The Spatial Orientation and Interaction of Cell Wall Polymers in Bamboo Revealed with a Combination of Imaging Polarized FTIR and Directional Chemical Removal

Jiawei Zhu

International Center for Bamboo and Rattan

Wenting Ren

Fujian Agriculture and Forestry University

Fei Guo

Fujian Agriculture and Forestry University

Hankun Wang

International Center for Bamboo and Rattan

Yan Yu (✉ yuyan9812@outlook.com)

Fujian Agriculture and Forestry University

Research Article

Keywords: Bamboo, Imaging Polarized FTIR, Cell Wall Polymers, Spatial Orientation, Polymers Interaction

Posted Date: December 29th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1076632/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Cellulose on March 8th, 2022. See the published version at <https://doi.org/10.1007/s10570-022-04506-9>.

Abstract

The mechanical and physical properties of lignocellulosic materials are closely related to the orientation and interaction of the polymers within cell walls. In this work, Imaging Polarized FTIR, combined with directional chemical removal, was applied to characterize the spatial orientation and interaction of cell wall polymers in bamboo fibers and parenchyma cells from two bamboo species. The results demonstrate the cellulose in bamboo fibers is nearly axially oriented whereas it is almost transversely arranged in parenchyma cells. Xylan and lignin are both preferentially oriented alongside cellulose, but with less orientation degree in the parenchyma cells. After lignin removal, the average orientation of xylan and cellulose is little affected, suggesting a strong interaction between cellulose and xylan. Meanwhile, the alkaline treatment significantly weakens the orientation of lignin in both fibers and parenchyma cells, and more significant for the latter, indicating the easy-degradable nature of lignin in parenchyma cells. And, it seemed the lignin and xylan in fibers were more difficult to be removed as compared to parenchyma cells, supporting the assumption that stronger interaction exists between lignin and xylan in the fibers. In a word, it was believed parenchyma cells are more suitable for biorefinery owing to its less ordered and relatively loose molecular assembly, as compared to fibers.

Introduction

Higher plants are featured with an extremely complex supramolecular cell wall assembled with cellulose, hemicellulose and aromatic polymers like lignin (Zhao et al. 2019; Tatjana et al. 2017). This structure provides the cell wall with mechanical strength (Dick et al. 2011), but also makes it inherently recalcitrant to enzymatical and chemical treatments in biorefinery scenario (Ji et al. 2017). Decades of efforts have been devoted to genetic engineering and chemical pretreatments, aiming at improving the accessibility and digestibility of lignocellulosic materials (Poovaiah et al. 2014; Vanholme et al. 2008; Loque et al. 2015). These works indeed improved the utilization efficiency of biomass. Nevertheless, our inadequate and incomplete understanding on the spatial arrangement and interaction of polymers, which significantly contribute to the properties like biomass recalcitrance and timber strength (Terrett et al. 2019), still delays the development of biorefinery industry.

The anisotropy of wood cell wall arises from the spatial orientation of cell wall polymers (Hinterstoisser et al. 2001). A well understanding of wood polymer spatial orientation has been established for wood fibers (Anne et al. 2011; Chang et al. 2014; Jasna et al. 2011) using polarized FTIR microscopy. As expected, the cellulose, in the form of microfibrils, was highly oriented along its cell axis, providing strong longitudinal mechanical strength for the fibers (Simonovic et al. 2011). The hemicelluloses (glucomannan in softwood and xylan in wood) have been shown to be highly associated with cellulose and therefore preferentially oriented along the direction of microfibrils (Jasna et al. 2011). Whereas, lignin was found to be much less oriented than glucomannan and xylan (Akerholm & Salmen 2003).

In addition, the spatial orientation of cell wall polymers is closely influenced by their interactions. Molecular dynamics simulation indicated there was abundant hydrogen bonds interaction between

cellulose microfibrils and hemicellulose (Zhang et al. 2015). Solid-state nuclear magnetic resonance (NMR) revealed a two-fold helical screw symmetry with a regular pattern of acetate or glucuronate substitutions in cellulose-bound xylan (Simmons et al. 2016; Kang et al. 2019). These evidences indicate that part of hemicellulose is attached to the surface of cellulose, which is responsible for the preferred spatial orientation of hemicellulose. The interaction between hemicellulose and lignin occurs via two different mechanisms (Terrett et al. 2019; Kang et al. 2019). Hemicellulose may incorporate into lignin by covalent bonding, usually with ferulic acid as the linkage, that is also named lignin carbohydrate complex (LCC) (Grabber et al. 1995; Jung et al. 2011; Rennie et al. 2014). As a supplement, there is electrostatic interaction between lignin and hemicellulose (Kang et al. 2019). Therefore, hemicellulose and lignin played an indispensable role in connecting cellulose microfibrils into aggregates, and had profound effect on cell wall properties and utilization, such as biomass recalcitrance and timber strength (Slabaugh 2014; Knox 2008).

