillus* spp. also cause cellulose hydrolysis. *Penicillum* species are known for their ability to produce extracellular enzymes including cellulose. Some species of *Penicillum* can also degrade pectin and xylan (Mansour et al, 2015). Certain molds such as *Aspergillus* or *Penicillium* have been observed to grow on the surface of liquids of tannery pits and tannery wastes since they have tannin-degrading systems. *Aspergillus* and *Penicillium* species have been used biodegradation of polyphenols (Bhat et al, 1998). Chrysin is a natural flavonoid and is belonging to the polyphenols family. That's why it is thought that Chrysin may be consumed by the mold fungi.

In this study, it was expected to obtain greater decay resistance with chrysin modification of wood for both basidiomycetes and mold fungi. Low concentration level of chrysin and a harsh leaching process of acetone after impregnation process which caused 7% weight loss in wood components could be possible reasons for the low efficiency in decaying tests. This study clearly showed more studies are needed for better understanding the fungal resistance of chrysin in wood. Higher concentration levels of chrysin, more moderate leaching process and different decaying organisms should be studied. Studying of these parameters to obtain optimum modification process is still under progressive by the authors.

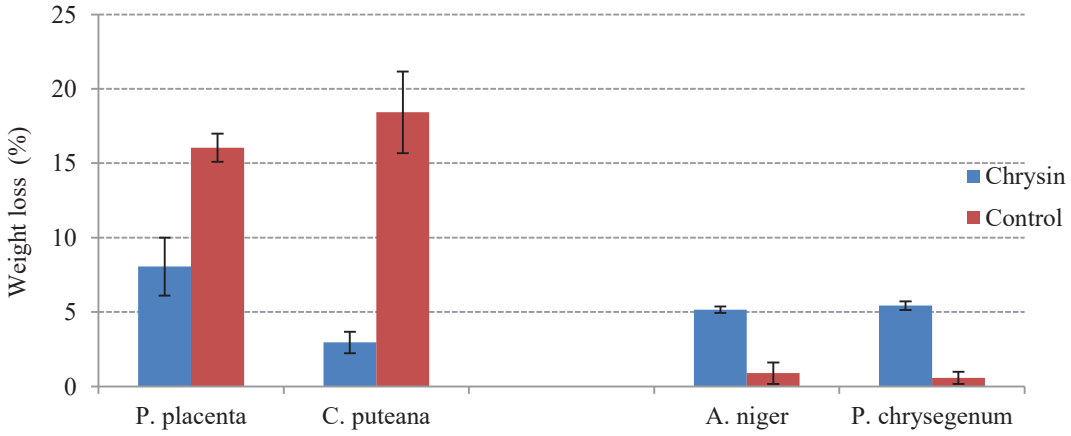


Figure 6. Weight loss (%) of wood samples after fungal decay.

4. Conclusion

In this study, the chemical modification method reported by Ermeydan et al. (2012) was upgraded. A natural hydrophobic flavonoid, chrysin, has been impregnated into the wood to improve durability of wood material. We inspired from heartwood formation and one step more used a natural hydrophobic flavonoid instead of hydrophilic flavonoids. Chrysin molecule contributed to a significant enhancement of the durability against brown rot fungi however the chemical did not have sufficient protection against mold fungi. The study which is the first part of the Tubitak project clearly showed more studies are needed by using different microorganisms since the chemical seemed to have not a broad spectrum of activity against wood decaying organisms.

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