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Mechanical properties and chemical composition of beech wood exposed for 30 and 120 days to white-rot fungi

Abstract: The effects of exposing specimens of Oriental beech [*Fagus sylvatica* subsp. *orientalis* (Lipsky) Greuter and Burdet] to the white-rot fungi *Pleurotus ostreatus* (Jacq.: Fr.) Kummer and *Trametes versicolor* (L.: Fr.) Pilát strain 325 have been studied concerning the mechanical properties and chemical composition in terms of carbohydrates, cellulose, and lignin. Biological decay tests were carried out in accordance with the EN 113 standard specifications for 30 and 120 days. *P. ostreatus* had nearly the same deteriorating effects on the mechanical properties and chemical composition as that caused by *T. versicolor*. High and significant correlations were found between some mechanical properties with chemical components; for instance, the correlation coefficient (R^2) between impact bending and carbohydrate content was about 0.96. The changes of components influence the various mechanical properties to a different degree. Incipient fungal decay caused severe changes for impact bending and carbohydrate loss. Several other properties declined at 120-day exposure time, such as the hardness, compression strength parallel to grain, and cellulose and lignin losses.

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Keywords: cell-wall components, chemical compositions, duration of exposure, fungal degradation, mechanical properties

DOI 10.1515/hf-2014-0057
Received February 23, 2014; accepted September 26, 2014; previously published online October 25, 2014

Introduction

Significant changes occur in the chemical composition of wood cell walls during incipient exposure to fungal attack (Schmidt 2006; Maresi et al. 2013). The changes also cause deteriorated mechanical properties even before a pronounced weight loss occurs (Curling et al. 2000). A close relationship between the degradation of hemicelluloses and worsening mechanical properties is well documented (Wilcox 1978; Winandy and Morrell 1993). The correlation is high between the changes in chemical composition and the strength properties of solid woods after treatment with fire retardants and preservatives (Winandy 1995; Winandy and Lebow 1996; Karimi et al. 2013). A more comprehensive knowledge on the relationship between chemical composition and mechanical losses at different stages of decay can provide a better concept for wood-preserving strategies and choosing an effective preservative (Gosh et al. 2012).

The present study aimed at analyzing the chemical composition of beech wood following a short (30 days) and a long (120 days) exposure time to two simultaneous white-rot fungi (Giles et al. 2012), namely, *Pleurotus ostreatus* (Jacq.: Fr.) Kummer and *Trametes versicolor* (L.: Fr.) Pilát strain 325. The decay patterns of these fungi species should be compared. The basic mechanical properties and essential chemical components of the unexposed control beech wood and the fungal degraded specimens should be determined, and mathematical correlations are sought for data correlation.

Materials and methods

The disks were cut from five trees of Oriental beech [*Fagus sylvatica* subsp. *orientalis* (Lipsky) Greuter and Burdet] at breast height and were then air-dried. For the fungal tests, the specimens with the nominal size of 5×2.5×1.5 cm^3 were cut from the disks in accordance with the EN 113 standard specifications. The specimens were cut for compression strength parallel to grain ($CS_{||}$) and hardness in accordance with the ASTM-D143-94 standard (nominal size of 5×2×2 and 6×2×2 cm^3, respectively). The size for unnotched impact bending specimens was 6×2×0.6 cm^3 in accordance with the ASTM-D256-04 standard. Five replicate specimens were prepared from different disks for each test. The conditioned specimens were exposed to fungi for 120 days and were then kept in a conditioning chamber [25°C and 40±3% relative humidity (RH)] for 4 weeks. The following calculations were carried out:

$$\text{Mass loss (ML\%)} = 100(M_2 - M_1)/M_1 \quad (1)$$

$$\text{Hardness (N·mm}^{-2}) = 1000 \cdot F_{max}/\pi r^2 \quad (2)$$

$$\text{Compression strength parallel to grain (CS}_{||}\text{; kN·mm}^{-2}) = F_{max}/A \quad (3)$$

$$\text{Unnotched impact bending (J×m}^{-2}) = F_{max}/A \quad (4)$$

where M_1 and M_2 are the initial weight and the weight after the fungal exposure, F_{max} is the maximum loading force, and A is the cross-sectional area of the specimens.

