INFLUENCE OF FUNGAL DECAY ON CHEMI-MECHANICAL PROPERTIES OF BEECH WOOD (*FAGUS ORIENTALIS*)

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The mechanical and chemical properties of beech (*Fagus orientalis*) heartwood and sapwood after incubation with rainbow white-rot fungus (*Coriolus versicolor*) (for 14 weeks by the Kolle flask method) were determined. Also, the weight loss of the samples was measured after exposure to the white-rot fungus. The results indicated that the highest weight loss (36.08%) was observed for sapwood samples and the lowest (32.98%) occurred in heartwood samples. The highest and the lowest compression strength parallel to the grain were noted in heartwood and sapwood decayed samples, respectively. The highest and the lowest Brinell hardness perpendicular to tangential and radial surfaces were observed in heartwood and sapwood decayed samples, respectively. The chemical analysis showed that the major changes occurred in lignin content, i. e. 54.4% and 47.10% lignin reduction for sapwood and heartwood, respectively. Also, alcohol-benzene solubility decreased, while 1% sodium hydroxide solubility and hot water solubility increased in the decayed wood samples.

Keywords: beech, rainbow white-rot fungus, heartwood, sapwood, lignin

INTRODUCTION

Changes in the chemical composition of wood, during biological decay, cause measurable reduction in weight and wood strength. The properties of wood are directly related to the chemical composition of wood, therefore yielding a positive or negative effect depending on end-use.[1] The mechanical properties of wood, including compression strength, bending stiffness, and torsion strength (which is used to measure shear strength) are adversely affected by wood decay fungi.[2] In timber, lignin plays the role that cement plays in concrete and is associated with compressive strength, while cellulose acts like sand and rubble and is linked with tensile and bending strength. Changes in the lignin and cellulose contents or topochemistry will undoubtedly affect these properties.[1] Winandy *et al*.[3] showed that a close relationship between degradation of hemicellulose components, such as arabinose and galactose, and the reduction of wood strength occurs. Changes in chemical composition appear to be similar in both the biological and chemical degradation system. Understanding the relation between chemical composition and strength loss may aid in developing a model to predict strength loss caused by a variety of biological, chemical and thermal agents. The sapwood of all species contains less extractives than the heartwood, while heartwood exhibits a greater degree of natural durability against microorganisms in most timbers.[4]

Wood-degrading fungi often belong to the Basidiomycetes class, which predominantly cause white-rot decay in wood. Apparently, white-rot fungi play a dominant role in the decomposition of lignocellulosic substrate and may be also involved in the decay of wood used for construction and building materials.[5] Furthermore, they have a common capacity to degrade lignin, as well as other wood components.

White-rot fungi also have a common ability to produce extra-cellular enzymes, which oxidize phenolic compounds related to lignin.[6]

An economic loss resulting from wood decay is actually due to reduction in the quality grade of timber and the requirement to replace the materials in the affected structural applications.[7]

In the present investigation, heartwood and sapwood of beech (*Fagus orientalis*) were exposed to *Coriolus versicolor* as a white-rot fungus to study the effect of the fungus on the mechanical properties, such as compression strength parallel to the grain, Brinell hardness perpendicular to tangential and radial surfaces, and chemical ones, including lignin and cellulose content of wood.

EXPERIMENTAL

Materials

All beech trees (*Fagus orientalis*) were randomly selected, taking into account stem straightness and absence of obvious decay, and beech wood was selected according to TS 2476,[8] as defect-free, clear, and normally grown (without zone lines, reaction wood, decay and insect damage, or fungal infection) wood from a forest in the north eastern city of Bandar Gaz in Iran. The trees were grown in a temperate climate at the elevation of 1230 meters above sea level with geographical direction of 36º31′ N and 53º55′ E. Three trees were cut from one single site (they were approximately the same age, 160 years, and had the same height, about 30 meters). The mean annual precipitation and temperature of this area ranges between 600-900 mm and 11.9-15.3 ºC, respectively. From each tree, a sample disc of 30 cm in length was taken at breast height level (1.3 m).

