

Mechanical Function of Lignin and Hemicelluloses in Wood Cell Wall Revealed with Microtension of Single Wood Fiber

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Abstract

The mechanical properties of wood are highly dependent on the structural arrangement and properties of the polymers in the wood cell wall. To improve utilization and manufacture of wood materials, there is an increasing need for a more detailed knowledge regarding structure/property relations at cellular level. In this study, Fourier-Transform Infrared (FT-IR) spectrometer and microtension technique were jointly adopted to track both changes in the chemical structure and cell wall mechanical properties of single tracheid fibers of Chinese Fir, which had been subjected to extraction treatments with sodium chlorite (NaClO₂) for delignification, as well as with sodium hydroxide (NaOH) at different concentrations for extraction of hemicelluloses. Measurements with FT-IR spectroscopy provided information about the chemical changes in cell wall during the extraction process, while the microtension experiments gave qualitative information about the micromechanical properties of the extracted cell wall. The micromechanical data indicated that lignin had little impact on air-dried tensile elastic modulus and strength of cell wall, while hemicelluloses affected these properties significantly. Our results underlined the key role of hemicelluloses to maintain the integrity of wood cell wall.

Keywords: hemicelluloses, lignin, cell wall, mechanical properties, microtension.

Introduction

Wood cell wall is a highly organized composite that may contain many different polysaccharides, proteins, and aromatic substances. Recent scientific and technological advances offer new possibilities to using plant cell walls in the production of cost-effective biofuels (Michael et al. 2007, Pu et al. 2011). However, the key obstacle for transitioning from plant cell wall to biofuels is the complicated structure of the cell wall, which is, by nature, resistant to breakdown-the recalcitrance problem. In view of this, fundamental understanding of structure and chemical components properties of cell wall is of great importance.

Cellulose, hemicelluloses and lignin are the main components of wood cell wall. The arrangement of chemical components, their interactions and their mechanical properties result in mechanical properties of the cell wall which finally affect the macroscopic properties of wood. Mechanical investigations of the wood polymers show that there are strong interactions between the hemicelluloses, xylan and glucomannan, and the other wood polymers, cellulose and lignin. Studies of the softening behavior of glucomannan and xylan suggest that xylan is more associated with lignin while the glucomannan is more associated with cellulose (Salmén and Olsson 1998, Åkerholm and Salmén 2001, Stevanic and Salmén 2009). However, most previous work of wood polymers in cell wall had been carried out by spectroscopic studies.

Microtension of single fibers belongs to a powerful tool for mechanical characterization of plant fibers. In this context, Jayne (1959) was one of the first who performed tensile experiments on single pulp fibers. Since then, mechanical properties of different kinds of plant fibers were tested. At the same time, sample preparation, alignment of fibers to tensile direction and cell wall area determination of this method were improved (Burgert et al. 2002, Yu Y et al. 2010, 2011).

This study used single-fiber-test technology to investigate the effects of chemical components on mechanical properties of single fibers. The techniques for isolating fibers mechanically and extraction treatments were introduced. FT-IR microscopy was applied to track the chemical changes in cell wall. A further aim was to gain insight into the arrangement of the polymer network from mechanical point.

Materials and Methods

Materials. Material was taken from the adult wood of a 42-year-old Chinese Fir (*Cunninghamia lancolata* (Lamb.) Hook) grown in Anhui Province, China. Tangential slices of 100- μ m-thick were cut with a microtome from never dried adult latewood. Some tangential slices were fixed under a light microscope and single fibers were isolated using very fine tweezers (Burgert et al. 2002). Others were treated with extraction methods which were NaClO₂ for delignification and NaOH at different concentrations for extraction of hemicelluloses. For delignification, three kinds of chemical solutions were chosen to delignify wood cell wall with different extent: A) an aqueous solution of 0.3% NaClO₂ buffered with glacial acetic acid at pH 4.4~4.8 for 4h at 80°C; B) an aqueous solution of 0.3% NaClO₂ buffered with glacial acetic acid at pH 4.4~4.8 for 8h at 80°C; C) an aqueous solution of 150ml distilled water, 1.0g NaClO₂ and 2.0ml glacial acetic acid for 8h at 80°C. On the delignified samples, a successive extraction of hemicelluloses

which allowed a reasonably specific degradation of hemicelluloses (Nelson 1961) was carried out by a treatment with (i) 6% NaOH, (ii) 6 and 8% NaOH and (iii) 6, 8 and 10% NaOH at 60°C for 2h each. Subsequently, fibers were washed carefully in deionized water several times and dried on glass slides at room temperature (25°C).