The specimens were dried at 103°C for 24 h and weighed with 0.01 g precision and then were then autoclaved at 120°C for 20 min. The specimens were exposed in Kolle flasks on 4.8% malt extract agar to the white-rot fungi: *P. ostreatus* (isolate 11) purified at the University of Sari[1] and *T. versicolor* mentioned in the standard for comparison. The isolates were reidentified by rDNA-internal transcribed spacer sequencing (Bari 2014). The specimens were incubated at 25°C and 65% RH for 120 days. ML was determined according to the standard. Ten replicate specimens were prepared for each treatment. After incubation, mycelia were removed and the specimens were dried at 103°C for 24 h and weighed.

After the mechanical tests, the sections from the decay zone of each specimen were cut and ground to pass a 40-mesh (420-µm) screen. The wood powder served for chemical determinations according to the TAPPI standard specifications. Lignin, cellulose, and a modified method for carbohydrate analysis (Davis 1998) were carried out based on T-222 om-98 (Tappi standard 1998), T-17 wd-70 (Tappi standard 1997), and T-249 cm-85 (Tappi standard 1992) procedures, respectively.

The Klason lignin was determined according to T-222 om-98 of TAPPI standard. One gram of sawdust (free from extractives) was mixed with 72% sulfuric acid (15 ml) for 2 h at room temperature (about 20°C). The mixture was then diluted with 560 ml of boiling distilled water for preparing a 3% solution of sulfuric acid, heated under reflux for 4 h, and filtered. The residue of Klason lignin was washed with hot distilled water and dried at 103°C to constant weight. The Klason lignin content was calculated using Eq. (5):

$$\text{KL (\%)} = (\text{Sd-KL})/(\text{Sd}) \times 100 \quad (5)$$

where Sd is the dried weight of sawdust and KL is the dried weight of extracted Klason lignin.

The amount of cellulose content was determined according to T-17 wd-70 of TAPPI. Based on this procedure, 2 g of sawdust (free from extractives) were mixed with 96% EtOH (100 ml) and 65% nitric acid (50 ml). The mixture was heated under reflux for 1 h and filtered. The residue of cellulose was washed with warm distilled water and dried at 103°C to constant weight. Finally, cellulose content was calculated by Eq. (6):

$$\text{Cellulose (\%)} = (\text{Sd-EC})/\text{Sd} \times 100 \quad (6)$$

where Sd is the dried weight of sawdust and EC is the dried weight of extracted cellulose.

The carbohydrate content was determined using a two-step hydrolysis digestion in H_2SO_4 followed by high-performance liquid chromatography (HPLC) analysis using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Briefly, 80–100 mg wood powder vacuum dried at 45°C for 24 h were accurately weighed into a test tube to which 1 ml of 72% (w/w) concentrated H_2SO_4 was added. The sample was placed in a 30°C agitating water bath for 1 h. Then, 28 mg of an aqueous fucose solution were added as a fluorescent internal standard (FIS) to reach a 4% acid solution. A secondary hydrolysis was subsequently performed by placing the capped tubes in an autoclave at 120°C for 1 h. After the samples are allowed to cool to 25°C, an aliquot was removed and diluted for injection onto HPLC for wood sugar determination. Total cellulose (wt.%) was calculated as the sum of the five major wood sugars (arabinose, galactose, glucose, xylose, and mannose) based on their anhydrous molecular weights.

The sections were cut from mini-blocks (8×8×8 mm) and stained with safranin (0.5% aqueous), Astra Blue (0.3% aqueous) solution and mixed in a 1:1 ratio. Staining continued for 3–5 min (Mohebby 2003; Schweingruber et al. 2011). The stained samples were washed with sodium hypochlorite for 1–3 min and then washed with 95% and absolute EtOH for 2–3 min followed by washing with xylol for 1–2 min. Finally, the samples were washed with absolute EtOH for 1 min. A drop of Mountalan glue was placed on the section with a cover glass pressed on top. To avoid buckling of the sample, a 50 g weight was placed on the cover glass edges while the slide was drying in an oven at 60°C for 12 h. The dried sections were microscopically inspected with magnifications of ×20 to ×1000 (Olympus camera E-450).

