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

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Chinese Fir wood (*Cunninghamia lanceolata* (Lamb.) Hook) was subjected to extraction treatments with sodium chlorite ($NaClO_2$) for delignification, as well as with sodium hydroxide (NaOH) at different concentrations for extraction of hemicelluloses. The wood was examined using a Fourier Transform Infrared (FT-IR) spectrometer and microtension technique to track changes in the chemical and the micromechanical properties of the cell wall. The results of the microtensile tests indicated that the hemicelluloses caused more damage to the mechanical properties of the cell wall than lignin. The micromechanical properties that occurred with degradation of chemical components underlined the key role of hemicelluloses in maintaining the integrity of the cell wall.

Keywords: Hemicelluloses; Lignin; Cell wall; Mechanical properties; Microtension

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INTRODUCTION

Wood cell walls are highly organized composites that contain many different polysaccharides, proteins, and aromatic substances. Recent scientific and technological advances offer new possibilities for the use of plant cell walls in the production of cost-effective biofuels (Himmel *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, *i.e.* it is recalcitrant. In view of this challenge, efforts to increase the fundamental understanding of the structure and the chemical composition of the cell wall are of great importance.

Cellulose, hemicelluloses, and lignin are the main components of the wood cell wall. The arrangement, interactions, and properties of chemical components decide 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). Duchesne *et al.* (2001) investigated the influence of cellulose and hemicellulose on fiber ultrastructure with FE-SEM and CP/MAS ^{13}C-NMR in combination with spectral fitting. They determined that increasing the hemicellulose

content was associated with changes in the ultrastructure of the fiber cell wall; they correlated this with differences in the mechanical properties of the pulps under consideration. Gindl *et al.* (2002) using UV microscopy and nano-indentation observed an increase in lignin content which contributed directly to the hardness of the wood cell wall.

Microtension measurements of single fibers provide a powerful tool for mechanical characterization of plant fibers. In this context, Jayne (1959) was one of the first researchers who performed tensile experiments on single pulp fibers. Since then, mechanical properties of different kinds of plant fibers have been tested. At the same time, sample preparation, alignment of fibers to the tensile direction, and cell wall area determinations associated with this method have improved (Burgert *et al.* 2002; Yu *et al.* 2010).

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

EXPERIMENTAL

Materials

Material was taken from the adult wood of a 42-year-old Chinese Fir (*Cunninghamia lanceolata* (Lamb.) Hook) grown in Anhui Province, China. Slices having dimensions of about 100 μm (tangential) x 10 mm (radial) x 35 mm (longitudinal) were cut with a microtome from never dried adult latewood (21 to 25 year rings). The tangential slices were randomly assigned for two treatments. The first part of tangential slices was not treated at all, and the second part was treated with selective extraction methods, which were sodium chlorite ($NaClO_2$) for delignification and sodium hydroxide solution (NaOH) at different concentrations for extraction of hemicelluloses. For delignification, three kinds of chemical solutions were chosen to delignify the wood cell wall to different extents: A) an aqueous solution of 0.3% $NaClO_2$ buffered with glacial acetic acid at pH 4.4~4.8 for 4 h at 80 ºC; B) an aqueous solution of 0.3% $NaClO_2$ buffered with glacial acetic acid at pH 4.4~4.8 for 8 h at 80 ºC; C) an aqueous solution (pH=2.7) of 150 mL of distilled water, 1.0 g $NaClO_2$ and 2.0 mL of glacial acetic acid for 8 h at 80 ºC. The removal of hemicelluloses was according to the method proposed by Nelson (1961). On the delignified samples (the delignification treatment C), a successive extraction of hemicelluloses was carried out by a treatment with (i) 6% NaOH, (ii) 6 and 8% NaOH and (iii) 6, 8, and 10% NaOH at 60 ºC for 2 h each. After all treatments the samples were washed with deionized water and stored in a refrigerator at 4 to 8 ºC. All single fibers were isolated from tangential slices using very fine tweezers under a light microscope (Burgert *et al.* 2002). The mechanically and chemically isolated fibers were gained from the first part and the second part samples, respectively, and were dried between two glass slides to avoid fiber twisting (Groom *et al.* 2002a).