Bamboo, consisting of stiff sclerenchyma fibers (account for 40% by volume) and soft parenchyma cells (account for 52% by volume) (Liese et al. 1980), is ideal renewable resource to fill the gap between wood consumption and supply. Bamboo fibers and parenchyma cells differ substantially in chemical composition and cell wall structure (Abe et al. 2010; Jin et al. 2019), optimized for their mechanical and physiological functions, respectively. Specifically, fibers possess excellent mechanical properties, while parenchyma cells are more suitable for biomass conversion (Jin et al. 2019). It was therefore speculated that fibers should be more orderly assembled than parenchyma cells, which serve more for mechanical buffer and nutrition storage.

In this work, the spatial orientation and interaction of cell wall polymers from two bamboo species were revealed with Imaging Polarized FTIR combined with directional chemical removal. The IR spectra of fibers and parenchyma cells at different polarization angles were respectively collected for polymer orientation assessment. Directional chemical component removal was applied to better explore their interactions. The aim of this work is to reveal the structural discrepancy of bamboo fibers and parenchyma cells at molecular scale, which will give new insights into their relationship of structure-property-function, as well as precise utilization.

Materials And Methods

Sample Preparation

The 3-years-old *Bambusa longispiculata* (HM) and *bambusa pervariabilis* (CG) used in this work were collected from the Hua'an Bamboo Garden in Fujian province, China. Bamboo blocks with sizes of approximately 10 (longitudinal) × 5 (tangential) × 5 (radial) mm³ were collected from the 1.5 m height location of a bamboo culm. After softening in water at 80 °C for 8 h, longitudinal sections with thickness of 20 and 35 µm. (20 µm sections for fibers spectra collection and the 35 µm ones for parenchyma cells) were prepared with a sliding microtome for Polarized FTIR microspectroscopy under transmission mode.

Six sections of each thickness from each bamboo species were made, with a total of 24 sections prepared.

Delignification and alkaline treatment

The prepared sections were divided into three groups and soaked in deionized water, 1% (w/w) acidified sodium chlorite (acidified to pH=4.5 with HAc) and 8% aqueous NaOH, respectively. They were kept in an incubator at 30 °C for 8 hours. After that, the sections were washed to pH=7.0 with deionized water. They were then sandwiched between two glass slides before being oven-dried at 60°C for 16 h.

Polarized FTIR measurement

Polarized FTIR measurements were carried out using a imaging FT-IR system (Spotlight 400, Perkin-Elmer Inc., Shelton, CT, U.S.A.) equipped with a gold wire grid polarizer under transmission mode. Before scanning, the mercury cadmium telluride (MCT) detector was cooled with liquid nitrogen and the instrument was stabilized for over 30 minutes (Jasna et al. 2011). The longitudinal sections were mounted on a sample stage, as parallel as possible to the orientation of the 0° polarization (Fig. 1). A CCD camera was used to locate the area of interest. And then, a 50 × 50 μm scanning area was selected with a spatial resolution of 6.25 × 6.25. The IR spectra were recorded with a resolution of 4 cm⁻¹ in the range of 720-1900 cm⁻¹. To improve the signal-to-noise (S/N) ratio, the scanning time was set at 64 and background signal was acquired for every sample. The IR radiation was polarized from 0° to 90° in relation to the fiber direction of samples with step intervals of 5°, therefore, 19 IR spectra for each sample were collected.

Data processing

Data processing were performed using the software MAGE-Spotlight developed by Perkin-Elmer, Inc and OMNIC 8.2.0.397 developed by Thermo Fisher Scientific, Inc. The corrections, including atmospheric correction, automatic baseline correction and baseline offset correction, were carried out to standardize the IR spectra.

The IR spectra recorded at 0° and 90° polarization angles were processed using the following Eq. 1 to produce average orientation spectrum.

$$S_o = S_{0^\circ} - S_{90^\circ} \quad (1)$$

where S_o is the orientation spectrum, implying the orientation of polymers. S_{0° and S_{90° are the absorption spectra recorded at 0° and 90° polarization angle, respectively.

