Nanoindentation of wood cell walls: Continuous stiffness and hardness measurements

W.T.Y. Tze a,*, S. Wang a, T.G. Rials a, G.M. Pharr b, c, S.S. Kelley a, d

a Forest Products Center, The University of Tennessee, Knoxville, TN 37996-4570, USA
b Department of Material Science, The University of Tennessee, Knoxville, TN 37996-2200, USA
c Metals and Ceramic Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6116, USA
d Department of Wood and Paper Science, North Carolina State University, Raleigh, NC 27695, USA

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Abstract

The objective of this study was to measure the mechanical properties of individual, native wood fibers using the continuous nanoindentation measurement technique. The indentation depth profile exhibited a small length-scale effect, which was confirmed using the size-effect index derived from the indentation loading curve. The hardness ($H_u$) or stiffness ($E_u$) values determined from indentation unloading were also examined for 10 different annual rings of a loblolly pine, with microfibril angles (MFA) between 14° and 36°. A predictable pattern of $E_u$ values was found as a function of MFA, and hence $E_u$ can at least be considered a relative measure of the longitudinal stiffness properties of wood cell walls. For $H_u$ values, a dependence on orientation was observed, and there is a preliminary indication that the dependence could be affected by cell-wall extractives. It is thus desirable, for cell-wall modification studies, to minimize any unintended variations by using samples that are from the same growth ring, so that any treatment-induced changes in the cell-wall hardness can be identified.

Keywords: A. Wood; B. Mechanical properties; Nanoindentation

1. Introduction

Nanoindentation testing is a technique that determines the mechanical properties of a material in the micron or sub-micron scale. The test involves penetrating a sample material using an indenter, while the penetration depth and load are recorded so that the stiffness and hardness of the indented location can be subsequently calculated. The indenter head can be 100 nm in radius (in the case of a Berkovich indenter), and the penetration can be up to 1 or 2 μm in depth, with the resulting indent having a linear dimension in the order of micrometers. This dimension is in the same order of magnitude of the wood cell-wall thickness. The wood cell walls were reported to be 5–6 μm and 9–13 μm in thickness, respectively, for earlywood and latewood of loblolly pine [1]. Therefore, the local mechanical properties of wood cell walls can be probed using nanoindentation tests. More specifically, the test detects the mechanical properties of the cell-wall S-2 layer, which constitutes about 80% of the total cell-wall thickness and is the major contributor to the mechanical properties of wood cell walls.

The micron spatial (lateral) resolution in nanoindentation tests renders the technique very useful in the investigation of the wood cell-wall level as a result of growth processes or utilization operations. To date, a few studies have used nanoindentation to investigate the effects of seasonal growth response (earlywood versus latewood) [2], cell-wall lignification [3], melamine modification [4], and adhesive bonding [5] on the mechanical properties of single...
cell walls. An attractive feature demonstrated in these studies is that the measurements were made without requiring chemical or mechanical modifications to isolate individual wood fibers as required in single-fiber tensile tests. These chemical and mechanical modifications change the mechanical properties of the wood fibers in poorly defined ways.

Accompanying the promise of nanoindentation for studying the mechanical properties of wood, there are also uncertainties in the analysis of the raw data. The first of these uncertainties is that the stiffness values (13–21 GPa; compiled in [6]) obtained from the nanoindentation test, which is usually performed in the longitudinal direction of wood, is less than half the value of the tensile modulus obtained from single wood-fiber tensile test (60–80 GPa) [7]. Gindl and Schönberl [6] attempted to identify the origin of the underestimation by modeling the longitudinal modulus of the cell-wall S-2 layer using the laminate theory, assuming that the S-2 layer is a composite of lignin-hemicellulose matrix reinforced with variations in the microfibril angle (MFA) of the crystalline cellulose. These researchers observed that the modeled stiffness values at an MFA of 25° approximated the experimental (nanoindentation) stiffness values. Gindl and Schönberl [6] attributed the observation to the loading angle (which is also 25°) owing to the geometry of a Berkovich indenter. This finding led the researchers to conclude that the nanoindentation test data is influenced by the anisotropy of wood, where in off-axis loading of the tracheid (either due to indenter face angle or cell-wall microfibril angle), the lower transverse modulus contributes to reducing the observed stiffness value in nanoindentation experiments.

