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Bio-Durability and Engineering Characteristics of Heat-Treated Poplar Wood

Biološka trajnost i tehnička svojstva toplinski modificirane topolovine

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ABSTRACT • *The aim of this study was to evaluate the effect of brown rot fungus Coniophora puteana activity on physical and mechanical properties as well as biological resistance of heat-treated poplar wood. Two poplar wood species (Populus deltoids and Populus nigra) were heat-treated by thermo-wood (Thermo-D) method. Control and heat-treated specimens were exposed to brown rot fungus C. puteana for 16 weeks. Physical and mechanical characteristics of specimens including density, compression strength parallel to the grain and impact strength were evaluated before and after exposure to fungus. Mass loss of specimens caused by fungal activity (MLF) was also calculated. In addition, the effect of thermal modification on laccase production by C. puteana was assayed. The highest mass loss due to fungal deterioration was observed in control specimens, coinciding with the highest substrate-enzyme interactions and constant decrease in detectable laccase levels. According to the results, thermal modification can be used effectively to protect poplar wood against brown rot fungus attack.*

KEYWORDS: *poplar; thermo-wood; fungus; physical and mechanical properties; biological resistance*

SAŽETAK • *Cilj ovog istraživanja bio je procijeniti utjecaj gljive smeđe truleži Coniophora puteana na fizička i mehanička svojstva te na biološku otpornost toplinski modificirane topolovine. Dvije vrste topolovine (Populus deltoids i Populus nigra) toplinski su modificirane postupkom thermo wood (Thermo D). Kontrolni i toplinski modificirani uzorci bili su izloženi 16 tjedana gljivi smeđe truleži C. puteana. Prije i nakon izlaganja gljivama određena su fizička i mehanička svojstva uzoraka uključujući gustoću, čvrstoću na tlak paralelno s vlakancima i čvrstoću na udarac. Također je izračunan gubitak mase uzoraka kao posljedica aktivnosti gljiva (MLF). Osim toga, ispitan je utjecaj toplinske modifikacije na stvaranje lakaze zbog djelovanja gljive C. puteana. Najveći gubitak mase, tj. najveća razgradnja nastala djelovanjem gljive zabilježena je na kontrolnim uzorcima, a to se podudara s najjačim međusobnim djelovanjem supstrata i enzima te s konstantnim smanjenjem detektirane razine lakaze. Prema dobivenim rezultatima, toplinska se modifikacija može učinkovito iskoristiti za zaštitu topolovine od napada gljiva smeđe truleži.*

KLJUČNE RIJEČI: *topolovina; thermo-wood; gljive; fizička i mehanička svojstva; biološka otpornost*

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1 INTRODUCTION
1. UVOD

Wood as a natural polymer has been widely used in structures for many years. However, some properties of this natural material such as moisture absorption, dimensional instability, biological degradation, weathering, etc., limit its use. So, numerous new methods of wood modification have been developed to remove these disadvantages and expand its functionality. Wood heat treatment is one of the modification methods, which is carried out by different processes. This kind of modification was first studied scientifically by Stamm and Hansen in the 1930s in Germany and in the 1940s by White in the United States. The most comprehensive research in this field has been carried out by VTT (International Thermo-Wood Association) in Finland, whose product is called thermo-wood. A temperature of 160-260 °C is usually used to produce thermo-wood (Militz, 2002). Thermal modifications have some positive and negative effects on wood. In some cases, thermal treatments lead to positive changes in chemical structure of wood, colour, dimensional stability, thermal insulation properties, biological resistance to biological degradation. However, these treatments also reduce some mechanical properties such as wood bending strength.

Heat treatment of wood in the temperature range of 140-260 ºC with a long holding time causes an irreversible reduction of moisture absorption (González-Peña *et al.,* 2004; Obataya and Tomita, 2002); it leads to dimensional stability (Krause *et al.,* 2004), less moisture movement (Militz and Tjeerdsma, 2001), and improved biological resistance (Welzbacher and Rapp, 2004; Farahani *et al.,* 2001). However, one of the major drawbacks of these treatments is the reduction of wood mechanical properties (González-Peña and Hale, 2007).