The relative absorption of a specific IR peak (that represented a specific polymer) were calculated using the following Eq. 2.

$$R_A = (I_p - I_{\min}) / (I_{\max} - I_{\min}) \quad (2)$$

where R_A is the relative absorption of a specific IR peak at a given polarization angle. I_p is the absorption intensity of a specific IR peak at a given polarization angle. $I_{\max}$ and $I_{\min}$ is the maximum and minimum absorption intensity of a specific IR peak between 0° to 90° polarization angles, respectively.

Results And Discussion

Polarized IR spectra of bamboo fibers and parenchyma cells

Polarized FTIR spectra were recorded at 0° and 90° with respect to the longitudinal cell axis of bamboo fibers and parenchyma cells, and the difference spectra were collected from the two directions as shown in Fig. 1. The dominant polymers in bamboo cell wall are cellulose, hemicellulose and lignin. The associated FTIR characteristic peaks of these polymers are displayed in Fig. 2. The band at 1161 cm^{-1} is associated with the C-O-C glycosidic bond vibration corresponding to backbone of cellulose and xylan, while the bands at 1426 cm^{-1} and 1373 cm^{-1} are associated with the C-OH and C-H wagging of cellulose side group (Akerholm et al. 2004; Simonovic et al. 2011). Previous studies demonstrated that the main hemicellulose in bamboo was xylan, which is substituted by arabinose and glucuronic acid as the side chains (Peng et al. 2011). Furthermore, the bamboo xylan is highly acetylated (Terrett et al. 2019). The peak at 1738 cm^{-1} is attributed to the C=O vibration of acetyl groups and carboxyl groups in xylan (Akerholm et al. 2001). Moreover, the band at 1461 cm^{-1} (C-H wagging) is also related to xylan (Marchessault 1962).

For lignin, the bands near 1506 and 1601 cm^{-1} are both assigned to the C=C stretching vibration of aromatic rings. In addition, 1601 cm^{-1} is also associated with the C=O asymmetric stretching vibration (Faix 1991). Furthermore, IR intensity changes of cellulose, hemicellulose and lignin from 0 to 90° can be clearly observed in Fig. 2, implying the distinct spatial orientation of these cell wall polymers.

Polymer orientation in the cell walls of bamboo fibers and parenchyma cells

Polarized IR produces spectrum at one given angle (Fackler et al. 2013), resulting in a stronger absorption for the function group that is more oriented with respect to the polarized IR light (Stevanic et al. 2009). Fig. 3 exhibited the average orientation spectra of all the three cell wall polymers for fibers and parenchyma cells from both HM and CG. In the average orientation spectra, the positive signals indicate the associated functional groups arranged in a preferred orientation to the longitudinal cell axis, while the negative signals indicate a more perpendicular orientation (Jasna et al. 2011; Peng et al. 2019). Furthermore, the intensity of average orientation spectra is positively correlated to the orientation degree of a specific function group when fibers and parenchyma cells were compared. It was interested to note the polymers of fibers and parenchyma cells possessed completely opposite orientation spectra, indicating that polymers orientation of these two cells was justly opposite, with the polymers in the former oriented more parallel to the longitudinal direction of bamboo culm, and those in the latter more

oriented to the transverse direction. Ahvenainen et al (2017) measured the microfibrillar angle (MFA) of bamboo parenchyma cells to be about 65° with wide-angle X-ray scattering, which well supported our deduction.

For fiber cellulose, the high and sharp positive peaks at 1161 cm^{-1} , 1426 cm^{-1} and 1373 cm^{-1} demonstrated that the cellulose was arranged in a highly parallel orientation to the fiber axis. On the contrary, the three negative cellulose peaks indicated the cellulose in the parenchyma cell wall was arranged more transversely. The peak at 1161 cm^{-1} corresponding to C-O-C cellulose backbone (Simonovic et al. 2011), therefore, indicates a parallel orientation of this function group to cellulose polymers. The peak at 1426 cm^{-1} and 1373 cm^{-1} corresponding to cellulose side group (Akerholm et al. 2004), should be perpendicular to the cellulose polymers. However, due to the high crystallinity of cellulose, cellulose chains will squeeze each other, forcing the side group parallel to the main chains. This squeezing effect was related to the cellulose crystal d-spacing. Our previous work demonstrated that the cellulose in fibers had smaller crystal d-spacing than that in parenchyma cells (Ren et al. 2021), which could give reasonable explanation for the stronger cellulose side group orientation in the fibers.