Preparation of test samples

Wood samples were randomly selected. The boards of 50 mm thickness were tangentially sawn and stored at 20 ºC and 65% relative humidity for 2 months to reach 12% final moisture content. The samples were then prepared separately from two portions of the trees – sapwood (SW) and heartwood (HW), to measure variations in the mechanical and chemical properties and natural durability (sapwood and heartwood recognition was done by color difference, though the color difference was very little). The heartwood samples were cut from a zone free of red heart and defect. Sapwood was cut from an area of 30-40 annual rings near the bark. Samples with the dimensions of 50 x 25 x 15 mm were prepared for weight loss and Brinell hardness (BH) measurement, and of 60 x 20 x 20 mm (longitudinal, tangential and radial directions) for compression strength (CS) tests, according to British Standard 838:1961[9] and ASTM D143-94[10] standard, respectively.

Decay test

The decay test was conducted in accordance with BS 838:1961,[9] after exposure to *Coriolus versicolor* (*C. versicolor*), for 14 weeks, by applying the Kolle flask method. Prior to inoculation, malt extract agar was used as an agent for the fungus culture. The agar medium was sterilized in an autoclave at a temperature of 120 ºC and pressure of 1.2 atm for 20 minutes. Subsequently, every Kolle flask was inoculated by a small plug of *C. versicolor* and set in 25 ºC for 7 days. During this time, its mycelium covered the entire surface of the agar. Next, sapwood and heartwood of beech specimens (50 x 25 x 15 mm and 60 x 20 x 20 mm) were dried at 103 ± 2 ºC for 24 hours, and oven-dried weight was determined. In the following stage, the samples were placed on the top of two small glass legs in Kolle flask to avoid contact of specimens with fungal mycelia (for each mechanical test, 20 samples of sapwood and 20 samples of heartwood were prepared).

Both heartwood and sapwood were incubated at 25 ºC and 70% relative humidity until the samples were acclimatized by the *C. versicolor* and were then transferred to an incubator for 14 weeks (under the same conditions). The samples were removed from the incubator and fungal mycelia were removed from the surface of the specimens. The samples were then placed in an oven at 103 ± 2 ºC for 24 hours to reach to constant weight and to determine weight loss for each individual sample, as follows:

$$\text{Weight loss} = [(W1-W2)/W1] \times 100$$

where W1 is the oven-dry weight of sample prior to exposure and W2 is the oven-dry weight of sample after exposure to fungus. To classify and determine durability, Findlay classification[11] was used in relation to wood species from a natural durability standpoint, as described by Olfat *et al*.[7] According to this method, the natural durability of all wood species are grouped according to the weight loss (Table 1).

Table 1
Typical service life criteria for the evaluation of durability on wood species and mass loss criteria of laboratory durability test (wood blocks)[11]

Natural durability or decay resistance class	Laboratory tests mass loss (%) Findlay (1985)
1. Very durable	0%, or negligible
2. Durable	<5%
3. Moderately durable	6-10%
4. Non-durable	11-30%
5. Perishable	>30%

Mechanical testing

The compression strength and Brinell hardness tests were carried out according to ASTM D2017-81[12] (for each portion of wood, i.e. sapwood and heartwood), used in the experiment, by a Schenck universal testing machine.

The test measures the force required to push a steel ball with a diameter of 11.28 millimeters (0.444 inches) into the wood to a depth of half the ball's diameter (the diameter was chosen to produce a circle with an area of 100 square millimeters). In the ASTM standardized test, the results were expressed in units of force (N or KN).

Chemical analysis

The decayed and control samples of beech heartwood and sapwood, which had been used in the mechanical testing, were milled and sieved using a 40 mesh screen. The wood meals (powder) from both sapwood and heartwood samples were prepared for chemical analysis, according to TAPPI T 264 om-88,[13] individually. The chemical analysis of the solubility values for 1% NaOH, hot water, and alcohol-benzene were done according to TAPPI T212 om-93,[14] TAPPI T207 om-93,[15] and TAPPI T204 cm-97[16] standard methods, respectively. The cellulose content was determined according to the Cross and Bevan method, as described by Oluwadare and Asagbara,[17] while the lignin content was determined according to TAPPI T222 om-98[18] standard method, using the extractive-free wood meal.