FT-IR Spectroscopy

FT-IR spectra of the untreated and treated samples were collected using a Nexus 670 spectrometer equipped with a MCT/A detector. Each spectrum was recorded over the

4000 to 400cm^{-1} range, with a resolution of 4 cm^{-1}, and derived from the average of 200 scans. The range from 800 to 1800cm^{-1} was selected as reference for the normalization using OMINIC software version 7.1. Only relative changes in the averaged spectra were evaluated. Characteristic wavenumbers of cell wall polymer bands that were used for evaluating the effects of the treatments are listed in Table 1.

Table 1. Characteristic Wavenumbers of Cell Wall Polymer Bands Used for Evaluating the Effects of the Treatments

	Wavenumber (cm^{-1})	Band Assignment	Reference
Lignin	1508	Aromatic skeletal vibrations	Schwanninger *et al*. 2004
	1264	C=O stretch	Åkerholm and Salmén 2003
Xylan	1600	C=O stretch, sidegroups	Åkerholm and Salmén 2004
	1730	C=O stretch, sidegroups	Åkerholm and Salmén 2004
Glucomannan	810	In-phase ring stretching mannose residue	Marchessault 1962
Cellulose	1368	C-H bending vibrations	Stevanic and Salmén 2009
	1316	CH_2 wagging vibration	Åkerholm and Salmén 2001

Microtensile Test

Tensile tests on single fibers were performed with a custom-built microtension tester (SF-Microtester I) (Fig. 1a) (Cao *et al.* 2010; Wang *et al.* 2011). A "ball and socket" type fiber gripping was adopted for microtensile testing (Fig. 1b, c). The gripping system comprises a pair of fiber clamps that can effectively grasp the resin droplets at the ends of fibers during tension. The θ and a values are the angle and the length of shorter side of the trapezoid, as shown in Fig. 1c. Both of them can be adjusted according to the length of fibers to be tested and the stiffness requirement for the clamps, minimizing the possible deflection during tension. Two resin droplets (cold-curing adhesive, HY-914), approximately 200 μm in diameter, were placed in the center portion of each fiber with an approximate spacing of 1.2 to 1.4 mm via a fine tweezers. The capacity of load cell used was 5N. The tensile speed was 0.8 μms^{-1}. Tests were carried out under an environment of 23 ºC and 25% relative humidity (RH).

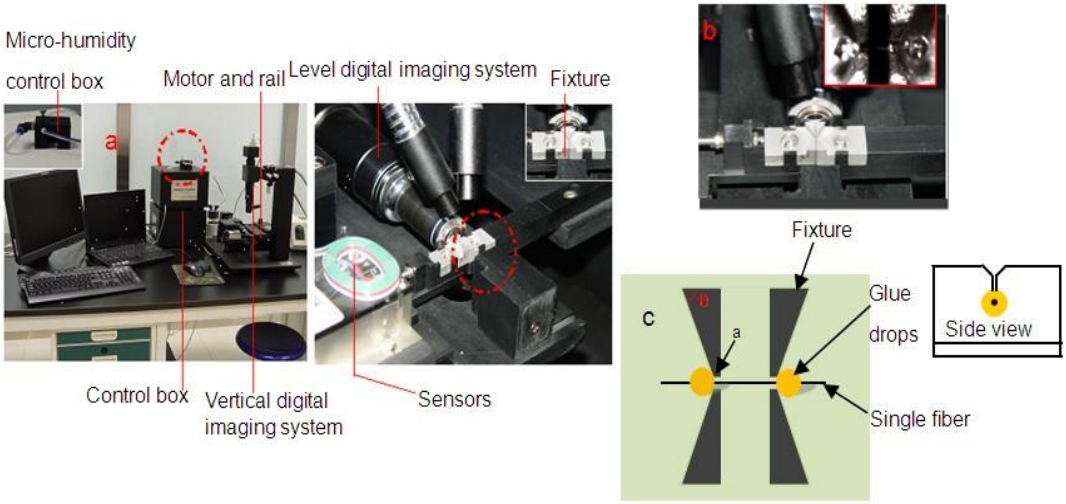

Fig. 1. Microtension test system, SF-Microtester I (a), "ball and socket" type fiber clamping (b, c)

To calculate the tensile strength and modulus of fibers, the cell wall area of every broken fiber was determined with a confocal scanning laser microscope (Meta 510 CSLM, Zeiss). More details for cell wall area measurement are found in Yu *et al.* (2010 and 2011).