In the case of xylan, two significant peaks at 1738 cm^{-1} and 1461 cm^{-1} were detected in Fig. 3. According to the literature (Marchessault 1962), the carbonyl group (1738 cm^{-1}) had a transition moment at an angle of 54° to the polymer backbone. The C-H in the xylan (1461 cm^{-1}) (Marchessault 1962) has a different vibrational energy of the C-H in cellulose (1373 cm^{-1}) (Simonovic et al. 2011), which makes it possible to selectively analyze the orientation of xylan and cellulose. Furthermore, it was reported that the C-H in xylan is also perpendicular to the main chain (Marchessault 1962). Hence, the negative peaks at 1738 cm^{-1} and 1461 cm^{-1} in fibers indicated that the xylan was arranged in a preferential orientation to the cellulose backbone. The xylan in the parenchyma cells also showed preferential orientation, but to a less extent as compared to that of fibers.

Lignin has two important vibration peaks (1505 cm^{-1} , 1601 cm^{-1}) suitable for analysis (Faix 1991). These vibrations occur parallel to the aromatic ring structure (Peng et al. 2019). The smaller but still distinct peaks at both 1505 cm^{-1} , 1601 cm^{-1} indicated lignin was also preferentially arranged in the cell walls of both fibers and parenchyma cells, but with less degree than the xylan. What is more, the lignin from fibers is more preferentially oriented than that from the parenchyma cells.

Figure 4 showed relative absorption change of the correlative peaks assigned to cellulose, xylan and lignin, with increased polarization angle. In this plot, the peaks with vibration oriented parallel to the cell wall axis will have stronger relative absorption at lower polarization angle, while the peaks with vibration oriented perpendicular to the cell wall axis are stronger at higher polarization angle (Peng et al. 2019; Anne et al. 2011). Compared with orientation spectra, the angular dependence of absorption intensity could be more clearly expressed (Peng et al. 2019; Chang et al. 2014). The relative absorption of all the three cell wall polymers showed distinct and stable angular dependence, valid for both fibers and parenchyma cells of the tested two bamboo species. This give stronger evidence for the preferential

orientation of cell wall polymers in bamboo. What is more, all the cell wall polymers from fibers and parenchyma cells exhibited opposite angular dependence, which further demonstrated the cell wall polymers of fibers are as a whole longitudinally oriented, while they are transversely oriented in the parenchyma cells. A closer view will further reveal the difference in angular dependence between bamboo fibers and parenchyma cells, with higher consistency for the former, indicating a more ordered polymer arrangement as a whole in the fibers, as compared to parenchyma cells.

Considering MFA is closely correlated to the cellulose orientation, the average MFA of both fibers and parenchyma cells can be estimated from Fig. 4. As shown in Fig. 4A, all the three cellulose peaks (1161 cm^{-1} , 1373 cm^{-1} and 1426 cm^{-1}) in fibers exhibited a consistent negative correlation between absorption intensity and polarization angle. Based on the high absorption of these three absorption peaks at small polarization angle ($0\text{-}10^\circ$) for the fibers and large angle ($80\text{-}90^\circ$) for the parenchyma cells, a small MFA ($\sim 10^\circ$) and a large MFA ($\sim 80^\circ$) could be estimated for the fibers and parenchyma cells, respectively. This result agrees well with the previous results by XRD measurement (Zhang et al. 2020).

Changes of polymer orientation after lignin removal

Cell walls of higher plant are featured with a tough and relatively rigid reinforced composite structure (Zhao et al. 2019). That structure could be analogous to reinforced concrete, in which cellulose fibrils act as reinforcing steel bar and hemicellulose-lignin matrix acts as the concrete (Simmons et al. 2016). Therefore, the interaction between these polymers played a significant role in the mechanical behavior of cell wall. In the present study, the orientation change of cell wall polymers after directional chemical removal was proposed to understand their interactions.

Acidified sodium chlorite is capable of directionally removing aromatic ring substances and has little effect on polysaccharides (Zhang et al. 2018). Fig. 5 shows the IR spectra of fibers and parenchyma cells (HM and CG) before and after lignin removal. The significant intensity reduction of the peaks at 1601 cm^{-1} and 1505 cm^{-1} (the C=C aromatic ring vibrations plus C=O stretch) demonstrated lignin was partially removed, while the high and sharp peak at 1738 cm^{-1} suggested xylan was much less affected. Fig. 6 presented the polymer average orientation spectra of fibers and parenchyma cells (HM and CG) after lignin removal. The peaks at 1601 cm^{-1} and 1505 cm^{-1} in the spectra of parenchyma cells almost disappeared after lignin removal. However, the residual lignin in the fibers still maintained a relatively stronger orientation, indicating interaction between residual lignin and xylan still remained in the fibers. This interaction facilitates the xylan-guided orientation of residual lignin. Therefore, it is reasonable to infer that a stronger interaction exists between lignin and xylan in bamboo fibers.