The mini-blocks were fixed overnight with 3% glutaraldehyde in 0.1 M phosphate cacodylate buffer and then were rinsed in the same buffer three times for 30 min. Postfixation was carried out with 1% osmium tetraoxide in 0.1 M phosphate cacodylate buffer for 4 h. Fixed specimens were washed with distilled water three times for 30 min each. The dehydration with an increasing EtOH percentage (10%, 30%, 50%, 70%, 80%, 90%, and three times 100%) was carried out for 30 min (each step). Critical point drying was applied with CO_2 at 42°C. The specimens were coated with Au/Pd at 0.4 Torr pressure and 20 mA for 3 min before examination with a Jeol JSM-6400 scanning electron microscope (SEM) instrument at 15–20 kV (Mohebby 2003).

Statistical analysis was conducted by means of a SAS software program version 9.2 (2010). A two-way analysis of variance (ANOVA) was performed to discern significant differences at the 95% level of confidence. Grouping was then made between treatments by the

1 Bari, E., Taghiyari, H.R., Schmidt, O., Ghorbani, A., Aghababaei, H. (2014) Effects of nano-clay on biological resistance of wood-plastic composite against five wood-deteriorating fungi. Submitted for publication to Maderas Cienc. Tecnol. 17(2):2015. DOI: 10.4067/S0718-221X2015005000020

Duncan's test. A hierarchical cluster analysis, including dendrograms based on the Ward's method with squared Euclidean distance intervals, was carried out with SPSS/18 (2010). Cluster analysis was performed to find similarities and dissimilarities between treatments based on more than one property simultaneously. Cluster analysis has the potential to evaluate the groupings based on more than two properties in a single run. The scaled indicator in each cluster analysis shows which treatments are similar or different; lower scale numbers show more similarities, while higher ones show dissimilarities (Ada 2013). The fitted-line and scatter plots were made by the Minitab software version 16.2.2 (2010).

Results and discussion

The average MLs after 30 days of exposure were about 15.6% and 18.2% for *P. ostreatus* and *T. versicolor*, respectively (Figure 1). The corresponding ML values after 120-day exposure were 26.7% and 26.4%, respectively. The severity of fungal attack after 120 days of exposure indicates that both fungi have similar ability to decay beech wood. It is well known that the fungi in focus belong to the so-called simultaneous rot type, where carbohydrates and lignin are almost uniformly degraded at a similar degradation rate during all decay stages (Liese 1970; Schwarze et al. 2004). The hyphae grow inside the cell lumen with close contact to the tertiary wall, and excrete the degrading agents, which are active only in direct proximity of the hyphae, and the wall thickness is gradually reduced (Schmidt 2006). Thus, the microscopic images displayed similar decaying patterns for both fungi (Figures 2a and b and 3). The degree of cell wall thinning was similar in the case of both fungi in the cross-sectional view (Figure 2a and b); the accumulation of hyphae as well as the rupture of cell walls (Figure 2c and d) was also nearly the same. In the tangential view, the overall number of cavities in cell and vessel walls was also similar, although the number and diameter of cavities caused by *T. versicolor* appeared to be greater (Figure 2e and f). *Trametes versicolor* caused decay with higher severity based on the microscopic appearance. This was also evident from carbohydrate

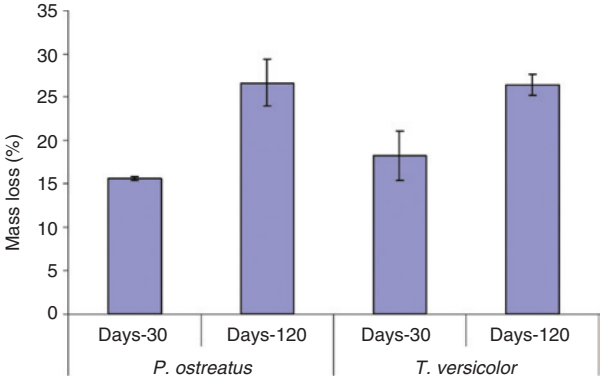

Figure 1 Average ML for beech wood specimens exposed to *P. ostreatus* or *T. versicolor* for 30 and 120 days (%) (n=5).