In total, 54 mechanically isolated fibers and 50 to 60 fibers for each chemical treatment were analyzed.

Scanning Electron Microscopy

Fracture surfaces were imaged with a FEI-ESEM X L-30 scanning electron microscope at 7 kV after air drying for 1 day and gold coating.

Lignin Quantification

The lignin contents of untreated and chemically treated 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 sample treatments based on semi-quantitative interpretation of the cell wall chemical components. FT-IR spectra of the untreated and treated fibers are shown in Fig. 2.

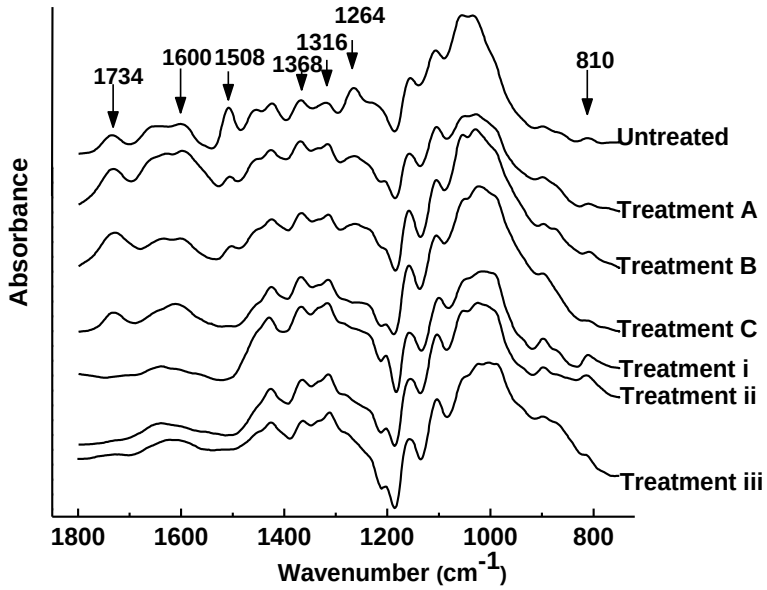

Fig. 2. IR-Spectra following different treatments

Table 2. Lignin Content after the Delignification Treatments

Sample	Lignin content /%
Untreated sample	33.61
The delignification treatment A	29.76
The deliginification treatment B	25.37
The deliginification treatment C	0.40

The 1508 cm^{-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 $NaClO_2$/glacial acetic acid, a significant reduction of the peak at 1508 cm^{-1} can be observed. This is in line with studies of lignin content determined by amounts of acid-insoluble lignin (Table 2), and the peak at 1508 cm^{-1} nearly disappeared when the lignin content decreased to 0.4% after the delignification treatment C. The same change of lignin band at 1264 cm^{-1} was also observed. The small variations seen in the spectra at bands of 1734, 1600, and 810 cm^{-1} indicated that the hemicelluloses (xylan and glucomannan) changed little during delignification treatments. After the additional treatment with NaOH, the removal of xylan was observed by the reduction of the bands at 1730 and 1600 cm^{-1}, and the degradation of glucomannan was revealed by the reduction of the band at 810 cm^{-1} (Fig. 2i, ii, iii). The subsequent treatment of the delignification treatment C sample with 6% NaOH removed the xylan. Upon treatment with 8 and 10% NaOH, a significant decrease of the peak at 810 cm^{-1} occurred. During all chemical treatments, a slight change of the bands at 1368 and 1316 cm^{-1} indicated that the cellulose was the least-changeable wood component relative to the treatments employed in this work.