However, the average orientation of xylan (1738 cm^{-1} and 1461 cm^{-1}) and cellulose (1161 cm^{-1} and 1461 cm^{-1}) is little affected after lignin removal (Fig. 6), suggesting a strong interaction between cellulose and xylan. That is consistent with several previous studies (Simmons et al. 2016; Zhang et al. 2015). Xylan, the most prevalent non-cellulosic polysaccharide, was crosslinked to lignin, and also bound intimately to cellulose microfibrils in the cell wall (Simmons et al. 2016; Terrett et al. 2019). Advanced 13C

solid-state magic-angle spinning (MAS) 2D NMR spectroscopy directly demonstrated xylan is spatially close to cellulose via its twofold screw configuration (Simmons et al. 2016). Molecular dynamics (MD) simulation further demonstrated xylan interacted with cellulose through three typical binding modes including bridge, loop and random (Zhang et al. 2015).

Changes of polymers orientation after hemicellulose removal

Alkaline treatment, an effective method for hemicellulose extraction, have various effects on cell wall polymers including cleaving the ether and ester linkages in lignin-hemicellulose complex, as well as the ester bonds between lignin and hydroxycinnamic acid (Wen et al. 2011). Fig. 5 showed the IR spectra of fibers and parenchyma cells (HM and CG) before and after alkaline treatment. The disappearance of the peak at 1738 cm^{-1} (C=O vibration in O=C-H of acetyl groups and O=C-O of carboxyl groups) demonstrated that *O*-acetyl groups and carboxyl groups in xylan were disrupted (Wen et al. 2011, 2015), and xylan was partially removed. However, the peak intensity at 1601 cm^{-1} and 1505 cm^{-1} only slightly decreased, suggesting lignin was much less affected by alkaline treatment. Fig. 7 showed the average orientation spectra of cell wall polymers from fibers and parenchyma cells (HM and CG) after alkaline treatment. The complete disappearance of the peak at 1738 cm^{-1} was attributed to the destruction of *O*-acetyl groups and carboxyl groups in xylan.

The complete disappearance of peaks at 1601 cm^{-1} and 1505 cm^{-1} in the parenchyma cell average orientation spectra indicated the residual lignin lost its orientation after alkaline treatment. That further demonstrated the orientation of lignin was largely dominated by xylan in the parenchyma cell wall, again strongly supporting the strong interaction between lignin and xylan. In fact, decades of efforts have been devoted to elucidating the interaction between lignin and polysaccharides (Terrett et al. 2019; Jung et al. 2011). This interaction can be roughly divided into two types. One was the lignin-carbohydrate complex (LCC), which is featured with various chemical bonds including phenyl glycoside (PhGlc), benzyl ether (BE) and ester linkages (Terrett et al. 2019; Poovaiah et al. 2014). The second interaction was proposed recently based on the evidence from advanced ^{13}C solid-state MAS 2D double-quantum (DQ) correlation NMR spectroscopy, which found cross peaks in S3/5 (C3/5 in syringyl)-X4, revealing the electrostatic interactions between lignin and xylan (Kang et al. 2019). Moreover, the weak electrostatic interactions between cellulose-lignin have also been proposed via this technology (Kang et al. 2019). Differing from parenchyma cells, the lignin in the fibers still maintained a certain orientation after alkaline treatment, indicating lignin in the fibers is more tough to alkaline treatment, and also implying the difference of lignin structure between the two types of bamboo cells. Our unpublished results indicate that the lignin in bamboo parenchyma cells had a higher content of β -*O*-4 substructures and a higher S/G ratio, but less β - β and β -5 linkages than the lignin in bamboo fibers. More β -*O*-4 linkages imply the lignin in parenchyma cells is more easier to be disrupted under alkaline treatment.

The cell wall structure evolution model after lignin and hemicellulose removal

Based on the above analysis, the cell wall structure evolution model after lignin and hemicellulose removal is presented in Fig. 8. As a whole, the cellulose in bamboo fibers is nearly axially oriented whereas it is almost transversely arranged in the parenchyma cells. In the native fibers and parenchyma cells, xylan and lignin are both preferentially oriented alongside cellulose, but with different extents. The xylan is more oriented than the lignin because the former is closely associated with cellulose. The formed framework by cellulose and xylan then guided the orientation of lignin that is deposited latterly. The delignification will not significantly change the orientation of both cellulose and xylan. However, the bamboo fibers and parenchyma cells responded differently to the same procedure of delignification with more lignin remained in the former, highlighting the stronger interaction between lignin and xylan in the fibers. On the contrary, the removal of hemicellulose (mainly xylan) significantly weakens the orientation of lignin in both fibers and parenchyma cells, and more significant for the latter. It is therefore inferred xylan and lignin plays distinct roles in the bamboo cell wall, where the former participated in the construction of the so called cellulose-hemicellulose framework in the cell walls, whereas the lignin mainly acts as a filler of this framework.