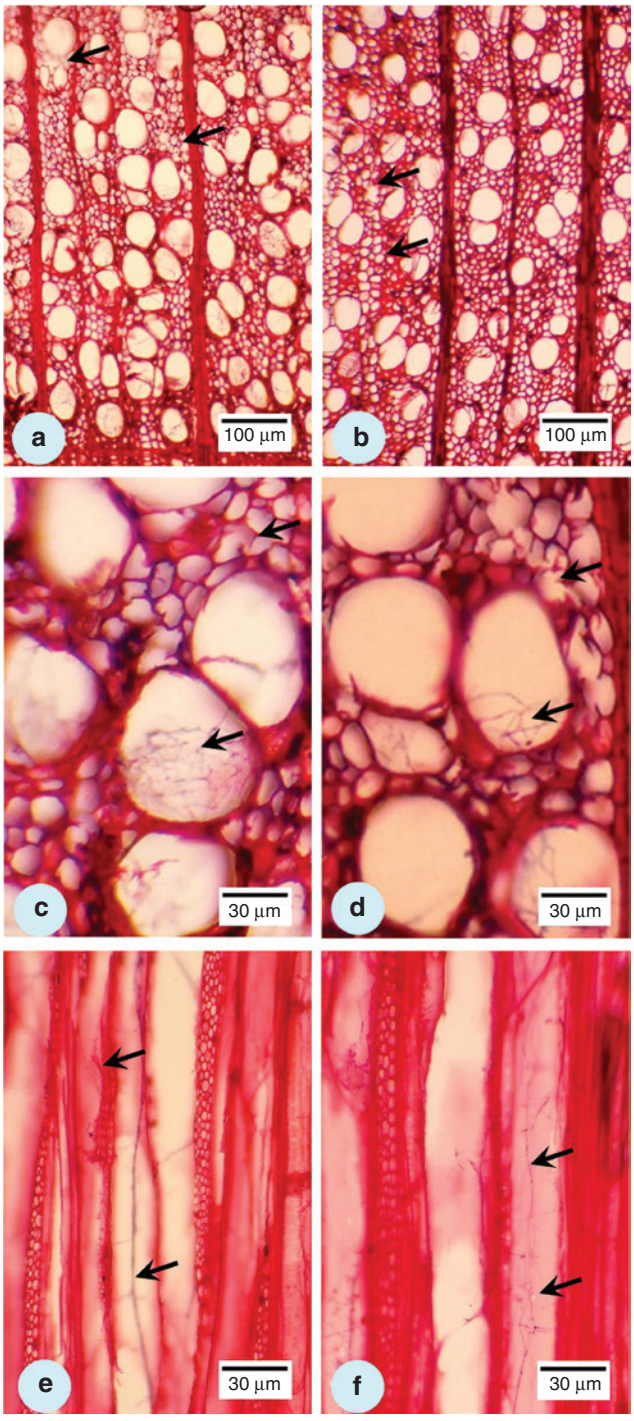

Figure 2 Microscopic images showing the decaying patterns by *P. ostreatus* (left) and *T. versicolor* (right) (a–d are cross-sectional views and e and f are tangential view).