Experience with materials other than wood also reveals that the nanoindentation test data are influenced by the penetration size effect—the variations of modulus or (more commonly) hardness with the penetration depth (area). This phenomenon, which is more pronounced in materials of low hardness, also occurs in isotropic materials especially those that are crystalline [8]. The size-effect phenomenon has been ascribed to several mechanisms related to sample polishing (work hardening and surface-microcracks formation), test artifacts (indenter bluntness and indenter/surface friction), and material properties [9]. The material-related size effects can be explained in the perspective of the strain gradients that exist in the vicinity of the indentation. The strain gradient (or the density of dislocations) is larger at a smaller indentation depth; the resulting increase in the effective yield strength is projected as an increased hardness near the surface [8]. The penetration size effect complicates the comparisons of indentation properties of different materials, and this issue has not been addressed for nanoindentation of wood.

The continuous measurement technique offers a tool to probe stiffness as a function of indentation depth in one single experiment [10]. Commonly, a discrete indentation loading, with a targeted penetration load or depth, provides only one value of hardness (from the maximum load) or stiffness (from the slope of the unloading curve) as shown in Fig. 1. In the continuous measurement technique, cycles of indentation, each of which consists of incremental loading and partial unloading, are performed until a final desired depth is attained. Each loading-and-partial unloading cycle provides a value of hardness and stiffness; hence as the penetration progresses, various hardness or stiffness values were determined as a function of the indentation depth. Such a feature is particularly attractive in examining graded materials, for instance multilayer films, where hardness or stiffness differs in different layers under the surface [10]. The continuous measurement technique is also potentially useful for investigating penetration size effects, and it has not been applied to the indentation studies of wood.

The objective of this study was to measure the mechanical properties of wood fibers using nanoindentation with the continuous measurement technique. The study also examines the effects of microfibril angle on the stiffness and hardness values determined from nanoindentation experiments.

2. Materials and methods

The wood samples were collected from a loblolly pine stem. This tree was part of a study of the relationship between the Near Infrared spectra and the MFA (from X-ray diffraction measurements), moduli of elasticity (MOE; from three-point bending experiments), and moduli of rupture (MOR; also from three-point bending) of wood [11]. Five different annual rings (number 2, 5, 16, 32, and 50; counted from the pith), with MFA values ranging from 15° to 36°, were selected from the portion of the tree stem that was 0.3 m above the ground. The latewood portion of these annual rings were cut to 2 mm × 5 mm × 5 mm in radial, tangential, and longitudinal dimensions, respectively.

The wood samples were soaked in the Spur epoxy resin [12], which is commonly used as an embedding medium for electron microscopy of biological samples. The soaking was performed in a desiccator and under vacuum for
overnight. On the next day, the resin-impregnated samples were removed from the desiccator and transferred to a flat mould, which was subsequently filled with freshly prepared epoxy resin. The mould containing both wood samples and epoxy resin was then placed in an oven at 70 °C for 12 h. The embedding epoxy, once cured, was intended to allow a smooth surface cut (ultra-microtoming), to insure the wood cellular structure to be intact during microtoming, and to keep the wood cell walls from buckling during the indentation of the cell walls.

The resin-embedded samples were glued to an acrylic block using a five-minute epoxy. The acrylic block was then mounted onto an ultra-microtome. The transverse (cross-sectional) surface of the wood was leveled using a glass knife, and then smoothed using a diamond knife. The smoothed samples were conditioned for at least 24 h at 21 °C and 60% relative humidity in a room that housed the nanoindenter.

To begin indenting, the indenter was positioned, using an attached microscope, onto the cell wall in the longitudinal direction of the tracheid. A continuous measurement was performed at a displacement rate of 2 nm/s until a final penetration depth of 300 nm. At this depth, the loading was held for 50 s prior to the ultimate unloading. The unloading was then performed at a rate of 100 nm/s until 90% of the load was removed. At the end of the indentation experiment, the sample was examined under the microscope to evaluate the position and quality of the indentation.