Brown rot fungi have an enzyme system that produces hydrolytic and oxidative enzymes, which act on cell wall component degradation such as lignin, cellulose and hemicelluloses (Baldrian and Gabriel, 2003). Cellulases can be classified into three types: endoglucanases, exoglucanases and 1, 4-β-glucosidases (Gielkens *et al.,* 1999). Lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) are the most widely distributed lignin-degrading enzymes, which are responsible for the biodegradation of lignin (Vicuna, 2000).

Evaluation of heat treatment effect on bio-durability of a wood species showed that it significantly increases wood durability against fungal action due to the reduction of hydroxyl groups (Mburu *et al.,* 2007). Kamperidou (2019) evaluated the biological durability of thermo-chemically modified pine and spruce wood against white and brown rot fungi. The results showed that thermal modification presented lower mass loss caused by used fungi compared to the unmodified wood. In other words, heat treatment increases the biological resistance of wood.

According to Gao *et al.* (2016), the colour of wood became darker and there was an improvement in dimensional stability as well as reduction in modulus of elasticity of heat-treated poplar wood at temperatures of 140 to 200 °C for 1-3 hours. In addition, bio-durability of heat-treated specimen was better against white rot fungus than brown rot fungus.

Furthermore, Corleto *et al.* (2020) investigated the effect of thermal modification on properties of padauk wood, indicating that heat treatment at high temperature caused significant loss in bending strength and bending stiffness of padauk wood. Moreover, the results of chemical analysis showed that cellulose and lignin proportion increased, while that of hemicellulose decreased substantially following thermal modification. Another study showed that durability and dimensional stability of European birch, European aspen, Norway spruce as well as Scots pine improved by thermal modification (Karlsson *et al.,* 2011; Militz and Altgen, 2014).

Kaygin *et al.* (2009) investigated the effect of mass loss of mechanical properties of heat-treated Paulownia at 160, 180 and 200 °C. The results showed that mechanical properties including compression strength, modulus of elasticity, bending strength and impact strength decreased with increasing of heat treatment temperature. In fact, it was determined that, due to thermal modification, the mass loss significantly affected mechanic properties of Paulownia wood.

Heat treatment can be used effectively against fungal attack for Scotch pine, oak and beech wood species (Ayata *et al.,* 2017). At high temperature of wood thermal modification, the lignin content increases because of autocondensation and its higher thermal stability. There is a slight decrease in the extractives content. Moreover, the cellulose and its DP decrease, while cross-linking reactions occur in the cellulose (**Čabalová** *et al.,* 2019).

Brown rot fungi have the ability to degrade cellulose by producing extracellular fungal cellulose degrading enzymes. The extracellular enzymes produced by these fungi are greatly affected by the type of substrate being decayed. Our study focuses on measuring oxidative enzyme laccases secreted by *C. puteana* in the presence of poplar woods (control and heat-treated specimens) as substrates. Therefore, this study aimed to find the effect of heat treatment on biological resistance of thermo-wood against degradation by brown rot fungus *Coniophora puteana*. Meanwhile, physical and mechanical properties of thermo-wood and control specimen after exposure to *C. puteana* were investigated. In addition, levels of laccase enzyme on both substrates (control and modified specimens) were assayed.

2 MATERIALS AND METHODS
2. MATERIJALI I METODE

The lumbers of two poplar wood species (*Populus deltoids* and *Populus nigra*) were prepared and treated in Mazand wood company. After kiln loading, the temperature was rapidly raised to a level of around 100 ºC and then increased steadily to 130 ºC. Thereafter, the temperature inside the kiln was increased to around 212 °C and remained constant for 3 hours. The final stage was cooling and moisture conditioning by using water spray system. When the temperature reached 80 ºC, re-moisturizing took place to bring the wood moisture content to a level of around 6 %. In order to evaluate biological resistance as well as physical and mechanical properties of treated timbers, six specimens were prepared in accordance with EN 113-2:2020 standard.