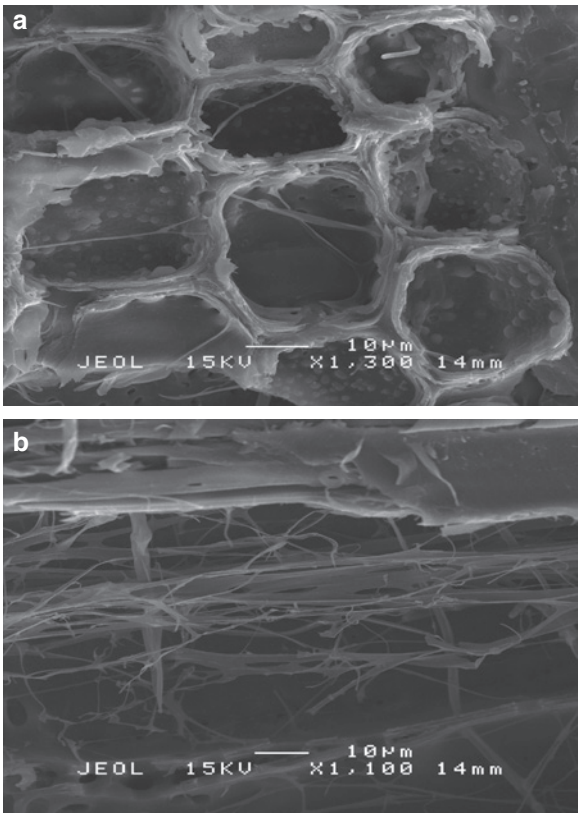

Figure 3 Field emission-SEM images of beech wood specimens infected by *P. ostreatus* (a and b).

loss as well as the mechanical properties. The chemical analysis showed that the fungi degraded the cell wall components to different degrees and rates. The carbohydrate content decreased rapidly within 30 days of fungal exposure of both fungi species (Figure 4a), while cellulose and lignin contents displayed a moderate but steady decline (Figures 4b and c).

Generally in white-rot fungi, the wood strength properties are reduced to a lesser extent than in brown-rot wood, because less cellulose is degraded at the same ML level (Schmidt 2006). An initial stage of fungal exposure resulted in a significant decrease in the modulus of rupture (MOR). Similar rapid decreasing effects on MOR and work to maximum load were previously reported to occur by the attack of brown-rot fungi (Curling et al. 2002). The rapid and severe effects were quite obvious for impact bending and carbohydrate loss after the first 30 days of fungal exposure. Other properties and chemical components, however, displayed a steady decrease as the time was extended to 120 days. The degradation curves of cellulose and lignin are similar to the declining curves of hardness and $CS_{||}$. Accordingly, under the conditions of this study, impact bending is more sensitive to fungal attack at the early stages of decay; however, hardness and $CS_{||}$ are more dependent on the total duration of fungal exposure.

Both fungi caused hardness decrement compared to the control specimens. The lowest hardness was observed in the specimens exposed to *T. versicolor* for 120 days (32% loss). The specimens exposed to *T. versicolor* displayed significantly lower hardness values after both 30 and 120 days (Figure 4d). $CS_{||}$ indicated nearly the same trend in the specimens exposed to the different fungi (Figure 4e). Both fungal species decreased rapidly the unnotched impact bending strengths (Figure 4f). The differences were not significant neither between the two fungal species nor between the two exposure times. The lowest impact bending was observed in the specimens exposed to *P. ostreatus* for 120 days (0.19 J m^{-2}), showing 95% decrease compared to the control specimens (4.09 J m^{-2}).

A comparison of curves of bending and carbohydrate content (Figures 4a and f) indicates a high similarity, that is, a high correlation between these data. A similar correlation is probably between hardness and $CS_{||}$ with cellulose and lignin losses. In Table 1 and Figure 5, the statistical data to these correlations are presented, which show the correlation among hardness, $CS_{||}$, and impact bending, on the one hand, and carbohydrates, cellulose, and lignin, on the other hand. The corresponding regression coefficients (R^2) are high. Impact bending correlates with carbohydrates with R^2=0.962; hardness and $CS_{||}$ correlate with cellulose with R^2 of 0.89 and 0.96, respectively. These results show that it would be rewarding to pay more attention to this type of correlation in the course of fungal decay.

Cluster analysis based on all mechanical properties as well as the chemical components shows that the control specimens are clustered differently to those exposed to both fungi because the control specimens are clustered on the 25th grade to other treatments based on the scale bar (Figure 6a). The differences are already obvious after 30 days of fungal exposition. The samples after 30- or 120-day exposition clustered together. This indicates that the properties of the samples (impact bending) and their chemical composition (carbohydrate content) are not too different, although the exposure time significantly affects the overall properties.