Five indents were made on the cell wall of each tracheid in its longitudinal direction. To reduce variability, only the cell wall at the tangential side of the tracheid was examined although a previous study [2] observed no differences in cell wall at the tangential side of the tracheid was examined in its longitudinal direction. To reduce variability, only the load was removed. At the end of the indentation experiment, the (effective) hardness or moduli of a sample, various approaches (Fig. 2) were used – taking the values corresponding to 100 nm ($H_{100}$ or $E_{100}$) and 200 nm ($H_{200}$ or $E_{200}$) indentation depth, or averaging the values for a depth of more than 200 nm ($H_{\text{ave} > 200}$ or $E_{\text{ave} > 200}$). In addition, the hardness and modulus values ($H_w$ or $E_w$) were also determined from the ultimate unloading curve (Fig. 1), an approach used by Gindl and co-workers in their studies on nanoindentation of wood [3–6].

The continuous measurement technique employed in this study produces a series of hardness and modulus values as a function of indentation depth. To express the (effective) hardness or moduli of a sample, various approaches (Fig. 2) were used – taking the values corresponding to 100 nm ($H_{100}$ or $E_{100}$) and 200 nm ($H_{200}$ or $E_{200}$) indentation depth, or averaging the values for a depth of more than 200 nm ($H_{\text{ave} > 200}$ or $E_{\text{ave} > 200}$). In addition, the hardness and modulus values ($H_w$ or $E_w$) were also determined from the ultimate unloading curve (Fig. 1), an approach used by Gindl and co-workers in their studies on nanoindentation of wood [3–6].

The moduli ($E_w$) derived from nanoindentation studies of the samples from different annual rings were then correlated to the theoretical value ($E_x$) of cell-wall longitudinal modulus. The $E_x$ value can be computed from the transformation of stiffness tensor for orthotropic materials

$$E_x = \frac{1}{E_1} \cos^4 \theta + \left( \frac{1}{G_{12}} - \frac{2v_{12}}{E_1} \right) \sin^2 \theta \cos^2 \theta + \frac{1}{E_2} \sin^4 \theta,$$

where $v_1$ and $v_2$ are the Poisson’s ratios of the specimen and indenter, respectively, while $E_i$ is the modulus of the indenter. Here, it is worth mentioning that the data reduction for hardness and stiffness mentioned above, which is commonly employed in nanoindentation studies, assumes an isotropic elastic–plastic behavior. Since wood cell walls exhibit inelastic and anisotropic behaviors, the moduli and hardness values determined in this study should be viewed as effective values, rather than the absolute material properties of the wood cell wall. These effective values provided the data that were used to compare different cell-wall materials in the present study.

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where in this case, $\theta$ is the microfibril angle of the wood sample, while $E_1$, $E_2$, $G_{12}$, and $v_{12}$ are elastic constants

$$E_s = \frac{(1 - v_1^2) (1 - v_2^2)}{E_1} \left( \frac{1}{E_t} \right)^{-1},$$

where $v_1$ and $v_2$ are the Poisson’s ratios of the specimen and indenter, respectively, while $E_t$ is the modulus of the indenter. Here, it is worth mentioning that the data reduction for hardness and stiffness mentioned above, which is commonly employed in nanoindentation studies, assumes an isotropic elastic–plastic behavior. Since wood cell walls exhibit inelastic and anisotropic behaviors, the moduli and hardness values determined in this study should be viewed as effective values, rather than the absolute material properties of the wood cell wall. These effective values provided the data that were used to compare different cell-wall materials in the present study.

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![Fig. 2. The depth-dependent longitudinal stiffness, and various approaches to express the sample stiffness.](image-url)
where \( t \) and \( r \), respectively, stand for tangential and radial directions [14].

2a Low values: 0.50:0.50 66.50 5.00 2.0 0.17

These values, in addition to the Poisson ratio (\( \nu \)) among the moduli (including Young’s and shear moduli) of cellulose and matrix.