Stress-Strain Curves

The typical stress-strain curves of single fibers with different treatments were presented in Fig. 3. For the individual fibers, the shape of stress-strain curve appeared to be linear during the test. No major differences were seen between the behavior of mechanically isolated and chemically treated fibers. This may be explained by the microfibrillar angle (MFA) of individual fibers. Groom (2002a) confirmed that the shape of the stress-strain curve of softwood fibers depended on MFA so 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° (Huang *et al* 2007).

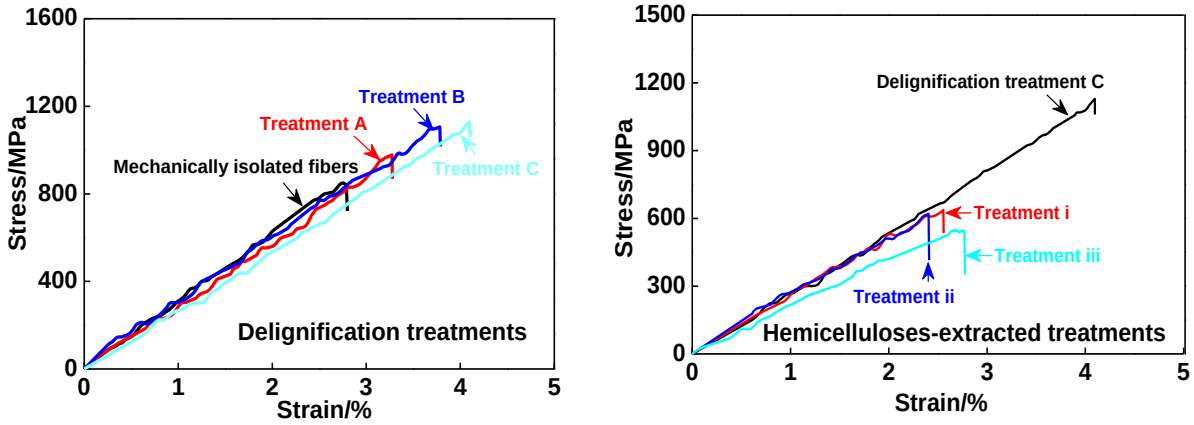

Fig. 3. Typical stress-strain curves of single fibers after different treatments

Mechanical Function of Lignin in Cell Wall

Figure 4 shows the average tensile modulus and strength values, determined from the micro-tension. A minor decreasing trend of the stiffness for single fibers was found, correlated with a decrease in the lignin content. The single fibers treated with delignification treatment C (lignin content 0.4%) method showed the lowest tensile modulus of 22.42 Gpa. Compared to that in mechanically isolated fibers, the average

tensile modulus of single fibers was reduced by 1.96, 4.74, and 5.10% with lignin content decreasing by 11, 25, and 99%, respectively (Fig. 4).

The average tensile strength of the single fibers showed an increasing trend when there was a decrease in the lignin content (Fig. 4). The average tensile strength of mechanically isolated fibers, *vs.* those isolated by treatment A, treatment B, and treatment C, was 577.43 MPa, 729.19 MPa, 775.05, and 815.21 MPa, respectively. This can be explained by the early views of Boyd (1982) concerning a lenticular microfibril arrangement, which is a structure with alternating close proximities or true aggregation of cellulose fibrils. Removal of some of the components of the matrix may cause increased cellulose aggregation (Salmén and Burgert 2009). This may be the reason that the modulus strength increased after delignification. However, in this study, the results that the tensile strengths of single fibers compared to that of mechanically isolated fibers, treated with delignification, were increased by 26.28, 34.22, and 41.18, respectively, which was beyond our expectations. The reason for this behavior is unclear. It is suggested that the mechanical isolation method may damage the inherent strength of single fibers.