Statistical procedure

Statistically significant differences in the mean strengths, chemical components content, and weight loss values between experimental methods for heartwood and sapwood against white-rot fungus were tested using the T-test method. The data obtained from the T-test were used to determine the least significant differences between methods. All comparisons were made at two statistical levels of $p < 0.05$ and $p < 0.01$. Statistical analysis was conducted by the SPSS software.

RESULTS AND DISCUSSION

The results acquired from the decay resistance and mechanical properties tests on sapwood and heartwood of *F. orientalis* after incubation with *C. versicolor* fungus for 14 weeks are summarized in Table 2. The mean weight loss values obtained from the decay test were 36.08% and 32.98% for sapwood and heartwood samples, respectively (Figure 1). The results of the T-test (Table 3) indicated that the mentioned white-rot fungus had a significant effect on the weight loss of sapwood and heartwood samples ($p < 0.05$). Because the mean weight loss of decayed heartwood and sapwood was above 30%, according to Findlay classification table (Table 1), both heartwood and sapwood samples of *F. orientalis* were placed into the perishable (5) wood class. Olfat *et al.*[7] reported that the weight loss of *F. orientalis* wood samples was of 42.20% after incubation with *C. versicolor* fungus for 16 weeks, therefore, the weight loss percent of the lignin content in the wood samples was well established.

Table 2

Average value for heartwood and sapwood decay resistance and mechanical test results of *F. orientalis*

Test samples	Weight loss (%)	Brinell hardness (N)		Compression strength (MPa)
		Tangential	Radial	
Heartwood (control)	0.0	5746.1	5597.9	55.3
Decayed heartwood	33.0	868.4	774.6	17.8
Sapwood (control)	0.0	5571.8	5419.5	50.9
Decayed sapwood	36.1	703.9	620.1	15.6

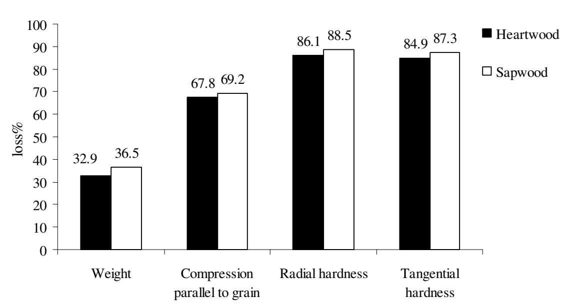

Figure 1: Mean reduction values of weight and mechanical strength

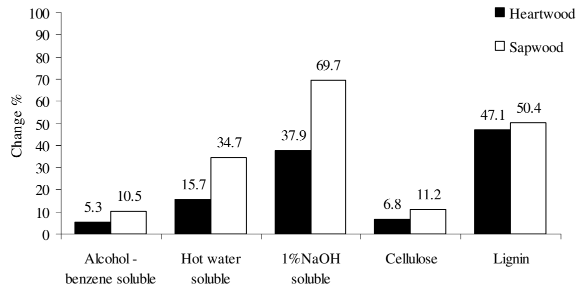

Figure 2: Mean reduction values of chemical components

Table 3
T-test analysis for decay test and compared mean mechanical test results of *F. orientalis* decayed heartwood and sapwood samples

Test type	Test samples	Mean	Number	Standard deviation	Degree of freedom	Significant	T
Weight loss (%)	Decayed heartwood	32.98	20	0.70	38	0.031	1.94
	Decayed sapwood	36.08	20	0.72			
Compression strength parallel to the grain (MPa)	Decayed heartwood	17.79	20	3.13	38	0.018	2.47
	Decayed sapwood	15.60	20	2.35			
Brinell hardness perpendicular to tangential surface (N)	Decayed heartwood	868.4	20	176.81	38	0.000	3.55
	Decayed sapwood	703.9	20	107.13			
Brinell hardness perpendicular to radial surface (N)	Decayed heartwood	774.6	20	156.09	38	0.000	3.9
	Decayed sapwood	620.1	20	83.66			

The mean compression strength parallel to the grain values of the control heartwood and control sapwood samples were calculated as 55.3 MPa and 50.9 MPa, respectively, while the mean compression strength parallel to the grain values of the decayed heartwood and decayed sapwood samples were of 17.8 MPa and 15.6 MPa, respectively.