\( \nu_{12} \)

1 – 0.45:0.55 63.96 9.51 2.4 0.33

\( \nu_{12} \)

2a High values: 0.60:0.40 102.9 11.58 2.5 0.17

\( \nu_{12} \)

2c High values: 0.60:0.40 102.9 11.58 2.5 0.16

\( \nu_{12} \)

a Model 1 was derived for radiata pine [14], while models 2a and 2b were used in [6] for spruce.

b The \( E_2 \) value for model 1 was averaged from the values of tangential and radial Young’s moduli tabulated in [14].
c The \( G_{12} \) value for model 1 was averaged from the values of \( G_{1t} \) and \( G_{1r} \), where \( t \) and \( r \), respectively, stand for tangential and radial directions [14].
d Refer to [6] for the two different sets of values (low and high) for the moduli (including Young’s and shear moduli) of cellulose and matrix.

These values, in additional to the Poisson ratio (\( \nu_{12} \)), were used in [6] to calculate the elastic constants (model 2a and 2b) of the laminate (S-2 cell wall) at \( f_{\text{cellulose}}/f_{\text{matrix}} = 0.50:0.50 \). The high moduli values of the cellulose and matrix were also used in the present study to determine the elastic constants (model 2c) of the S-2 cell wall at \( f_{\text{cellulose}}/f_{\text{matrix}} = 0.60:0.40 \).

for the S-2 cell wall. Four different sets of elastic constant values (presented in Table 1) were used in this study. These elastic constants were determined by various researchers [6,14], using the laminate theory, with the assumption that the cell-wall layer consists of cellulose embedded in a matrix of hemicellulose and lignin. The laminate calculation requires input of elastic constant values of cellulose, hemicellulose, and lignin, which had been estimated in different reports, some of which were compiled by Bergander and Salmen [15]. Using different input values and adopting different cellulose:matrix volume fraction ratio, different sets of cell-wall elastic constants would be resulted. The effects of these input values and volume fraction will be discussed in the following section.

3. Results and discussion

3.1. Approach comparisons

The variability of the data was first examined to ascertain that any potential differences observed in the longitudinal hardness and moduli could be genuinely attributed to differences between the samples. A mean value was obtained from the longitudinal hardness or moduli of five locations on a tracheid S-2 cell wall. The mean values for five adjacent tracheids were compared using single-factor analyses of variance (ANOVA). The analyses detected no significant differences (at a confidence level of 0.95) in either hardness or modulus values among the five tracheids of the same growth ring regardless of the estimation approach. An exception was observed for wood ring 32, where tracheid-to-tracheid differences within the growth ring were pronounced for cell-wall hardness or moduli expressed at 100- and 200-nm indentation depth, but not when the values were determined from the ultimate unloading curve (\( H_u \) or \( E_u \)). This particular sample had a high surface roughness compared to the other samples, as observed under the microscope. It is also noteworthy that for the (rough) sample ring 32, data from the “unloading” approach (\( H_u \) or \( E_u \)) exhibits coefficients of variation or CV (up to 14%; Table 2) that are smaller than other approaches (CV up to 21%; Table 2). On the other hand, when the sample surfaces were smooth, the \( H_u \) or \( E_u \) data exhibit low CV values (2–8%; Tables 2 and 3) that are quite

Table 2

<table>
<thead>
<tr>
<th>Ring no.</th>
<th>Ring no. 5</th>
<th>Ring no. 16</th>
<th>Ring no. 32</th>
<th>Ring no. 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H_{100} )</td>
<td>0.54 (3) A</td>
<td>0.50 (5) A</td>
<td>0.44 (6) A</td>
<td>0.42 (21) #</td>
</tr>
<tr>
<td>( H_{200} )</td>
<td>0.53 (2) A</td>
<td>0.48 (5) B</td>
<td>0.44 (5) A</td>
<td>0.40 (17) #</td>
</tr>
<tr>
<td>( H_{100,200} )</td>
<td>0.53 (1) A</td>
<td>0.48 (4) B</td>
<td>0.44 (5) A</td>
<td>0.40 (16) #</td>
</tr>
<tr>
<td>( H_e )</td>
<td>0.46 (2) B</td>
<td>0.42 (4) C</td>
<td>0.38 (5) B</td>
<td>0.34 (14)</td>
</tr>
</tbody>
</table>

Note: Values were averaged from the mean hardness of five tracheids. The coefficient of variation (in bracket; units in percentage) represents the variability of the mean hardness of the five tracheids. The values assigned with the same letter in a column are indications that the approaches provide hardness values that are insignificantly different at a confidence level of 0.95. The hardness value followed by a “#” symbol had significant within-ring data variability, and hence no comparisons of the approaches were made for ring no. 32.