Cluster analysis based solely on hardness, impact bending, and $CS_{||}$ shows the different clusters compared to that of the control (Figure 6b). The specimens exposed to *T. versicolor* for 30 days and *P. ostreatus* for 120 days are

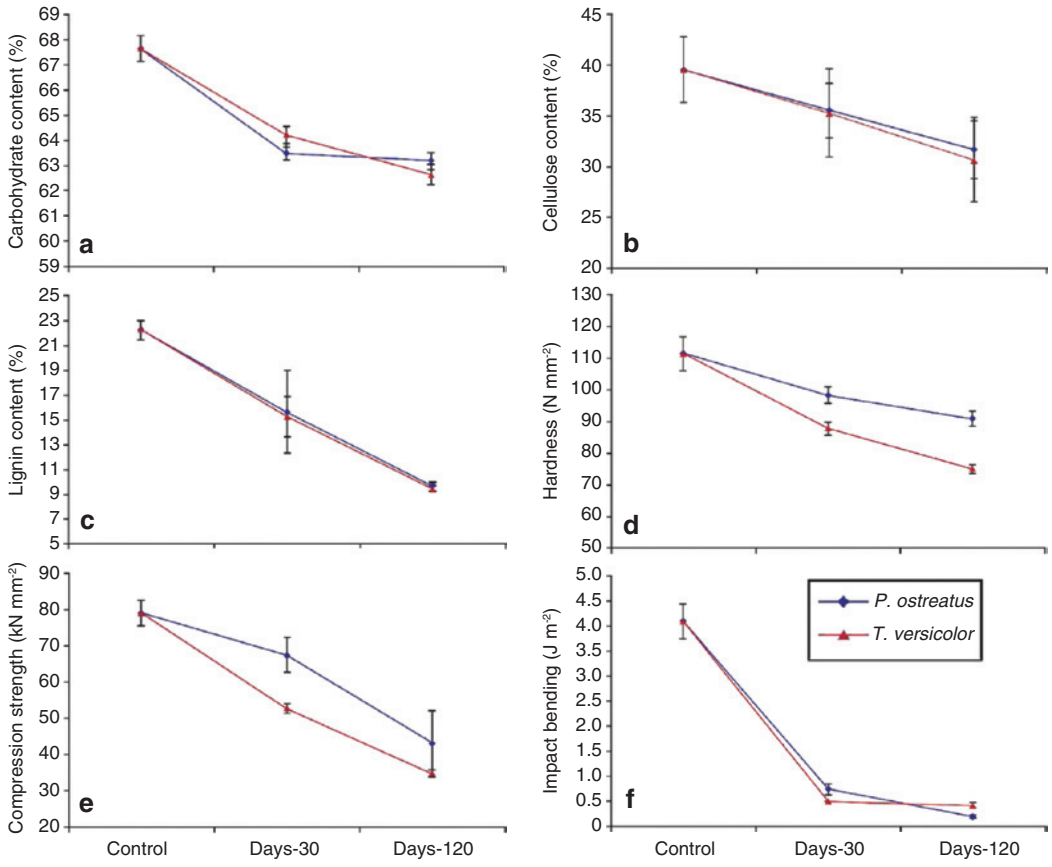

Figure 4 Average mechanical properties and chemical components for beech wood specimens exposed to *P. ostreatus* or *T. versicolor* for 30 and 120 days (n=5).

similarly clustered because of the high intensity of chemical degradation by *T. versicolor* in the first 30 days. Cluster analysis based on the contents of carbohydrates, cellulose, and lignin shows nearly the same clustering as that of the mechanical and chemical compositions (Figure 6c). Accordingly, the chemical composition seems to be more sensitive to exposure time than the metabolism of these two fungi species.

Table 1 Regression analysis among the mechanical properties and chemical compositions analyzed in beech wood specimens exposed to *P. ostreatus* or *T. versicolor* for 30 and 120 days.