In this study, brown rot fungus *Coniophora puteana* was used to evaluate biological resistance of control and modified specimens. Modified and control specimens were exposed to fungal mycelium in Kolle-flasks. As a nutrient medium for fungal mycelium, Malta Extract Agar (Merck KGaA, Darmstadt, Germany) was used. After preparing nutrient medium and sterilization in autoclave (temperature 120 °C, time 20 minutes, pressure 1.5 kg/cm^2), small pieces of fungal mycelium were inoculated in Kolle flasks. Then the flasks with nutrient medium and mycelium were incubated in the climate chamber at a temperature of 22 °C and 65±5 % relative humidity for 2 to 3 weeks until the entire surface of the nutrient medium was overgrown by mycelium (as shown in Figure 1). Six modified and control specimens were placed in each flask and were incubated in the same climate chamber for another 16 weeks according to EN 113-2: (2020) standard (Figure 1). After 16 weeks, all Kolle flasks were taken out from the climate chamber.

Ligninolytic enzyme laccase was extracted at 25 ºC in two extraction cycles, 5 and 24 hours, with 50 mM sodium acetate buffer (pH=5.5) with 0.1 g/L Polysorbate 20 (Tween® 20; from Sigma Aldrich: Steinheim, Germany). It should be noted that two extraction cycles of 5 and 24 hours were used to obtain the optimal time for maximum enzyme extraction. An amount of 1.5 gr chips prepared from decayed specimens were first soaked in 50 mL extraction buffer for 5 hours. The second extraction was performed with 25 mL extraction buffer for 24 hours. For this purpose, six repetitions were considered. Then, the supernatants collected from all extractions were filtered, centrifuged at 5.000 rpm for 15 minutes and used to test enzyme activity.

Laccase activity was assayed by measuring the oxidation of 2,6-DMP. An aliquot of enzyme solution was incubated in 1 ml of 5 mM of 2,6-Dimethoxyphenol (2,6-DMP) in 0.1 M sodium acetate buffer (pH= 3.6) at 30 ºC. Absorbance was monitored at 469 nm in a spectrophotometer. One unit of laccase activity was defined as the amount of the laccase that oxidized 1 µmol of 2,6-DMP per minute at 30 ºC (Field *et al.,* 1993).

In order to evaluate the durability of wood specimens against brown rot fungus, the mass loss was calculated. After 16 weeks of incubation, the wood specimens were taken out of the Kolle flasks and carefully cleaned from the mycelium and then oven dried for 24 hours at the temperature of 103±2 °C until the constant mass was reached, and then weighed. The mass loss