FT-IR Spectroscopy. FT-IR spectra of the treated and control samples were collected using a Nexus 670 spectrometer equipped with a MCT/A detector. Each spectrum was recorded over the 4000 to 400 cm^{-1} range, with a resolution of 4 cm^{-1} , and derived from the average of 200 scans. The range from 800 to 1800 cm^{-1} was selected as reference for the normalization using OMNIC software version 7.1.

To investigate the effect of treatments on the chemical components of cell wall, specific wavenumbers of cell wall polymer absorption bands of cellulose, hemicelluloses, that is, xylan and glucomannan, and lignin were examined in the area from 800 to 1800 cm^{-1} . Three vibration bands of cellulose, that is, the C-H deformation at 898 cm^{-1} , the C-H bending vibrations at 1368 cm^{-1} and the CH₂ wagging vibration at 1316 cm^{-1} were used (Stevanic 2009, Åkerholm and Salmén 2001). For hemicelluloses, two bands of xylan, namely, the C=O stretching vibration at 1734 and 1600 cm^{-1} were used. For glucomannan, the band at 810 cm^{-1} (Marchessault 1962), attributed to vibrations caused by equatorially aligned hydrogen on the C₂ atom in the mannose unit, was observed. The band of aromatic skeletal vibrations in lignin at 1508 cm^{-1} (Schwanninger et al. 2004) and the vibration of the guaiacyl ring, together with the C=O stretch at 1264 cm^{-1} (Åkerholm and Salmén 2003) were observed.

Microtensile Tests.

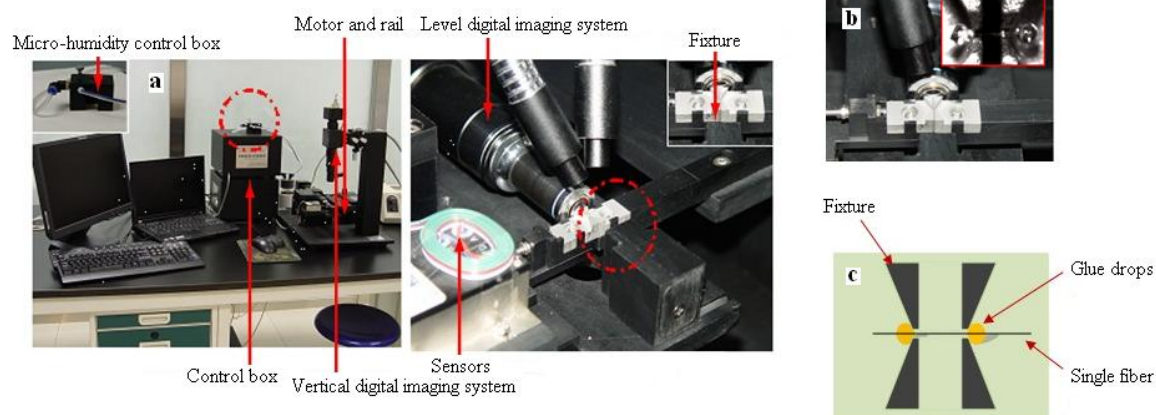


Fig. 1 Microtensile testing system.
SF-Microtester I (a), “ball and socket” type fiber clamping (b, c).