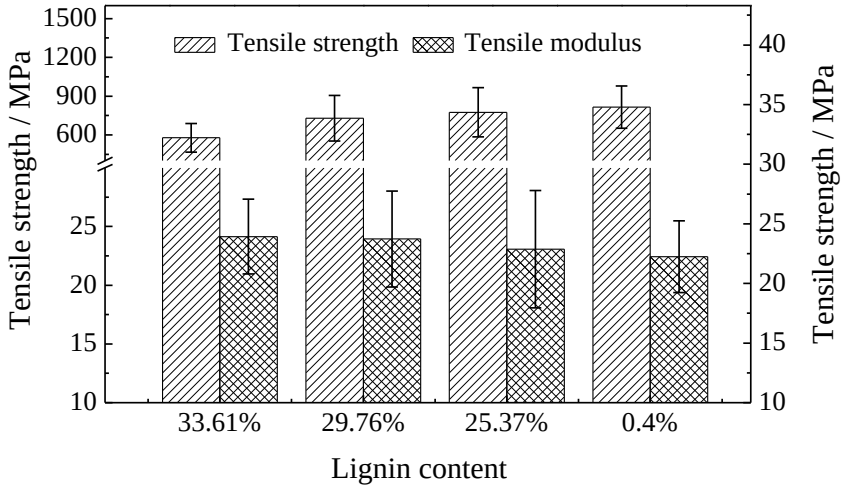

Fig. 4. Tensile modulus and strength of single fiber with delignification treatments

Mechanical Function of Hemicelluloses in Cell Wall

The tensile modulus and strength in the samples successively extracted of hemicelluloses are shown in Fig. 5. After treatment with NaOH, a clear decrease in tensile modulus and strength was apparent, compared to that in delignification treatment C samples (lignin content 0.4%). Furthermore, no major change was observed for a treatment with 6% NaOH, with 6 and 8% NaOH, and with 6, 8, and 10% NaOH. The tensile modulus obtained was 9.55% (6% NaOH), 11.08% (6+8% NaOH), and 11.57% (6+8+10% NaOH) lower in average than that obtained from delignification treatment C fibers (Fig. 5). The tensile strength of single fibers was reduced by 29.36, 30.71, and 32.15% for the hemicelluloses-extracted at 6% NaOH, 6+8% NaOH, and 6+8+10% NaOH, respectively. Thus, removal of the hemicelluloses, especially the xylan, caused more damage to the mechanical properties of the cell wall than removal of the lignin when the samples were evaluated in an air-dry condition.

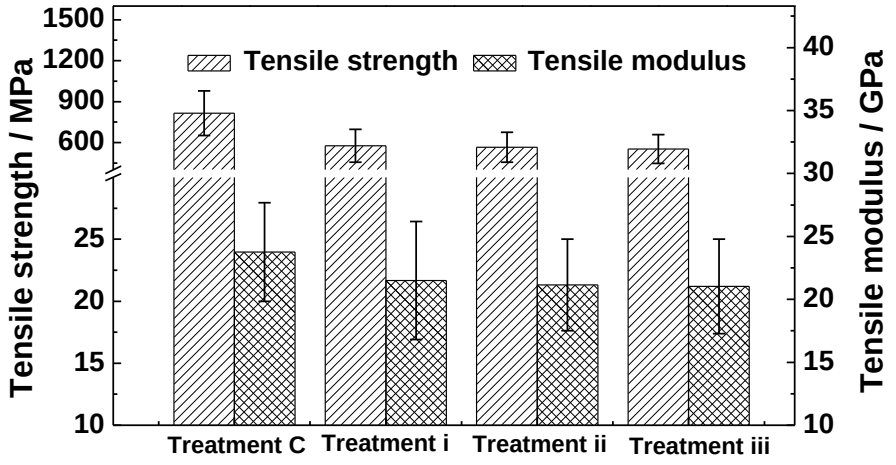

Fig. 5. Tensile modulus and strength of single fiber with hemicelluloses-extracted treatments

In the cell wall of softwood tracheids, the xylan is more closely associated with a less-condensed type of lignin, while the glucomannan is associated more with the cellulose and a condensed type of lignin (Salmén and Burgert 2009; Lawoko *et al.* 2005). It was assumed that there was progressive degradation of the hemicelluloses, that is, a loss of the glucuronic acid unit of xylan and the decomposition of glucomannan due to NaOH treatments (Fig. 2i, ii, iii). Furthermore, the xylan was degraded initially after 6% NaOH treatment (Fig. 2i). This resulted in a weakening of the flexible connections of the polymers in the cell wall, although the structure of the cellulose fibrils was rather intact. This may have been the cause of the gradual decrease in tensile modulus when there was a successive chemical treatment.