Furthermore, our work found little discrepancy between HM and CG, indicating that the cell wall polymer arrangement is not related to the specific bamboo species. The different polymer arrangement characteristics between bamboo fibers and parenchyma cells should be attributed to their different cell wall functions. In particular, the stiff fibers provide mechanical support for bamboo stem, while the soft parenchyma cells contributes much less to axial mechanical strength (Malanit et al. 2011), but function as nutrition storage (Abe et al. 2010). Therefore, all the polymers in the bamboo fibers are more orderly arranged in the longitudinal direction to achieve better mechanical properties, while they were oriented perpendicular to the cell axis in the parenchyma cells with relatively weaker polymer interaction, especially between lignin and xylan.

Conclusion

In this work, Imaging Polarized FTIR microspectroscopy, combined with directional chemical removal, were applied to reveal the polymer orientation and interaction in the cell walls of two bamboo species, and significant differences were observed between fibers and parenchyma cells. All the polymers in the cell walls of bamboo are preferentially oriented but with different extents. Cellulose, as expected, is the mostly oriented polymer, followed by xylan and lignin in order. All the polymers in bamboo fibers is as a whole axially oriented whereas it is almost transversely arranged in parenchyma cells. Furthermore, the orientation degree of xylan and lignin in the parenchyma cells are not so strong as that in fibers. It seemed the lignin and xylan in fibers were more difficult to be removed as compared to parenchyma cells, according to the orientation analysis after directional chemical removal. That gives indirect but rational evidence to support the assumption that stronger interaction exists between lignin and xylan in the fibers. The results also supported the viewpoint that cellulose and xylan are combined to form the framework in the bamboo cell wall, with lignin acting as fillers. Briefly, it was believed the cell walls of fibers are more orderly and compactly assembled in bamboo, as compared to parenchyma cells, which can give reasonable explanation on the well observed higher biomass recalcitrance of bamboo fibers.

Declarations

Funding

This work was financially supported by the National Natural Science Foundation of China (31770600)

Conflict of interest The authors declare that there is no conflict of interest and that the manuscript has been approved by all authors.

References

1. Abe K, Yano H (2010) Comparison of the characteristics of cellulose microfibril aggregates isolated from fiber and parenchyma cells of Moso bamboo (*Phyllostachys pubescens*). *Cellulose* 17(2):271–277
2. Ahvenainen P, Dixon PG, Kallonen A, Suhonen H, Gibson LJ, Svedstr MK (2017) Spatially-localized bench-top x-ray scattering reveals tissue-specific microfibril orientation in moso bamboo. *Plant Methods* 13(1):5
3. Akerholm M, Hinterstoisser B, Salmen L (2004) Characterization of the crystalline structure of cellulose using statistic and dynamic FT-IR spectroscopy. *Carbohydr Res* 339:569–578
4. Akerholm M, Salmén L (2001) Interactions between wood polymers studied by dynamic FT-IR spectroscopy. *Polymer* 42:963–969
5. Akerholm M, Salmen L (2003) The oriented structure of lignin and its viscoelastic properties studied by static and dynamic FT-IR spectroscopy. *Holzforschung* 57:459–465
6. Anne O, Ingela B, Lorenz G, Lennart S (2011) Ultra-structural organisation of cell wall polymers in normal and tension wood of aspen revealed by polarisation FTIR microspectroscopy. *Planta* 233:1277–1286
7. Burton RA, Gidley MJ, Fincher GB (2010) Heterogeneity in the chemistry, structure and function of plant cell walls. *Nat Chem Biol* 6(10):724–732
8. Chang SS, Salmén L, Olsson AM, Clair B (2014) Deposition and organisation of cell wall polymers during maturation of poplar tension wood by ftir microspectroscopy. *Planta* 239(1):243–254
9. Dick M, Zhang Y, Hayes J, Salazar A, Zabolina OA, Hong M (2011) Structure and interactions of plant cell-wall polysaccharides by two- and three-dimensional magic-angle-spinning solid-state NMR. *Biochemistry* 50(6):989–1000
10. Fackler K, Thygesen LG (2013) Microspectroscopy as applied to the study of wood molecular structure. *Wood Sci Technol* 47(1):203–222
11. Faix O (1991) Classification of lignins from different botanical origins by FTIR spectroscopy. *Holzforschung* 45:21–27
12. Gierlinger N, Schwanninger M, Reinecke A, Burgert I (2006) Molecular changes during tensile deformation of single wood fibers followed by Raman microscopy. *Biomacromolecules* 7:2077–