Tensile tests on single fibers were performed with a custom-built microtension tester (SF-Microtester I) (Figure 1, a). “Ball and socket” type fiber gripping was adopted for microtensile testing (Figure 1b, c). Two resin droplets (cold-curing adhesive, HY-914) approximately 200 μm in diameter were placed in the center portion of each fiber via a fine tweezers. The capacity of load cell used was 5N. The tensile speed was 0.8 μms^{-1} . Tensile testing of the dried fibers was carried out in an environment of 20 \pm 5°C and

20±5% RH. The cell wall cross-sections of every broken fiber were measured with a confocal scanning laser microscope (Meta 510 CSLM, Zeiss) (Yan Yu et al, 2010, 2011). In total, 54 mechanically isolated fibers and 50-60 fibers for each chemical treatment were analyzed.

Lignin Quantification. The lignin contents of native and chemically altered fibers were determined by acid-insoluble lignin (GB/T 2677.8-94).

Results and Discussion

Selective Removal of Lignin and Hemicelluloses. The FT-IR measurements were used to assess the effectiveness of the treatments on the samples which provide a reliable characterization for the semiquantitative interpretation of cell wall chemical components. FT-IR spectra of native and treated fibers were analyzed, see Figure 2. Only changes in the relative intensities of specific bands were evaluated.

The 1508cm⁻¹ peak is often taken as a reference for lignin since it results purely from an aromatic skeletal vibration (C=C) in lignin. With the removal of lignin by NaClO₂/glacial acetic acid, a significant reduction of the peak at 1508cm⁻¹ can be observed. The relative intensity of the lignin band at 1508cm⁻¹ showed decreased obviously with the decrease of lignin content. And the peak at 1508cm⁻¹ nearly disappeared when the lignin content decreased to 0.4% after the delignified treatment of method C (Table 1). The same change of relative intensity of lignin band at 1264cm⁻¹ was also observed.