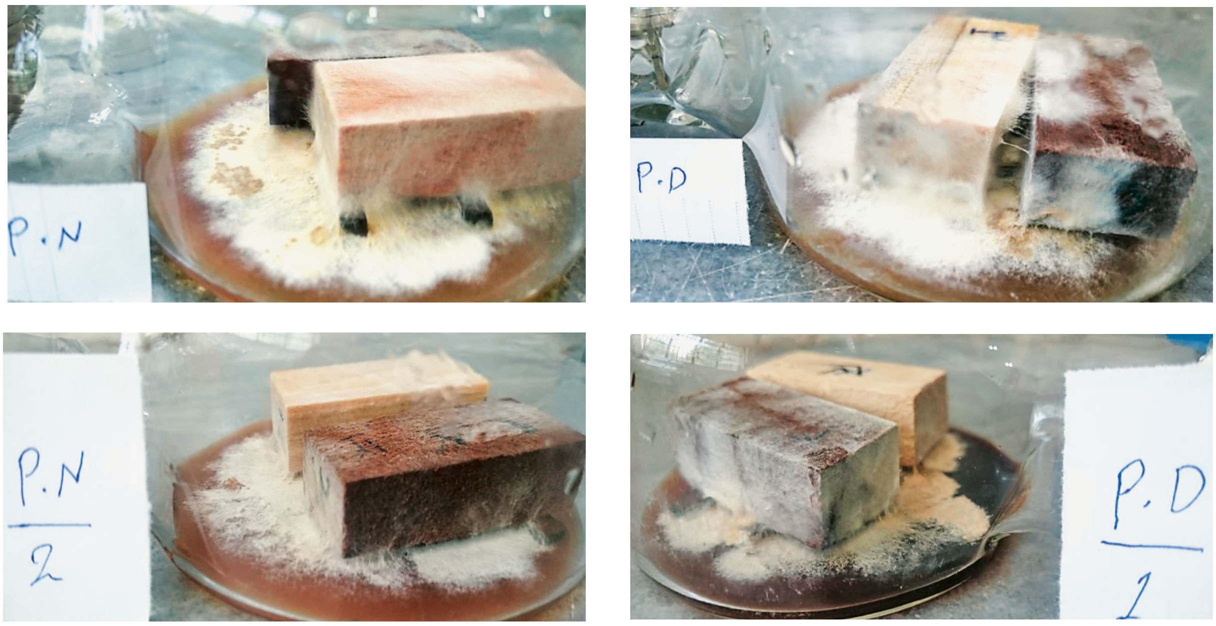

Figure 1 Control and heat-treated specimens after exposure to brown rot fungi (Up: after 3 weeks, Down: after 16 weeks)
Slika 1. Kontrolni i toplinski modificirani uzorci nakon izlaganja gljivama smeđe truleži (gore: nakon tri tjedna, dolje: nakon 16 tjedana)

and the repellent effect of thermal modification on *MLF* of each specimen were calculated according to equation 1 and 2, respectively:

$$M.L = ((D_1 - D_2)/D_1) \times 100 \quad (1)$$

Where:

M.L – mass loss (%)

D_1 – oven dry mass of specimen before exposure to fungi (g)

D_2 – oven dry mass of specimen after exposure to fungi (g)

$$R.E = ((M.L_1 - M.L_2)/ M.L_1) \times 100 \quad (2)$$

Where:

R.E – repellent effect of thermal modification on *MLF* (%)

$M.L_1$ – mass loss of control specimen caused by fungi (%)

$M.L_2$ – mass loss of heat-treated specimen caused by fungi (%)

In this research, only the properties that could be determined based on the specimens that can be placed in Kolle flasks, based on standard dimensions, were considered. The physical and mechanical properties of control and heat-treated specimens were tested according to ASTM D 143-09. Also, the standard ASTM D256 was used to measure impact strength, because the dimensions of the specimens in this standard were suitable to be placed in the Kolle flask. Moreover, the standard EN 113 was used to evaluate bio-durability. It should be noted that 6 replications were considered for each parameter. The obtained data from the tests were statistically analyzed based on One-Way ANOVA method. This method was used to determine any statistically significant differences between the means of the two groups (control and modified specimens) for each wood species separately.

3 RESULTS AND DISCUSSION
3. REZULTATI I RASPRAVA

In this research, the physical and mechanical properties of two wood species (*P. deltoids* and *P. nigra*) were evaluated in two stages: the first after thermal modification and the second after fungal degradation to show the effect of heat treatment on fungal functionality as well as physical and mechanical properties. Table 1 summarizes the results of statistical analysis of One-Way ANOVA test for physical and mechanical properties of two poplar wood species, specifying significant levels. The numbers presented in Table 1 show whether the heat treatment had a significant effect on the investigated properties.

3.1. Laccase activity assessment
3.1. Procjena pojave lakaze

The results of laccase evaluation of specimens after 16 weeks of exposure to *C. puteana* showed that the laccase value in controls was lower than in heat-treated specimens in both poplar wood species (Figure 2). In fact, the reduction of this enzyme in controls compared to thermo-wood indicates greater consumption and effectiveness of this enzyme in untreated specimens. As heat treatment reduces the amount of hemicellulose and holocellulose, hydroxyl groups, which are an active site for chemical reactions, are reduced. As a result, the activity and effectiveness of laccase enzyme produced by brown rot fungi in modified wood is reduced (Gaff *et al.,* 2019; Mburu *et al.,* 2007; Wentzel *et al.,* 2019). Faraz *et al.* (2003) reported that, when *Eucalyptus grandis* wood chips were exposed to *Ceriporiopsis subvermisporaon*, the greatest mass loss was directly associated with a significant reduction of laccase enzyme, which supports the results of this study.