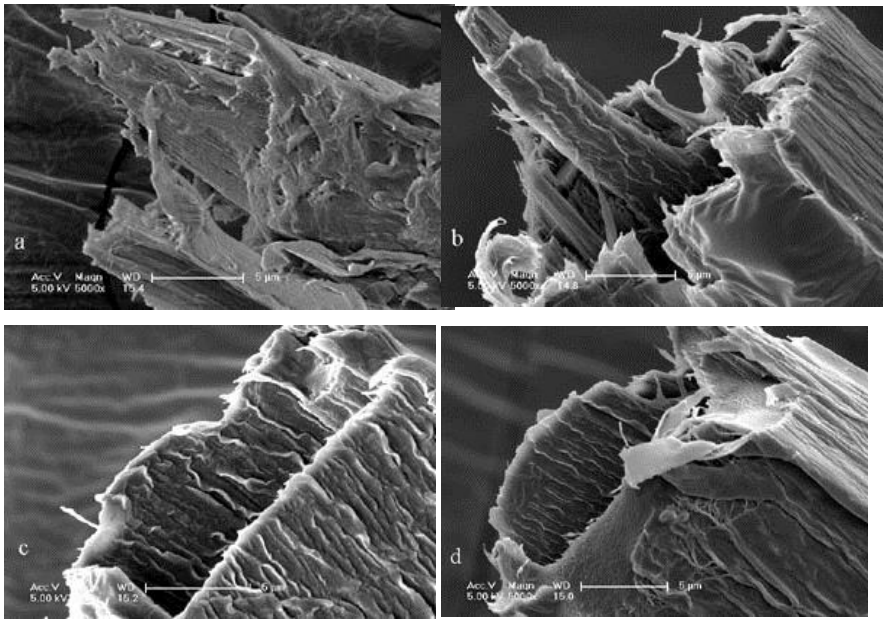

Fig. 6. Scanning electron microscope pictures of fracture surfaces of single wood fibers; a: mechanically isolated fiber; b: delignification treatment isolated fiber, c: 6% NaOH treated isolated fiber, d: 6%, 8%, and 10% NaOH treated isolated fiber

Fracture Surface Morphology of Single Fibers with Different Treatments

As shown previously (Beismann *et al.* 2000; Köhler and Spatz 2002), the character of the fracture surface is an indication of the micromechanical processes. We investigated the fracture characteristics of dry mechanically isolated fibers and different chemical treated isolated fibers, as shown in Fig. 6. Mechanically isolated fiber in the air dry state showed a rough fracture surface. The image shows an obvious example of ductile fracture failure. In contrast the fracture surface of the hemicellulose-extracted fiber was more typical of a brittle fracture. The hemicellulose-extracted fiber showed a fracture surface that was even smoother than that of mechanically isolated fiber, while the fracture surface of the fibers subjected to delignification treatment was intermediate.

CONCLUSIONS

The targeted modification method that changed the chemical components of individual fibers, while retaining the integrity of the entire cell wall was used in this study. The following changes were observed when using FT-IR spectra: a progressive degradation of lignin, xylan, and glucomannan. These chemical changes could account fully for the changes observed in the tensile modulus and tensile strength of single fibers. Microtensile tests on individual fibers showed that the decreasing tensile modulus in samples chemically treated was consistent with the degradation of lignin and hemicelluloses. The tensile strength of single fibers was increased with the lignin degradation while reduced with the hemicelluloses degradation. The removal of hemicelluloses, especially xylan, caused more damage to the mechanical properties of the cell wall than the removal of lignin. Further studies are needed to optimize the modification at the individual cell level in order to achieve a larger number of modified fibers and to investigate the mechanical performance of individual components and the cell wall structure.

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