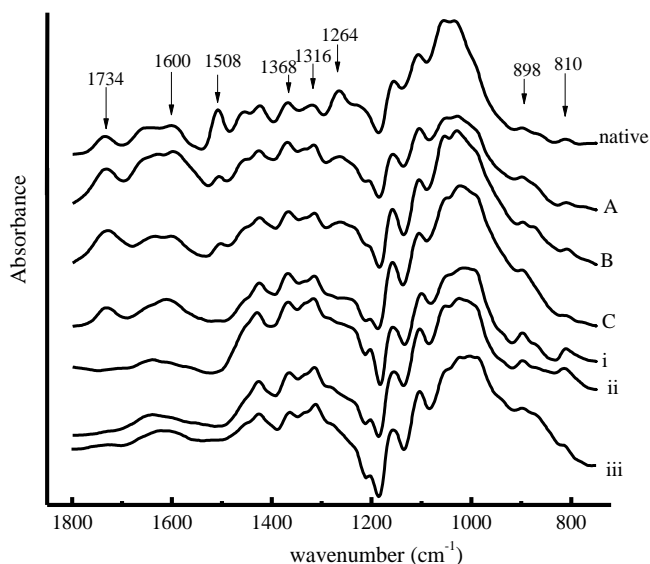


Fig. 2 IR-Spectra of different treatments

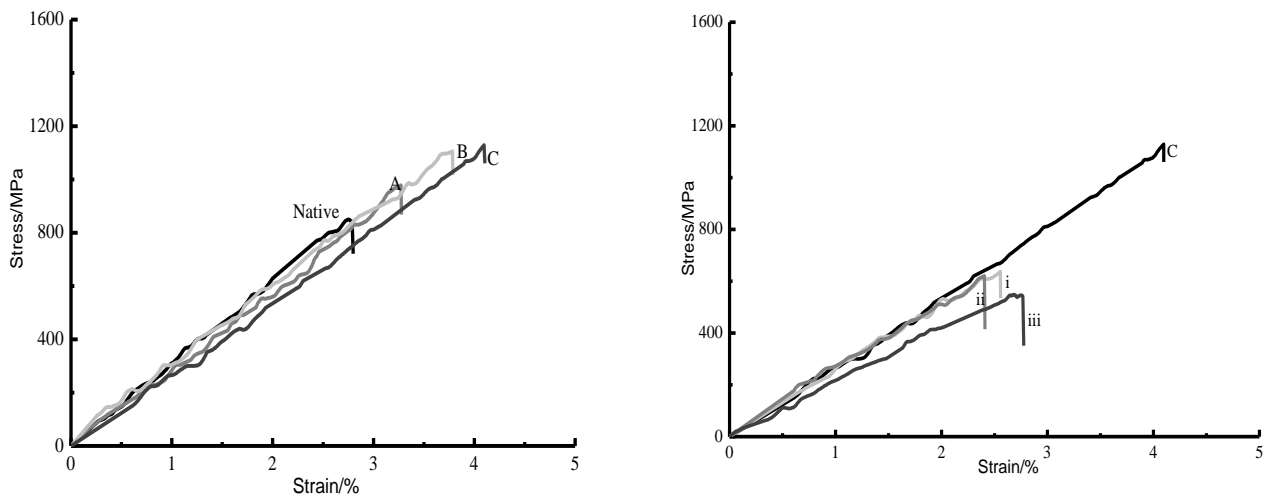
The relative intensity of hemicelluloses (xylan and glucomannan) bands at 1734, 1600 and 810cm⁻¹ showed little changes due to delignification. After a successive treatment with 6% NaOH on delignified samples, the bands at 1734 and 1600cm⁻¹ disappeared (Figure 2i). This indicated that xylan was almost removed. The intensity of glucomannan band at 810cm⁻¹ showed minor change after successive treatments with 6% and 6%+8% NaOH (Figure 2i, ii). However, the peak at 810cm⁻¹ nearly disappeared after treatment with 10% NaOH, which implied glucomannan was almost removed (Figure 2iii).

Table 1 Lignin content after delignified treatments

Sample	Lignin content (%)
Native sample	33.61
A	29.76
B	25.37
C	0.40

The small variations of the absorption spectra bands at 1368 and 1316 cm^{-1} for native and treated fibers indicated that the cellulose underwent least change during the process of lignin and hemicelluloses extraction.

Stress-strain curves. The typical stress-strain curves of single fibers after different treatments were presented in Figure 3. All the fibers tested showed a linear stress-strain behavior to failure, which indicated that chemical components changes did not affect the tensile behavior of single fibers. Groom (2002a) found the shape of the stress-strain curve of softwood fibers depended on microfibrillar angle (MFA) that Individual fibers with MFAs less than 20° appeared to be full linear during the test. In this study, the MFAs of wood fibers were around 10° , explained why all the wood fibers display linear stress-strain behavior.



(a) delignified treatments

(b) hemicelluloses-extracted treatments

Fig. 3 Typical stress-strain curves of single fibers after different treatments

Mechanical Function of Lignin in Cell Wall. Figure 4 and Figure 5 showed the effect of lignin on the tensile modulus and strength of fiber cell wall. The tensile modulus was reduced by 1.96%, 4.74%, and 5.10% with lignin content decreasing by 11%, 25%, and 99%, respectively (Figure 4). However, the average tensile strength of single fibers showed an increasing trend when there was a decrease in the lignin content (Figure 5). That was increased by 26.28%, 34.22%, and 41.18% for delignification reduced by 11%, 25%, and 99%, respectively.

In the cell wall of softwood tracheids, cellulose is the main structural component working as a framework substance. Since the stiffness of cellulose (167.5GPa) is more than 80 times that of lignin (2.0GPa), even a twofold increase of the lignin content should not noticeably alter the stiffness of the composite cell wall. And the results were consistent with the measurements made by Duchesne (2001) on kraft pulp fibers by FE-SEM and CP/MAS 13C-NMR.