13. Grabber JH, Hatfield RD, Ralph J, Amrhein N (1995) Ferulate cross-linking in cell walls isolated from maize cell suspensions. *Phytochemistry* 40:1077–1082
14. Hinterstoisser B, Åkerholm M, Salmén L (2001) Effect of fiber orientation in dynamic FTIR study on native cellulose. *Carbohydr Res* 334:27–37
15. Jasna S, Jasna S, Daniela D (2011) Anisotropy of cell wall polymers in branches of hardwood and softwood: a polarized ftir study. *Cellulose* 18(6):1433–1440
16. Ji G, Han L, Gao C, Xiao W, Zhang Y, Cao Y (2017) Quantitative approaches for illustrating correlations among the mechanical fragmentation scales, crystallinity ad enzymatic hydrolysis glucose yield of rice straw. *Bioresource Technol* 241:262–268
17. Jin K, Kong L, Liu X, Jiang Z, Tian G, Yang S (2019) Understanding the xylan content for enhanced enzymatic hydrolysis of individual bamboo fiber and parenchyma cells. *Acs Sustain Chem Eng* 7(22):18603–18611
18. Jung HG, Mertens DR, Phillips RL (2011) Effect of reduced ferulate mediated lignin/arabinoxylan cross-linking in corn silage on feed intake, digestibility, and milk production1. *J Dairy Sci* 94:5124–5137
19. Kang X, Kirui A, Widanage MD, Mentink F, Cosgrove DJ, Wang T (2019) Lignin-polysaccharide interactions in plant secondary cell walls revealed by solid-state nmr. *Nat Commun*, 10(1). DOI: 10.1038/s41467-018-08252-0
20. Knox JP (2008) Revealing the structural and functional diversity of plant cell walls. *Curr Opin Plant Biol* 11:308–313
21. Li MF, Chen CZ, Sun RC (2014) Effect of pretreatment severity on the enzymatic hydrolysis of bamboo in hydrothermal deconstruction. *Cellulose* 21(6):4105–4117
22. Liese W (1980) The anatomy of bamboo culms. Inbar Technical Report 1980
23. Loque D, Scheller HV, Pauly M (2015) Engineering of plant cell walls for enhanced biofuel production. *Curr Opin Plant Biol* 25:151–161
24. Malanit P, Barbu MC, Frühwald A (2011) Physical and mechanical properties of oriented strand lumber made from an Asian bamboo (*Dendrocalamus asper* Backer). *Eur J Wood Wood Prod* 69:27–36
25. Marchessault RH (1962) Application of infra-red spectroscopy to cellulose and wood polysaccharides. *Pure Appl Chem* 5:107–130
26. Peng H, Salmén L, Stevanic JS, Lu J (2019) Structural organization of the cell wall polymers in compression wood as revealed by ftir microspectroscopy. *Planta* 250:163–171
27. Peng P, Peng F, Bian J, Xu F, Sun RC, Kennedy JF (2011) Isolation and structural characterization of hemicelluloses from the bamboo species *phyllostachys incarnata* wen. *Carbohydr Polym* 86(2):883–890