Table 3

<table>
<thead>
<tr>
<th>Ring no.</th>
<th>Ring no. 5</th>
<th>Ring no. 16</th>
<th>Ring no. 32</th>
<th>Ring no. 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E_{100} )</td>
<td>16.3 (2) A</td>
<td>15.7 (5) C</td>
<td>13.8 (8) B</td>
<td>12.7 (19) #</td>
</tr>
<tr>
<td>( E_{200} )</td>
<td>16.0 (1) AB</td>
<td>16.2 (5) AB</td>
<td>14.2 (7) A</td>
<td>12.8 (15) #</td>
</tr>
<tr>
<td>( E_{avg,200} )</td>
<td>15.9 (2) B</td>
<td>16.2 (4) AB</td>
<td>14.3 (7) A</td>
<td>13.0 (13) A</td>
</tr>
<tr>
<td>( E_e )</td>
<td>15.9 (3) B</td>
<td>16.3 (5) A</td>
<td>14.3 (8) A</td>
<td>13.3 (10) A</td>
</tr>
</tbody>
</table>

Note: Values were averaged from the mean moduli of five tracheids. The coefficient of variation (in bracket; units in percentage) represents the variability of the mean moduli of the five tracheids. The values assigned with the same letter in a column are indications that the approaches provide moduli values that are insignificantly different at a confidence level of 0.95. The moduli value followed by a “#” symbol was not included in the comparisons of the approaches due to the significant within-ring variability.
\[ P = B h^n \]

where \( B \) is a constant and \( n \) is the size-effect index [16]. The parameter \( n \) bears the value of 2 if there are no indentation size effects (Fig. 4). Hence, the amount of deviations of \( n \) from the value of 2 would indicate the extent of the size-effect contributions. For polymeric materials (which includes wood cell walls), the deviation from the ideal relation of \( P = B h^2 \) could also be contributed by viscoelastic effects such as stress relaxation (or creep) [17], which result in a low load \( (P) \) value at a given displacement \( (h) \), as compared to the case of \( n = 2 \) (Fig. 4).

Table 4

<table>
<thead>
<tr>
<th>Ring no. 2</th>
<th>Ring no. 5</th>
<th>Ring no. 16</th>
<th>Ring no. 32</th>
<th>Ring no. 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n ) from ( Eq. (5) )</td>
<td>1.74 ± 0.02</td>
<td>1.79 ± 0.05</td>
<td>1.81 ± 0.05</td>
<td>1.75 ± 0.06</td>
</tr>
</tbody>
</table>

Note: Values were averaged from five tracheids. The ± sign indicates the 95% confidence interval of the respective data sets.

A close examination of the hardness and stiffness measurements at different depths suggests that there is little if any indentation size effect. The hardness values below 100-nm indentation were highly variable (Fig. 3) due to surface roughness. Table 2 shows that in most cases \( H_{100} \), \( H_{200} \), and \( H_{ave,>200} \) are not statistically different. The depth-dependent changes in stiffness were generally small and not consistent (Table 3). To further examine the penetration size effect, a power-law equation was used to relate \( (P) \) to the indentation depth \( (h) \):

\[ P = B h^n \]

Fig. 3. An example of the depth-dependent longitudinal hardness.