3.2. Mass loss caused by fungi (*MLF*)
3.2. Gubitak mase zbog djelovanja gljiva (*MLF*)

The results revealed that heat treatment had a significant effect on the decay action of brown rot fungi, leading to mass loss (Table 1). The *MLF* in thermo-wood specimens were significantly lower than in control specimens. This indicates the inhibition of heat

Table 1 Summarized statistical results of One-Way ANOVA for physical and mechanical properties of treated poplar wood
Tablica 1. Sažeti statistički rezultati jednosmjerne ANOVA-e za fizička i mehanička svojstva toplinski modificirane topolovine

S.V	Wood species *Vrste drva*	After thermal modification *Nakon toplinske modifikacije*			After fungal degradation *Nakon razgradnje uzrokovane gljivama*			
		Density **Gustoća**	**Compression strength parallel to grain** *Čvrstoća na tlak paralelno s vlakancima*	**Impact strength** *Čvrstoća na udarac*	**Mass loss** *Gubitak mase*	**Density** *Gustoća*	**Compression strength parallel to grain** *Čvrstoća na tlak paralelno s vlakancima*	**Impact strength** *Čvrstoća na udarac*
Heat treatment *toplinska modifikacija*	*Populus deltoids*	0.008**	0.017*	0.025*	0.020*	0.284 ns	0.122 ns	0.016*
	Populus nigra	0.024*	0.231 ns	0.028*	0.001**	0.386 ns	0.200 ns	0.034*

ns, * and ** – Non-significant and significant at 5 % and 1 % probability levels, respectively / *nije značajno i značajno je pri razinama vjerojatnosti od 5 %, odnosno 1 %*

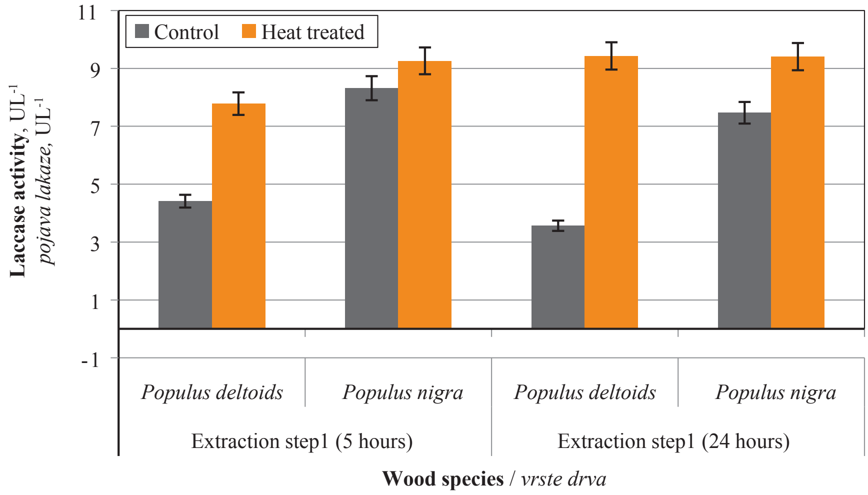

Figure 2 Laccase activity in heat-treated and control wood specimens during decay by *Coniophora puteana*
Slika 2. Pojava lakaze u toplinski modificiranim i kontrolnim uzorcima drva tijekom truljenja uzrokovanoga gljivom *Coniophora puteana*

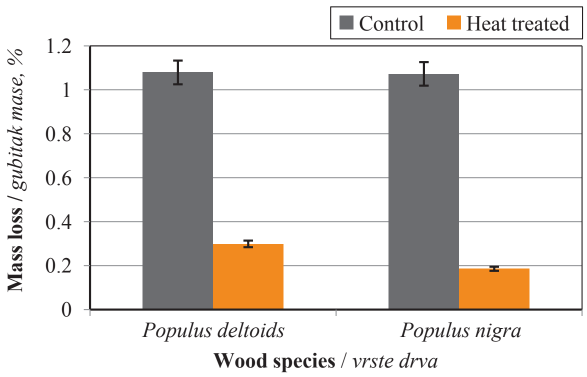

Figure 3 Effect of thermal modification on MLF in two wood species
Slika 3. Utjecaj toplinske modifikacije na MLF za dvije vrste drva