The results of T-test (Table 3) indicate that fungal decay had a significant effect on compression strength parallel to the grain of sapwood and heartwood samples of *F. orientalis* ($p < 0.05$).

The Brinell hardness values perpendicular to tangential and radial surfaces of the control and decayed heartwood and sapwood samples were compared. The Brinell hardness values perpendicular to tangential surface of the control and decayed samples for heartwood and sapwood were 5746.1 N, 5571.8 N, 868.4 N, and 703.9 N, respectively (Table 2). According to the results of the T-test (Table 2) applied to these variables, the mean values of Brinell hardness perpendicular to radial surface of the control and decayed samples for sapwood and heartwood were 5597.9 N, 5419.5 N, 774.6 N, and 620.1 N.

The Brinell hardness values of the heartwood in the control and decayed specimens were higher than those of the control and decayed sapwood. A statically significant difference was found between both decayed heartwood and sapwood in radial and tangential directions to the levels of 0.01 and 0.05 (Table 3). Table 2 shows that tangential Brinell hardness was higher than radial Brinell hardness both in the control and decayed specimens. Since the sapwood of *F. orientalis* contains less extractive material than the heartwood, the heartwood exhibits a greater degree of natural durability and mechanical properties against *C. versicolor*.

The activity of *C. versicolor* fungus caused loss in the weight, strength, mechanical properties of the samples of decayed heartwood and sapwood after 14 weeks of exposure (Figure 1). Similarly, Hosseini Hashemi *et al.*[19] reported such decreases in the mentioned mechanical properties of poplar wood samples both in the untreated and treated ones with boric acid preservatives. In their study,[19] mechanical properties of *Populus nigra* such as compression parallel to grain, Brinell hardness perpendicular to tangential and radial directions, and weight loss were reported to decrease due to *C. versicolor* fungus exposure for 14 weeks down to 59.07%, 78.80%, 68.18% and 28.6%, respectively.

Chemical analysis

The results of the chemical analysis performed on the control samples of *F. orientalis* and also on the specimens taken after incubation with *C. versicolor* fungus for 14 weeks are given in Table 4. The mean value of alcohol-benzene soluble extractives content in the control heartwood samples (2.85%) was higher than that of the control sapwood samples (2.29%). The mean extractives content value decreased in decayed heartwood and sapwood down to 2.70% and 2.05% after incubation.

Figure 2 shows that the decrease in the alcohol-benzene solubility of the extractives in the decayed sapwood samples (10.48%) was more pronounced than that in decayed heartwood (5.26%). A similar observation was made by Silk *et al.*[20] on the extractives soluble in alcohol-benzene of beech wood during fungal degradation: wood components were degraded by fungi, styrel esters and waxes being hydrolyzed into fatty acid and sterol moieties, which serve as a source of carbon for the growth of the fungi.

This could probably explain the decrease in the content of extractives soluble in alcohol-benzene. The extractives soluble in 1% NaOH and hot water in the control and decayed heartwood and sapwood samples were recorded as 22.85%, 20.46%, and 31.50%, 34.71%, respectively. Thus, a 37.85% and 69.65% increase of the extractives soluble in 1% NaOH, and a 15.73% and 34.65% increase of the extractives soluble in hot-water were observed for decayed heartwood and sapwood samples after 14 weeks of fungal exposure time (Fig. 2). Such an increase is similar to that reported by Istek,[21] who noted an increase in 1% NaOH solubility and hot-water solubility of extractives in *Populus tremula* (*P. tremula*) due to exposure to the *Phanerochaete chrysosporium* (*P. chrysosporium*) fungus for 8 weeks of 46% and 10%, respectively.