Fig. 4. Loading curve for illustrating size effects based on Eq. (5): \( P = B h^n \). Note: the curve for \( n < 2 \) was plotted from one of the data set in the present study.

indices for indentation size effects
3.2. Examination of longitudinal stiffness: relations to microfibril angles (MFA)

It was acknowledged, in the methodology section, that the current practice of deducing stiffness and hardness values from nanoindentation data does not result in absolute material properties for anisotropic materials such as wood cell walls. Recognizing this constraint, no specific efforts were made to compare (or match) \( E_u \) or \( H_p \) with the “true” moduli or hardness values. Instead, attempts were made to compare the trends between the effective values (from nanoindentation) and the reference (modeled) values as a response to changes in microfibril angle (MFA). More relevantly, the subsequent effort of this report is to identify if the effective values (\( E_u \) or \( H_p \)) can be considered a reasonable relative measure of the S-2 longitudinal moduli and hardness.

To better discern the changes in \( E_u \) values as a response to microfibril angle changes, five more samples (MFA values ranged from 14° to 30°) were added to the study. These samples (annual rings 2, 5, 9, 16, and 32; counted from the pith) were cut from a wood disk (labeled as disk 2) obtained from the portion of the stem five meters above the pith (and V also implies that at a large (effective) loading angle, the longitudinal stiffness would decrease. The effective moduli from nanoindentation show a somewhat similar trend. This trend, however, exhibits a gentler slope (at a given MFA value) compared to the modeled values (curves II and III; Fig. 5), indicating that altering the MFA values would result in a change in the longitudinal stiffness, but the change would be smaller than that predicted by either model 1 (for radiata pine) or model 2b (for spruce) described in Table 1.

It has been discussed in [6] that when MFA is 0°, each of the three faces of the Berkovich indenter will form an angle of about 25° with the longitudinal axis of the load-bearing microfibrils to result in a 25° off-axis loading. For an MFA value larger than 0°, however, the individual faces of the indenter will form different angle values in relation to the longitudinal axis of the microfibrils [6], hence the resultant off-axis loading becomes complicated to analyze. While the use of 3D finite element analyses seems to be a sensible way to tackle this complication, there are however, according to our experience in applying the technique for nanoindentation [21], uncertainties in assuming the boundary conditions. Such uncertainties present an even bigger challenge for the case of wood cell walls, whose micron-scale properties are not well understood, and so we opt to keep finite element analyses for a detailed study in the future. For now, it is hypothesized that an effective loading angle that is larger than the MFA was responsible for influencing the cell-wall longitudinal moduli observed in the present study. To pragmatically represent this situation, an effective loading angle was determined by increasing the cell-wall MFA value by an arbitrary 20°, i.e. a horizontal shift along the x-axis of the stiffness–MFA plot (Fig. 5). As an example, the modulus value plotted for curve IV at 10° MFA was calculated, based on Eq. (4), using an effective (loading) angle value of 30°. The resultant curves (IV and V in Fig. 5) exhibit trend lines that are very similar in shape to the experimental data trend. Indeed, a reasonable expression (curve VI) for the data trend can be obtained by performing an upward shift of curve V. This observation agrees with our hypothesis that the orientation dependence of the cell-wall stiffness from nanoindentation is influenced by an effective (off-axis) loading angle that is larger than the MFA value. The similar trend line exhibited by curves IV and V also implies that at a large (effective) loading angle, the predicted dependence of the nanoindentation stiffness on MFA was not noticeably different for using models that were adopted for different wood species (radiata pine versus spruce). Based on the predictable response of \( E_u \) on MFA, it is inferred that \( E_u \) can be considered relative quantities to discern the stiffness properties of one cell wall from another.

To expand the findings reported in the preceding paragraph, it is desirable to investigate some factors that potentially reconcile the predicted modulus values with the experimental values. First, the spruce cell-wall model (model 2a) was plotted (curve B in Fig. 6) using effective loading angle values that are 20° larger than the corresponding MFA, as described in the preceding paragraph. Then, high values of elastic constants were used for the cell-wall polymer (model 2b) in determining the S-2 proper-