treatment on fungal activity (Figure 3). As indicated in Figure 3, the repellent effect of thermal modification on MLF obtained 72.28 and 82.69 % in *P. deltoids* and *P. nigra*, respectively. On the other hand, as shown in Figure 2, laccase activity in heat-treated specimens is greater than in controls, which is a sign of thermo-wood resistance against fungal degradation. Considering the substrate-enzyme interactions, it seems that in the control specimens, laccase enzyme had more interaction with the substrate and therefore the mass in these specimens was reduced to a greater extent compared to the treated specimens. According to the results of this study, there are other reports that show the deep adsorption of laccases in substrates suitable for their degradation (Tu *et al.,* 2009). Moreover, improvement of bio-durability of thermo-wood against fungal degradation is related to the reduction of hydroxyl groups due to heat treatment (Mburu *et al.,* 2007).

3.3. Density
3.3. Gustoća

This physical property was evaluated as follows.

3.3.1 After thermal modification
3.3.1. Nakon toplinske modifikacije

The results showed that heat treatment had a significant effect on the density (Table 1), so that the thermo-wood process reduced the density of both wood species (Figure 4). The reduction of density due to heat treatment in *P. deltoids* and *P. nigra* was 8.81 and 5.17 %, respectively. The change of wood chemical structure at high temperature causes mass loss, which leads to density decrease. The reduction of density due to heat treatment is related to destruction and change of chemical structure of wood, which intensifies at high temperatures (Militz, 2002).

3.3.2 After fungal degradation
3.3.2. Nakon razgradnje gljivama

After density assessment of control and heat-treated specimens in section 3.3.1, these specimens were exposed to brown rot fungus *C. puteana.* The results

Table 2 Average reduction values of physical and mechanical properties due to fungal degradation
Tablica 2. Prosječne redukcijske vrijednosti fizičkih i mehaničkih svojstava zbog razgradnje gljivama

Property *Svojstvo*	**Density** / *Gustoća, %*		**Compression strength parallel to grain, %** *Čvrstoća na tlak paralelno s vlakancima, %*		**Impact strength, %** *Čvrstoća na udarac, %*	
Wood species / *vrste drva*	*P. deltoids*	*P. nigra*	*P. deltoids*	*P. nigra*	*P. deltoids*	*P. nigra*
Control / *kontrolni uzorak*	3.27	5.94	6.04	11.71	4.71	7.45
Thermo-wood / *toplinski tretirano drvo*	2.23	5.52	3.69	9.28	6.41	21.88

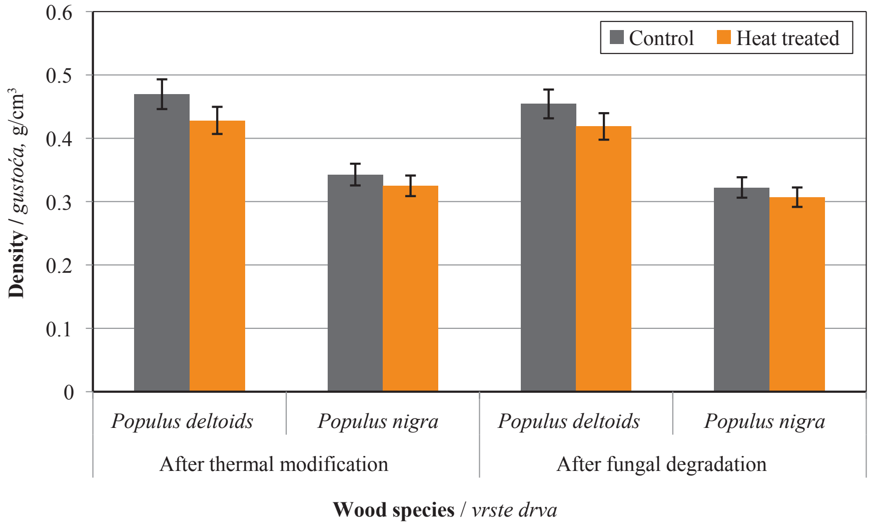

Figure 4 Effect of thermal modification on wood density and its variation under fungal attack
Slika 4. Utjecaj toplinske modifikacije na gustoću drva i promjene gustoće zbog napada gljiva