28. Poovaiah CR, Nageswara M, Soneji JR, Baxter HL, Stewart CN (2014) Altered lignin biosynthesis using biotechnology to improve lignocellulosic biofuel feedstocks. *Plant Biotechnol J* 12:1163–1173
29. Ren W, Guo F, Zhu J, Cao M, Wang H, Yu Y (2021) A comparative study on the crystalline structure of cellulose isolated from bamboo fibers and parenchyma cells. *Cellulose* 28:5993–6005
30. Rennie EA, Scheller HV (2014) Xylan biosynthesis. *Curr Opin Biotechnol* 26:100–107
31. Simmons TJ, Mortimer JC, Bernardinelli OD, Pppler AC, Brown SP, Deazevedo ER (2016) Folding of xylan onto cellulose fibrils in plant cell walls revealed by solid-state nmr. *Nat Commun* 7:13902
32. Simonovic J, Stevanic J, Djikanovic D, Salmen L, Radotic K (2011) Anisotropy of cell wall polymers in branches of hardwood and softwood: a polarized FTIR study. *Cellulose* 18:1433–1440
33. Slabaugh E (2014) Cellulose synthases: new insights from crystallography and modeling. *Trends Plant Sci* 19:99–106
34. Stevanic JS, Salmén L (2009) Orientation of the wood polymers in the cell wall of spruce wood fibres. *Holzforschung* 63(5):497–503
35. Tatjana D, Sivakumar P, Markus G, Nicolai D, Malcolm O, Katharina SG, Philipp MG (2017) Insights into cell wall structure of *Sida hermaphrodita* and its influence on recalcitrance. *Carbohydr Polym* 168:94–102
36. Terrett OM, Dupree P (2019) Covalent interactions between lignin and hemicelluloses in plant secondary cell walls. *Curr Opin Biotechnol* 56:97–104
37. Vanholme R, Morreel K, Ralph J, Boerjan W (2008) Lignin engineering. *Curr Opin Plant Biol* 11:278–285
38. Wen J, Xiao L, Sun Y, Sun S, Xu F, Sun R (2011) Comparative study of alkali-soluble hemicelluloses isolated from bamboo (*bambusa rigida*). *Carbohydr Res* 346:111–120
39. Wen JL, Sun SL, Xue BL, Sun RC (2015) Structural elucidation of inhomogeneous lignins from bamboo. *Int J Biol Macromol* 77:250–259
40. Wen JL, Sun ZJ, Sun YC, Sun SN, Xu F, Sun RC (2010) Structural characterization of alkali-extractable lignin fractions from bamboo. *J Biobased Mater Bio* 4:408–425
41. Zhang B, Guo Y, Liu X, Chen H, Yang S, Wang Y (2020) Mechanical properties of the fiber cell wall in *bambusa pervariabilis* bamboo and analyses of their influencing factors. *BioResources* 15(3):5316–5327
42. Zhang N, Xiong LM, Hong Y, Chen Y (2015) Cellulose-hemicellulose interaction in wood secondary cell-wall. *Model Simul Mater Sc* 23(8):085010
43. Zhang X, Liu M, Wang HK, Yu Y (2018) Ultralight, hydrophobic, anisotropic bamboo-derived cellulose nanofibrils aerogels with excellent shape recovery via freeze-casting. *Carbohydr Polym* 208:232–240
44. Zhang X, Li J, Yu Z, Yu Y, Wang H (2017) Compressive failure mechanism and buckling analysis of the graded hierarchical bamboo structure. *J Mater Sci* 52(12):6999–7007
45. Zhao Y, Man Y, Wen J, Guo Y, Lin J (2019) Advances in imaging plant cell walls. *Trends Plant Sci* 24(9):1819

Figures

Figure 1

Micrograph of a longitudinal section of bamboo illustrating the polarisation direction of measurements. Longitudinal section of bamboo fiber (A, C) and parenchyma cell (B, D). The polarization angle is shown in C.

Figure 2

Polarized FTIR spectra of fibers and parenchyma cells from M and CG at polarization angles of 0° and 90°. CF: CG fibers, CP: CG parenchyma cells, HF: H fibers, HP: H parenchyma cells. Note: Due to the IR absorption in the wavenumber interval between 1000 cm⁻¹ to 1120 cm⁻¹ being too high, this area has been masked off in the figure.

Figure 3

The average orientation spectra of HF (A), HP (B), CF (C), CP (D). Absorption peaks, characteristic for different cell wall polymers, are indicated in each spectrum; cellulose-1161 cm⁻¹, 1373 cm⁻¹ and 1426 cm⁻¹; xylan-1461 cm⁻¹, 1738 cm⁻¹; lignin-1505 cm⁻¹ and 1601 cm⁻¹.

Figure 4

The relative absorption of IR characteristic absorption bands related to cellulose (A, B), hemicellulose (C, D), lignin (E, F), plotted against the polarisation angle for HF, HP, CF, CP.

Figure 5

FTIR spectra of fibers and parenchyma cells from M (A) and CG (B) before and after delignification and alkaline treatment. Note: Due to the IR absorption in the wavenumber interval between 1000 cm^{-1} to 1120 cm^{-1} being too high, this area has been masked off in the figure.

Figure 6

The average orientation spectra of HF (A), MP (B), CF (C), CP (D) after delignification.

Figure 7

The average orientation spectra of HF (A), HP (B), CF (C), CP (D) after alkaline treatment.

Figure 8

Schematic illustration showing the cell wall evolution of bamboo fibers and parenchyma cells after lignin and hemicellulose (mainly xylan) removal. All the polymers in fiber are nearly axially oriented while they are almost transversely arranged in parenchyma cells. After lignin and hemicellulose removal, more lignin and xylan can be remained in fibers, indicating a stronger interaction between them, as compared to parenchyma cells.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Graphicalabstract.doc](#)