TECHNICAL NOTE: EFFECT OF EPOXY EMBEDMENT ON MICROMECHANICAL PROPERTIES OF BROWN-ROT-DECAYED WOOD CELL WALLS ASSESSED WITH NANOINDENTATION

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Abstract. Mechanical properties of brown-rot-decayed wood cell walls were evaluated using a nanoindentation technique. Epoxy resin is a typical medium for the sample embedding process in nanoindentation. It is assumed that the embedding process does not affect cell wall properties or that any effects are similar for different samples. As part of an investigation of microscale mechanical effects of brown-rot in wood, we applied nanoindentation to cell walls of decayed and nondecayed pine wood samples. For epoxy-embedded samples, there were no differences in modulus and hardness for control and decayed samples. However, for unembedded samples, significant differences were found between control and decayed samples. These results indicate that the epoxy-embedding process may confound micromechanical testing results. We speculate that in this case, epoxy resin penetrated and reinforced the cell wall of decayed samples.

Keywords: Brown-rot decay, softwood, nanoindentation, resin embedding.

INTRODUCTION

Nanoindentation is a technique to measure mechanical properties at the micrometer scale by applying controlled loads to the surface of materials. Load and displacement are monitored during loading and unloading cycles, and hardness and reduced modulus are calculated from unloading curves using equations derived from elastic contact theory (Oliver and Pharr 1992).

Nanoindentation has been used to study earlywood and latewood cell walls (Wimmer et al 1997), cell wall microfibril angle (Gindl and Schöberl 2004; Gindl et al 2004a; Tze et al 2007; Jakes et al 2008; Konnerth et al 2009), cell wall lignification (Gindl and Gupta 2002), adhesive bondlines (Gindl et al 2004b; Konnerth et al 2007), and melamine-modified wood cell walls (Gindl and Gupta 2002). Except for Jakes et al (2008), who used unembedded wood specimens prepared with a diamond knife, samples are usually embedded in epoxy resin to provide mechanical support during sample preparation and a smooth surface for indentation after ultramicrotoming (Wimmer et al 1997; Gindl et al 2004a; Tze et al 2007; Konnerth et al 2009). It has been documented that penetration of epoxy resin is limited because of the compact structure of the woody cell wall (Jayme and

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Fengel 1961; Fengel 1967; Wimmer et al 1997; Gindl and Gupta 2002; Zimmermann et al 2006). However, Meng (2010) reported that the Young's modulus and hardness of loblolly pine increased with epoxy embedding by 14% and 32%, respectively.

During early stages of decay, brown-rot fungi are thought to use nonenzymatic oxidative mechanisms because the pore size of sound wood cell walls is too small for even the smallest enzymes to penetrate (Cowling and Brown 1969; Flournoy et al 1991). The chemistry of this proposed mechanism has been extensively investigated (Goodell 2003), and the dramatic impacts on macromechanical properties by brown-rot fungi have been well documented (Cowling 1961; Winandy and Morrell 1993; Schwarze et al 2000; Curling et al 2002). However, spatial and temporal dynamics of brown-rot decay at the cellular level are not as well understood. Micromechanical techniques may prove to be useful in the study of wood deterioration processes; however, in this article, we report on a sample preparation issue we experienced in applying nanoindentation to brown-rot-decayed southern pine wood.

MATERIALS AND METHODS

Sample Preparation

A strip of southern pine (*Pinus* spp.) sapwood (20 × 300 × 5 mm thick) was cut into a series of 1- × 1-mm specimens that came from the same annual ring band. Specimens were steam-sterilized and placed on malt extract/agar plates with samples of the brown-rot fungus *Postia placenta*. After 4 wk exposure, the wood samples were dried at 65°C for 24 h to stop fungal activity. Mass loss of 1 cm^3 samples exposed at the same time was about 12%.

Nanoindentation

Samples (1 × 1 × 5 mm) were embedded in Spurr's epoxy embedding medium (Electron Microscopy Sciences, Hatfield, PA), which comprises 10 g of cycloaliphatic epoxide (ERL-4221), 6 g of diglycidyl ether (DER 736), 26 g of nonenyl succinic anhydride, and 0.4 g of 2-(dimethylamino)ethanol. Embedded samples were submerged in freshly mixed epoxy, vacuum was applied for several minutes to promote penetration, and then the epoxy was cured at 70°C in an oven for 8 h. Unembedded samples were also prepared. Freshly mixed epoxy was first placed in a conical embedding capsule and cured at 70°C for 1.5 h to increase viscosity and molecular weight of the resin. The capsule tip was cut off, samples were inserted in the partially cured resin, and the assembly was cured for another 6.5 h. No vacuum was applied to unembedded samples, and no resin was observed in the cell's lumina under an optical microscope. Lumina of embedded samples were filled with resin. For both embedded and unembedded samples, care was taken to ensure that sample orientation was perpendicular to indentation surface. A smooth surface was prepared using a glass knife followed by a Microstar Technologies (Huntsville, TX) diamond knife using a Reichert, Inc. (Buffalo, NY) OMU-3 Ultramicrotome. Surfaced samples were placed in a sample holder and then magnetically clamped to the indenter stage. Nanoindentation experiments were performed with a Hysitron Inc. (Minneapolis, MN) TriboIndenter system equipped with a three-sided pyramid diamond indenter (Berkovich type). The indentation cycle was as follows: after a set point load of 2 μN was reached between sample and tip, the loading cycle started at 30 μN/s up to a maximum load of 150 μN. Maximum load was held for 5 s and then unloaded at 30 μN/s. For each specimen, nanoindentations were made on the cross-sectional surface of the S_2 layer of latewood cell walls. Cell wall edges were avoided. For each specimen, at least three different scanning areas were selected, and for each scanning area, at least three indentations were made on multiple cells, therefore 11-64 indentations were made.