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![Fig. 5. The S-2 cell-wall longitudinal stiffness as a function of microfibril angles: experimental and modeled values. Note: curve II was from model 1 (refer to Table 1), and curve III was from model 2b (Table 1). Error bars refer to the 95% confidence intervals of the respective data points.](image-url)
ties. A considerable vertical shift was observed as a consequence, i.e. the predicted longitudinal moduli become higher (curve C), and relatively closer to the experimental data points. When the cellulose:matrix volume ratio is increased from 50:50 to 60:40 (model 2c), the vertical shift (curve D) is small. At this point, it is in order to mention that the cell-wall polymer elastic constants, which are used as input values for the calculation of S-2 cell-wall properties, were derived in the literature for 12% moisture content level. In contrast, the wood sample used in the experiment of this present study had an initial moisture content of 8.0%. Vacuum (impregnation) and subsequent heating in an oven (with the presence of epoxy resin) would reduce the moisture content level. The moisture change, though difficult to determine for wood samples that were finally embedded in the cured epoxy, should be consistent for all the samples, given that they were prepared using the same protocol. With a conservative assumption of 4% as the moisture content level of wood during the nanoindentation test, the longitudinal moduli were corrected from 12% MC to 4% MC using the following formula [22]:

$$E_x = E_i \left(\frac{E_{12}}{E_g}\right)^{M_x/M_{12}},$$

where $E$ and $M$, respectively, represents the stiffness (or $E_u$; in GPa) and moisture content (MC) level (in %) of the cell-wall sample, with $E_i$ referring to the $E$ value (at $M_i = 12\%$) that needs to be adjusted to $E_x$ (at $M_x = 4\%$). The symbol $M_x$ refers to the fiber saturation point (21% for loblolly pine; [22]) at which mechanical properties begin to change when wood is dried from a higher MC level. The ratio of cell-wall stiffness values at air-dry condition (MC = 12%) in relation to the green condition, $\left(\frac{E_{12}}{E_g}\right)$, was estimated from the values [22] of bending moduli of loblolly pine wood, i.e. $\left(\frac{MOE_{12}}{MOE_g}\right) = 1.27$. The resulting values, plotted as curve E, exhibit a substantial upward shift from curve D (12% MC). Hence the cell-wall polymer elastic constant values and moisture content levels are among the important factors that are responsible for the differences in the experimental and predicted values.

To put things into perspective, it should be reinstated that the present study did not attempt to quantitatively match nanoindentation data with modeled values that themselves are determined from estimated input values. Instead, the aforementioned discussions were meant to show that the modeled values would indeed be different if different values were used for the cell-wall polymer elastic constants. Additionally, the moisture content effects need to be taken into account in the modeling before fair comparisons between the modeled and nanoindentation values can be made. More relevantly, our preceding discussion concludes that the longitudinal moduli from nanoindentation, though not absolute in values, can be considered relative quantities. It follows these moduli ($E_u$) values can potentially be used for comparisons aimed at probing cell-wall mechanical changes associated with plant growth and processing.

3.3. Examination of longitudinal hardness: relations to microfibril angles

Corresponding to the 10 wood samples in the longitudinal stiffness measurements, 10 values of longitudinal hardness were obtained from nanoindentation. At first glance, the S-2 longitudinal hardness appears to be influenced by the microfibril angle (Fig. 7). This behavior seems to agree with the reported observation at the macroscopic level, where the Brinell hardness of wood decreases with the increase in grain angles in accordance with the
Hankinson-type formula [23]. The Hankinson-type formula has been commonly used in the field of wood science to express the (two-dimensional) orientation dependence of wood properties. In the present study, the same formula was used to perform a curve fitting on the plot of cell-wall (effective) longitudinal hardness as a function of MFA [22]:

\[ H_\theta = \frac{(H_L)(H_T)}{H_L \sin^2 \theta + H_T \cos^2 \theta}, \]

(7)

where \( H \) is the (effective) cell-wall longitudinal hardness (or \( H_a \)), \( \theta \) is the microfibril angle (MFA), \( H_L \) is the (effective) hardness value at 0° MFA, \( H_T \) is the (effective) hardness value at 90° MFA, and \( N \) is an empirically determined constant which ranges between 2 and 2.5 for compressive strength properties [22]. The fitted curve (Fig. 7) demonstrates a tendency of longitudinal hardness to decrease when MFA increases, with several exceptional data points deviating from the trend line. This behavior suggests that although there are some unaccounted variations in the data, the effective longitudinal hardness values from nanoindentation tests are generally orientation dependent. Whether this dependence is entirely on MFA or on its interaction with the indenter faces is not evident because the trend line was obtained by mere curve fitting, instead of theoretical calculations.