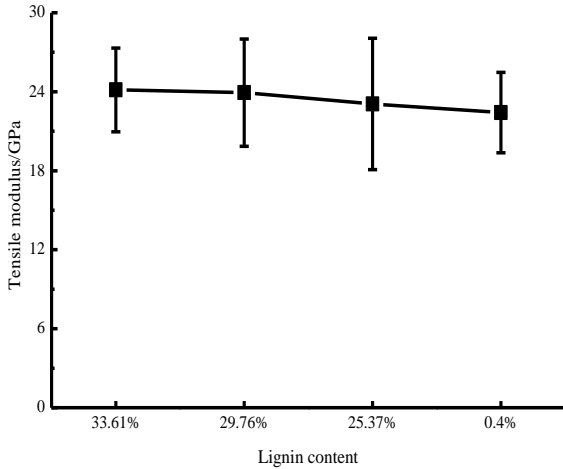


Fig. 4 Tensile modulus of single fiber with delignified treatments

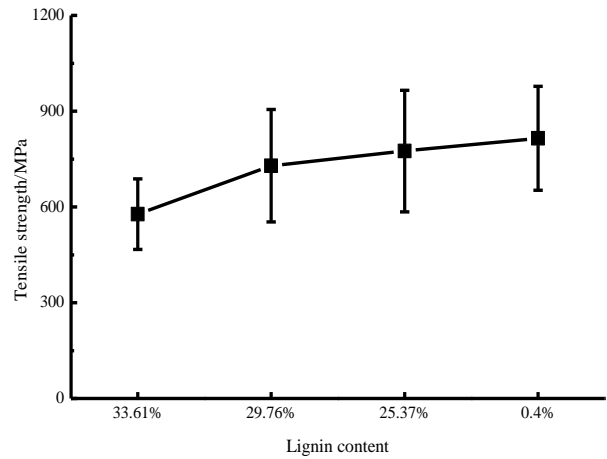


Fig. 5 Tensile strength of single fiber with delignified treatments

Mechanical Function of Hemicelluloses in Cell Wall.

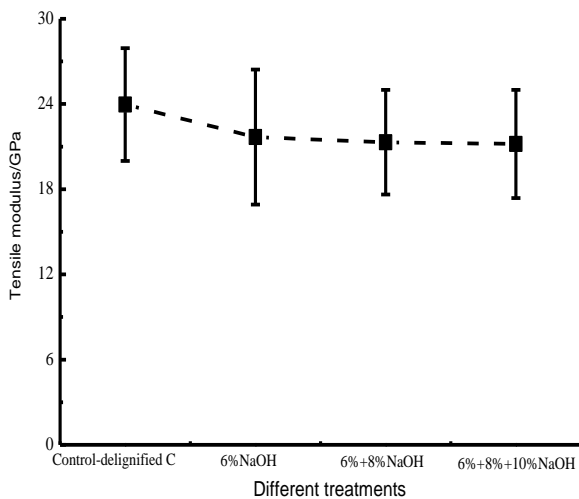


Fig. 6 Tensile modulus of single fiber with hemicelluloses-extracted treatments

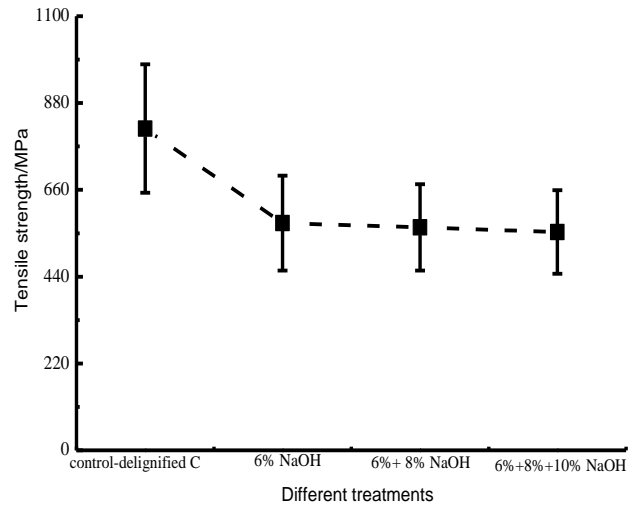


Fig. 7 Tensile strength of single fiber with hemicelluloses-extracted treatments

The effect of successive extractions of hemicelluloses on the tensile modulus and strength of fiber cell wall were presented in Figure 6 and Figure 7. The tensile modulus and strength of delignified fibers treated with increasing concentration NaOH showed an obvious decreasing. The tensile modulus was reduced by 9.55%, 11.08%, and 11.57%, while the tensile strength of single fibers was reduced by 29.36%, 30.71%, and 32.15% for hemicelluloses-extracted at 6% NaOH, 6+8% NaOH, and 6+8+10% NaOH, respectively. Compared to lignin, the effect of hemicelluloses on tensile modulus was more significant than that of lignin in dry condition.