					Regression coefficients (R^2)	
Properties	**Hardness**	**Impact**	**$CS_{||}$**	**Carbohydrates**	**Cellulose**	**Lignin**
Hardness	1	0.805[a]	0.944[b]	0.838[a]	0.892[b]	0.867[a]
Impact		1	0.793[a]	0.962[c]	0.841[a]	0.876[a]
$CS_{||}$			1	0.819[a]	0.960[c]	0.948[b]
Carbohydrates				1	0.908[b]	0.922[b]
Cellulose					1	0.955[c]
Lignin						1

[a]Not significant.
[b]Significant at the 5% level (two-tailed).
[c]Significant at the l% level (two-tailed).
All correlations were positive among the mechanical properties and chemical compositions.

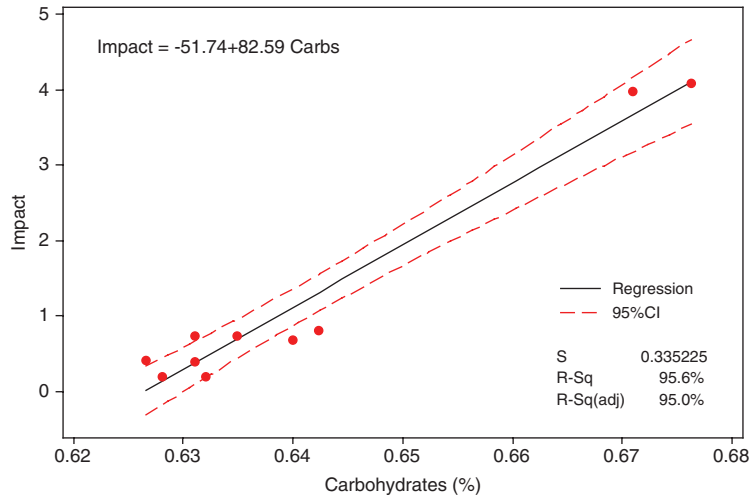

Figure 5 Fitted-line plot between unnotched impact bending and carbohydrate content.

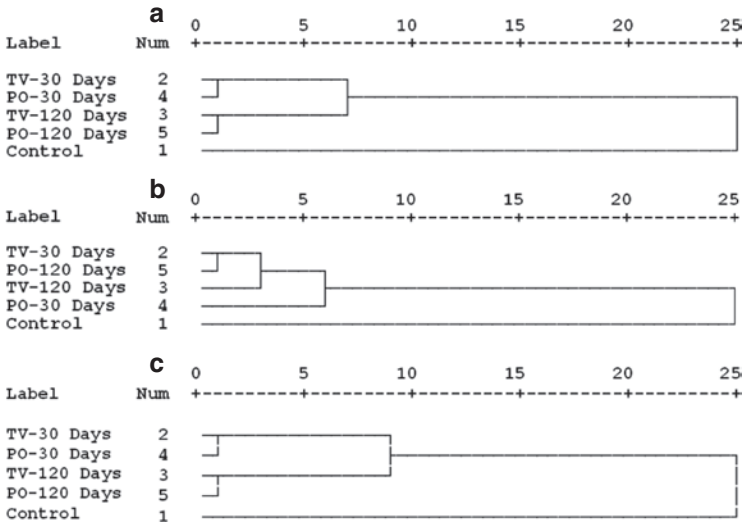

Figure 6 Cluster analysis for beech wood specimens exposed to *P. ostreatus* (PO) or *T. versicolor* (TV) for 30 and 120 days: (a) cluster analysis based on all mechanical properties and chemical components, (b) cluster analysis based on only mechanical properties, and (c) cluster analysis based on chemical components.

Conclusions

Beech wood specimens were exposed to the white-rot fungi *P. ostreatus* and *T. versicolor* for 30 and 120 days. Both fungi led to nearly the same rate of deterioration measured as ML. The mechanical properties and content of cell wall components changed at different rates during 30 and 120 days of exposure. After the first 30 days, the impact bending and carbohydrate loss were considerable. In the case of prolonged exposition, hardness, $CS_{||}$, cellulose, and lignin contents were further decreased. The results direct the scientific attention to the degradation degrees at 15-day exposition time.

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