The fact that the solubility of cellulose in 1% NaOH increased as a result of white-rot fungus exposure indicates that small (soluble) fragments were produced during depolymerization of cellulose and hemicelluloses.

The degradation of lignin is more pronounced than that of cellulose. A reduction from 24.01% to 12.71% (Table 4) for heartwood samples and from 21.11% to 10.47% for sapwood samples was recorded in the experiment. The greatest decrease in lignin content occurred in the decayed sapwood and heartwood with an amount of about 47.06% and 50.40% relative to their control samples (Fig. 2). The amount of lignin reduction is significant in pulp and paper making, since the goal of pulping and bleaching is to reduce and/or remove lignin from the cell wall to release the fiber.

Table 4
Chemical analysis of heartwood and sapwood samples of *F. orientalis* before and after incubation with *C. versicolor* white-rot fungus

Chemical components	Heartwood		Sapwood	
	Control	Decayed	Control	Decayed
Alcohol-benzene soluble extractives (%)	2.85	2.70	2.29	2.05
Hot-water soluble extractives (%)	5.34	6.18	4.70	6.33
1% NaOH soluble extractives (%)	22.85	31.50	20.46	34.71
Cellulose (%)	39.12	36.46	40.30	35.79
Lignin (%)	24.01	12.71	21.11	10.47

Istek[21] stated that 8 weeks of fungal treatment on *P. tremula* with *P. chrysosporium* resulted in an 18.24% decrease in lignin. Such decreases are similar to those reported by Hakala *et al.*[22] In this report, a 39% decrease in the amount of lignin (*Norway spruce* wood) was recorded, which is probably due to *Physisporinus rivulosus* fungus exposure for 10 weeks. This difference may be the result of the size of the materials and fungus species type used in the experiment.

The amount of cellulose for control and decayed (after 14 weeks) heartwood and sapwood samples was of 39.12%, 40.30% and 36.46%, 35.75%, respectively, the weight loss for heartwood and sapwood samples being of about 6.79% and 11.19%, respectively (Fig. 2). In addition, the decrease of the amount of sugar in the wood is due to fungal treatment. Regarding the treated samples, the fungus affected some important changes in the cell structure components of the wood, i.e. this structure was weakened and the cell walls were thinned and, by using a microtome, the layers of the treated samples were easily separated.[5]

Kleman *et al*.[23] reported that the DPn (number-average degree of polymerization), DPw (weight-average degree of polymerization) and DPv (degree of polymerization inferred from viscosity) of cotton cellulose reduced due to *P. chrysosporium* white-rot fungus exposure from 1057, 2009 and 1925 to 984, 1570 and 1511, respectively, and a weight loss of cellulose was observed, whereas the solubility in 1% NaOH increased (40%) after 7 weeks of exposure to the fungus. Their results indicate changes in the molecular size distribution of cellulose during decay by white-rot fungus, suggesting that the attack generated several hundred fragments of glucose units along with lower DP (Degree of polymerization) values. Therefore, white-rot fungus attacks the cellulose microfibrils, consuming the cellulose as it is degraded.

CONCLUSION

The heartwood and sapwood of *F. orientalis* present low decay resistance when exposed to white-rot fungus (*C. versicolor*). However, the heartwood shows higher resistance, compared to the sapwood. The toxic extractives in the heartwood of *F. orientalis* have a slight effect on natural durability against the *C. versicolor* fungus.

Chemical analysis showed that cell wall components, such as lignin and cellulose, decreased by 50.4%, 47.1% and 11.2%, 6.8% for decayed sapwood and heartwood samples respectively, after fungus treatment for 14 weeks. As a result of continuous decay progress, the wood becomes discolored losing more lignin rather than cellulose. The result is related to the employed extra cellular enzymatic action of white-rot fungi, selectively removing lignin and allowing cellulose fibers to be obtained. The lignin loss caused the weight loss and strength loss of wood.

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