showed that heat treatment had no significant effect on fungal functionality in reduction of density in both poplar wood species (Table 1). As shown in Figure 4, the density difference between the control and thermo-wood specimens is little in both poplar species. However, heat treatment has been able to reduce the activity of brown rot fungus in both wood species, so that the density reduction due to fungal action in thermo-wood specimen was less than in controls (Table 2). This indicates that heat treatment has a repellent effect on fungal activity.

3.4. Compression strength parallel to grain
3.4. Čvrstoća na tlak paralelno s vlakancima

This mechanical property was evaluated as follows.

3.4.1 After thermal modification
3.4.1. Nakon toplinske modifikacije

The results showed that thermal modification had no significant effect on the compression strength parallel to the grain of *P. nigra* but it did in *P. deltoids* (Table 1). However, as indicated in Figure 5, heat treatment has improved this mechanical property in both wood species, so that the value of compression strength parallel to the grain in *P. deltoids* and *P. nigra* was 27.46 and 11.21 %, respectively (Figure 5). As a matter of fact, wood chemical structure changes at high temperature during thermal modification. Consequently, heat treatment causes cross-linking of lignin as well as increasing of lignin and cellulose values in wood structure, which leads to the improvement of compression

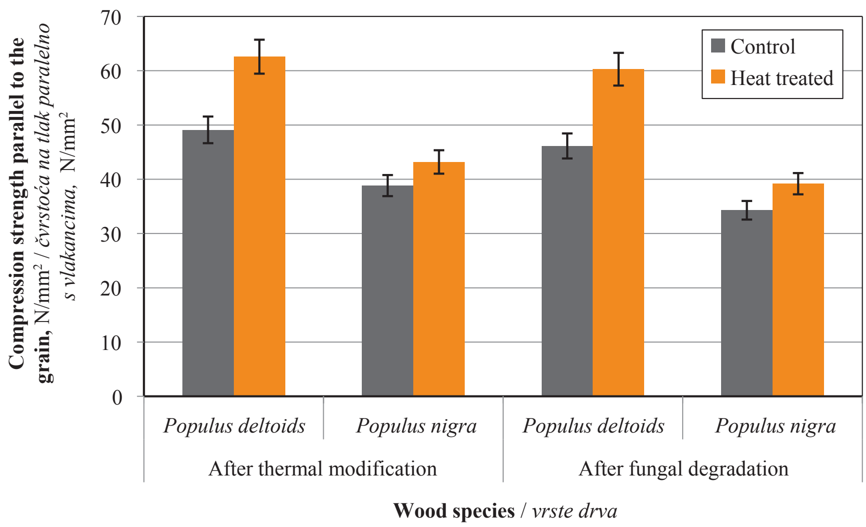

Figure 5 Effect of thermal modification on compression strength parallel to grain and its variation under fungal attack
Slika 5. Utjecaj toplinske modifikacije na čvrstoću na tlak paralelno s vlakancima i promjene čvrstoće zbog napada gljiva