A subtle decrease in the average indentation hardness with increasing MFA (values ranged from 0° to 50°) was also observed by Gindl et al. [24]. The researchers, nevertheless, concluded from the lack of statistical differences that the indentation hardness is governed by the yielding of the cell-wall matrix (i.e. lignin and hemicelluloses), instead of the property of the microfibrils (or their alignment). These previous findings do not necessarily contradict our observations because the yielding of the cell-wall matrix, if indeed influential for nanoindentation test data, could partially explain the deviation of data points from the trend line in the present study. It is worth mentioning that the three outlying data points above the trend line (Fig. 7) are in correspondence to the three samples collected from the inner most (heartwood) region (rings 2 and 5), which are known to be rich in extractives. On the other hand, the remaining data point, lying below the trend line in the present study, is of ring 50, the outermost (sapwood; normally less extractive) region of the wood disks examined in this study. Extractives in the wood cell walls have been ascribed [25] to contribute to the compression properties of wood, as evidenced from the decreased maximum crushing strength upon removal of extractives, even when the accompanying density changes had been taken into account. A co-occurrence was also observed between a lowered (cold water soluble) extractive content and the reduced parallel-to-grain compression strength property of fire-impacted loblolly pine wood [26]. Based on these findings, it is reasonable to expect that the yielding of the cell-wall matrix in the present study would vary depending on the extractive concentration in the cell wall. The variation in yielding should affect the indentation hardness in different extents, according to the classic work of Tabor [27] and the postulation of Gindl et al. in Ref. [24]. The net effect is hence an additional source of variation that influences the dependence of \( H_a \) on MFA.

Overall, an orientation dependence of \( H_a \) values was observed, and there is a preliminary indication that the dependence could be affected by variations in extractive contents. This postulated, additional source of variation, once verified in future studies, would add merits to using nanoindentation hardness as a relative parameter to probe the mechanical changes of cell walls as a response to physiological changes in living trees. In the context of cell-wall modification for enhanced wood utilization, any unintended variations can be minimized by using starting materials that are similar for both control and treatment experiments. One example is to use, for both the control and treated samples, wood of the same growth ring to minimize the effects of MFA and possibly the influence of cell-wall extractive materials (extractives), so that any treatment-induced changes in the cell-wall hardness can be clearly identified from the nanoindentation data.

4. Conclusions

This study reports the continuous nanoindentation measurement of longitudinal stiffness and hardness of wood cell walls as the indentation progresses. The indentation depth profile exhibited a small length-scale effect, which was confirmed using the size-effect index derived from the indentation loading curve. The ultimate unloading curve was used to determine the (discrete) modulus and hardness values \( (E_a \text{ and } H_a) \) for different wood annual ring samples. A predictable pattern of \( E_a \) values was found as a function of MFA, but the \( E_a \) values were lower than the corresponding moduli estimated from the cell-wall models. Further observations indicate a high possibility that the longitudinal stiffness determined by nanoindentation was influenced by an effective (off-axis) loading angle that is larger than the MFA value of the corresponding wood sample. While 3D finite element analyses are recommended for the future to verify this phenomenon, it is sufficient to conclude at present that \( E_a \) can be considered a relative measure of the wood cell-wall longitudinal stiffness. Results from additional analyses also suggest that the differences in the experimental \( (E_a) \) and predicted (modeled) values were largely influenced by the input values of the models (e.g. the elastic constant values of cell-wall constituents) and the moisture content levels of the samples during indentation. For cell-wall longitudinal hardness \( (H_a) \), a dependence on orientation was observed, and there is a preliminary indication that the dependence could be affected by variations in extractive contents. Further investigations are needed to verify the extractive effects. Meanwhile, for cell-wall modification studies, it is desirable to minimize any unintended variations by using samples that are from the same growth ring, so that any treatment-induced changes in the cell-wall hardness can be identified from nanoindentation data.
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References