Xylan and glucomannan are the two main kinds of hemicelluloses in the cell wall of softwood tracheids. Glucomannan is more closely associated with cellulose as it is more difficult to separate from cellulose than xylan. Extraction treatments on delignified samples with NaOH allow degradation of xylan firstly (Figure 2i). In cell wall,

hemicelluloses act as an interfacial coupling agent between the highly ordered cellulose of the microfibrils and lignin. And hemicelluloses play an essential role in the maintenance of the cell wall assembly. Therefore, the removal of hemicelluloses would destroy the integrity of cell wall. This may have been the cause of the obvious decrease in tensile modulus and strength when there was a degradation of hemicelluloses.

A Modified Cell Wall Model. The tensile properties of single fibers are highly dependent on the structural arrangement of the polymers in the fiber cell wall. As the results above, we established a new cell wall model, see Figure 8. It showed that cellulose was the main structural component in cell wall, the source of cell wall strength. Hemicelluloses (xylan and glucomannan) connected with the highly ordered cellulose of the microfibrils and lignin, and most of xylan contacted with glucomannan and lignin. Glucomannan and cellulose in close contact within the cell wall, and the force between xylan and glucomannan was less than that between glucomannan and cellulose. Xylan acted as an interfacial coupling agent between highly ordered cellulose of the microfibrils and lignin, which was important to maintain the integrity of cell wall mechanics. Lignin linked to xylan, but the connection was easy to destroy. To a certain extent, its existence enhanced the mechanical properties of cell wall.

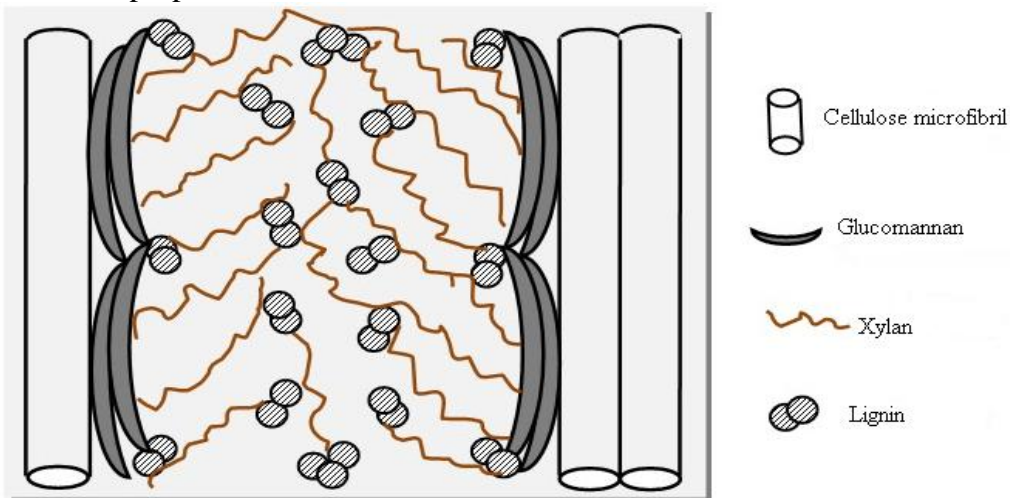


Fig. 8 Schematic diagram of interaction between chemical components in cell wall

Conclusions

The nanostructural organisation of wood cell wall after established extraction treatments method was studied by microtension experiments in combination with FT-IR spectroscopy. Different degrees of structural degradation were observed upon delignification and subsequent treatment with increasing concentrations of NaOH. The chemical changes might have reduced linkages between polymers in the cell wall. These chemical components changes could account fully for the changes observed in tensile properties of single fibers. The obvious decreasing tensile modulus and tensile strength in fibers treated with sodium hydroxide (NaOH) at concentrations of 6% were consistent with the xylan degradation. The findings suggest that xylan may contact with glucomannan and lignin in cell wall, and is important to maintain the integrity of cell wall mechanics.

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