RESULTS AND DISCUSSION

Prepared surfaces of the various samples were similar when examined using the scan mode of the nanoindenter. The mean value of the reduced modulus of embedded, nondecayed controls was similar to that of unembedded controls (Fig 1a) and close to previously reported values (Tze et al 2007; Konnerth et al 2009). Average reduced moduli of epoxy-embedded, brown-rot-decayed cell walls were actually greater than those of undecayed controls (p value < 0.001, analysis of variance and Tukey's honestly significant difference procedure for multiple comparisons) despite the long decay exposure period for samples and the decay weight losses observed. In contrast, unembedded, decayed samples showed significantly lower average reduced moduli values. Hardness values showed a similar pattern to reduced moduli data (Fig 1b).

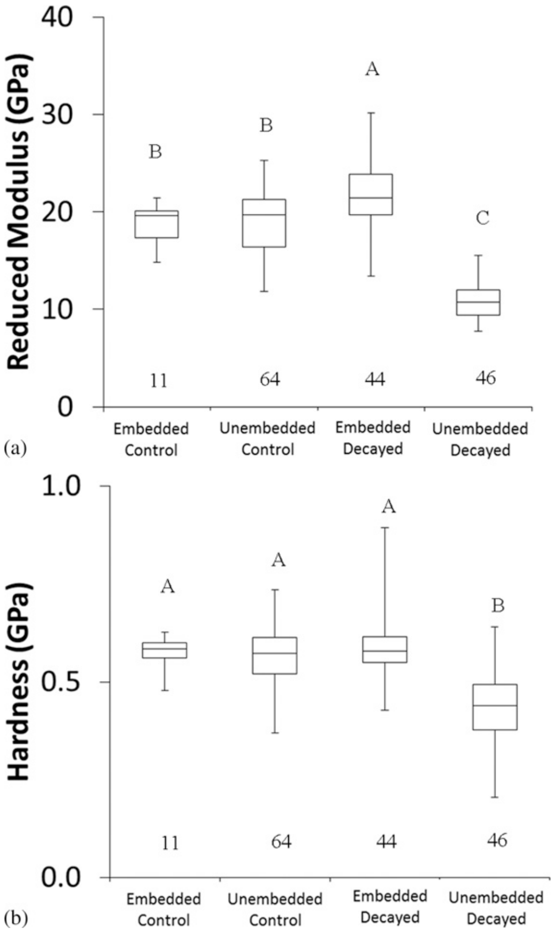

Figure 1. Reduced modulus (a) and hardness (b) of unembedded and embedded specimens decayed for 4 wk with brown-rot fungi. Numbers under the box plot are total number of indentations made, and letters indicate statistical difference ($p < 0.05$ from analysis of variance and Tukey's honestly significant difference procedure for multiple comparisons).

These results suggest that micromechanical properties of control (nondecayed) wood cell walls were not affected by epoxy embedding, which could have been caused by limited penetration of epoxy resin into sound wood cell walls (Gindl and Gupta 2002; Zimmermann et al 2006). However, embedding apparently masked mechanical property decreases in brown-rot-decayed specimens. This may have been caused by epoxy resin penetrating and reinforcing cell wall openings that were created during incipient decay by brown-rot fungus (Flournoy et al 1991; Chirkova et al 2006). Given that mechanical properties of epoxy are much lower than bulk cell wall material, it is interesting that embedded, decayed samples actually had significantly higher modulus values. As a result of decay, noncrystalline cell wall components were probably preferentially degraded, leaving more crystalline cellulose residues (Howell et al 2009). Noncrystalline cell wall components may have lower mechanical values than the epoxy used for embedding. Replacement of these components with epoxy could result in a composite with mechanical properties greater than that of the original cell wall.

Hardness values were not different for the epoxy-embedded decayed and control samples. The nanoindentation hardness of cell walls is dominated by yield processes in the matrix lignin (Gindl et al 2004). Brown-rot tends to modify, but not remove, lignin. This may explain why the embedding process did not increase hardness of the decayed sample. The natural, pre-existing variations in wood properties of each sample may have affected the results. Furthermore, only a few of the many cells in each sample were tested and the selection process may have inadvertently introduced bias. However, care was taken to ensure that samples were as similar as possible initially and a number of cells, from various locations within each sample, were chosen to have the average of values obtained be truly representative of the treatments. However, further study is required to determine the true extent of this epoxy reinforcement phenomenon of brown-rot-decayed wood.

CONCLUSION

Nanoindentation data (reduced modulus and hardness) obtained from brown-rot-decayed and nondecayed samples can be affected differently by epoxy embedding. Possible confounding effects of embedding media should be considered when samples with altered cell wall structures are analyzed by nanoindentation.

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