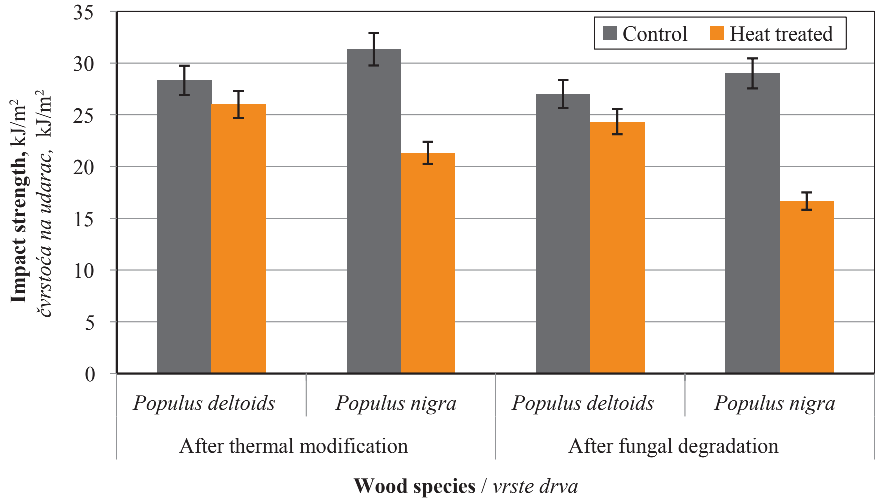

Figure 6 Effect of thermal modification on impact strength and its variation under fungal attack
Slika 6. Utjecaj toplinske modifikacije na čvrstoću na udarac i promjene te čvrstoće zbog napada gljiva

strength parallel to the grain. In other words, the change of wood chemical components due to the heat treatment is an effective agent for changing physical and mechanical properties of wood (Gaff *et al.,* 2019; Wentzel *et al.,* 2019).

3.4.2 After fungal degradation
3.4.2. Nakon razgradnje gljivama

The results revealed that heat treatment had no significant effect on fungal efficiency in terms of compression strength parallel to the grain (Table 1). However, comparison of the results in Figure 5 shows that this kind of modification was able to reduce the activity of brown rot fungi in both wood species. Consequently, the reduction of compression strength parallel to the grain due to fungal activity in thermo-wood specimen was less than that in the control, which indicates a repellent effect of heat treatment on fungal activity (Table 2). As shown in Figure 2, laccase activity in heat-treated specimens was more than that in the control group, which indicates bio-durability of thermo-wood. Heat treatment of wood at high temperatures reduces hydroxyl groups, which leads to the reduction of enzymatic hydrolysis (Mburu *et al.,* 2007).

3.5. Impact strength
3.5. Čvrstoća na udarac

This mechanical property was evaluated as follows:

3.5.1 After thermal modification
3.5.1. Nakon toplinske modifikacije

The results revealed that heat treatment had a significant effect on impact strength of both wood species (Table 1). Thermal modification had negative effect on this mechanical property in both species, as shown in Figure 5. The reduction of impact strength was 8.24 and 31.91 % in *P. deltoids* and *P. nigra*, respectively (Figure 6). The reduction of impact strength of thermo-wood might be related to brittle structure of heat-treated specimens. In fact, the reduction of this mechanical property is directly related to chemical structure changes, while mass loss of specimens occurs due to heating (Kaygin *et al.,* 2009).

3.5.2 After fungal degradation
3.5.2. Nakon razgradnje gljivama

The results showed that heat treatment had a significant effect on fungal action in terms of impact strength (Table 1). Although thermal modification can reduce the activity of the brown rot fungi, the reduction of impact strength in thermo-wood exposed to brown rot fungi was greater than in control group (Table 2). The effect of heat treatment on structural changes of wood as well as destruction of cellulose and holocellulose by brown rot fungi can be effective agents in the reduction of impact strength of thermo-wood specimens.

4 CONCLUSIONS
4. ZAKLJUČAK

The results of the present study revealed that the degradation of two poplar wood species (*P. deltoids* and *P. nigra*) by *C. puteana* could be affected by thermal modification. Although the activity of *C. puteana* and laccase production occurred in both control and heat-treated specimens, the lowest detectable laccase levels were observed in controls, coinciding with the highest mass loss. Considering the substrate-enzyme interactions, it seems that in the control specimens, laccase enzyme had more interaction with the substrate and therefore the mass in these specimens was reduced to a greater extent compared to the treated specimens. Therefore, the result of laccase activity assessment also showed that the laccase value in controls was less than that in modified specimens. Overall, the results revealed that thermal modification improves bio-durability of both poplar wood species; however, it reduces some mechanical properties. On the other hand, this kind of modification prevents the reduction of mechanical properties due to biological degradation.

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