Structural organisation of the wood polymers in the wood fibre structure


*Corresponding author: lennart.salmen@innventia.com

Abstract: The organisation of the major polymers in the wood fibre structure has a large impact on the properties of the structure. Numerous studies have been devoted to the cellulose microfibril arrangement, the structure providing the longitudinal strength of the fibre, while less is known regarding structural organisation of the other components, the hemicelluloses and the lignin. For the hemicelluloses, as being part of the cellulose aggregation process, indications of a strong coupling to the cellulose structure have been shown. For lignin, being lay down after the other components have been structured, no clear picture has been shown. Here the orientation of lignin vis-à-vis the cellulose orientation was examined for a number of different fibre structures. It was shown that the lignin in the middle lamella region seems to be non-oriented thus more resembling an isotropic material, while the lignin in the secondary wall of fibres is to some extent oriented. The orientation of this lignin in the secondary wall is less pronounced than the orientation of cellulose but has a preferential alignment in the direction of the fibre axis. The reason could be that lignin is only deposited in space remaining after the initial forming of the structured cellulose/hemicellulose fibrillar structure.

Keywords: cellulose, lignin, middle lamella, orientation, wood fibres.
Introduction

The structural organisation of the wood polymers within the cell wall is highly complex and still awaiting its detailed description on the ultrastructural level. The organisation and properties of the wood polymers to a large extent determines the properties of fibres and wood and the understanding of the interaction between these polymers is a key to the genetic development of improved wood and fibre quality.\[1]\n
An important aspect of the wood polymer organisation is the orientation of the different wood polymers within the mayor cell wall, the S\(_2\) wall, of the fibres. The majority of studies regarding orientation have only focused on the cellulose microfibril orientation, which dominates how the mechanical properties in the fibre direction are perceived. However, the organisation of the remaining wood polymers to a large extent influences transversal properties \[2\]. This organisation is also of importance when understanding the cell wall formation during growth. During the process of cell wall formation the hemicelluloses are deposited simultaneously with the organisation of the cellulose microfibrils \[3-4\]. Thus a high degree of association and orientation parallel to the cellulose microfibrils have been shown \[5-7\]. For the lignin, as laid down later in spaces remaining, it may be anticipated that structural restrictions may impose or restrict its organisation \[8-9\]. However, so far the picture seems not clear, with some data supporting orientation while others do not show such a clear case \[10-12\]. Thus in order to shed some more light on this question, a more in depth analyses of lignin orientation across the cell wall structure including the middle lamella as well as the secondary wall was performed using polarized FTIR. A comparison to measurements on different wood fibre structures was also made.

Polarised FTIR-microscopy has, in particular, been utilized for studies on different wood tissues for investigating the orientation of the wood polymers.\[6\] Measurements have been performed in transmission mode on microtome cut samples in the longitudinal-tangential direction of the wood as well as on single wood fibres isolated by various means. Signals specific for the main components of cellulose, lignin, glucomannan and xylan were identified and used to monitor the orientation of the polymers in relation to the main axis of the fibre.

Experimental

Materials

Wood fibre materials form different species, both of softwood and hardwood were used; softwood: Norwegian spruce \((Picea abies (L.) Karst.)\) and Serbian spruce \((Picea omorika (Panč) Purkyne);\) hardwood; maple \((Acer sp.)\) and hybrid aspen \((Populus tremula x tremuloides)\).

Norwegian spruce

A radial microtome cut of 20 \(\mu m\) in thickness from the mature part of a 40 year old spruce was prepared in order to have a section with pure middle lamella regions together with double cell wall regions. For comparison, single fibres taken from the first stage of a TMP production and treated mechanically in a disintegrator to remove the outer layers of the fibre walls, i.e. ML, P and S\(_1\) were used \[6\]. These ‘native’ fibres were thus mainly containing the S\(_2\) and S\(_3\) cell wall layers.

Serbian spruce

Fibre material was prepared by disintegration from the mature part of straight branches, free from compression wood. \[11\]

Maple

Fibre material was prepared by disintegration from the mature part of straight branches, free from tension wood. \[11\]
Hybrid aspen

Microtome sections (tangential x longitudinal) were made from the outer part of the stem of 1.5 m high hybrid aspen (Populus tremula x tremuloides; T89) trees grown in greenhouse.\textsuperscript{[12]}

Methods

FTIR microscopy measurements were carried out using a Spectrum Spotlight 400 FTIR Imaging System (Perkin Elmer Inc, Shelton, CT, USA). The area of interest was first displayed, using a visible CCD camera, to locate the cell wall area, which was then irradiated using mid-IR light. The scanning was carried out in imaging mode using an array detector, providing a pixel resolution of 6.25 µm x 6.25 µm (for the hybrid aspen only an average area of a resolution of 100 µm x 100 µm was examined), a spectral resolution of 4 cm\textsuperscript{-1} and a spectral range from 1800 cm\textsuperscript{-1} to 720 cm\textsuperscript{-1}.

Two absorption peaks were specifically selected as markers for the polymers; for lignin the 1500 cm\textsuperscript{-1} characteristic of the aromatic ring \textsuperscript{[13]} and for cellulose the 1160 cm\textsuperscript{-1} as characteristic of the carbohydrate glucosidic bond and the glucose ring \textsuperscript{[14]}. The cellulose 1425 cm\textsuperscript{-1} peak, characteristic of the C-OH bending vibration of the CH\textsubscript{2}-OH group absorbing in the direction along the chain was also chosen for comparison \textsuperscript{[15]}.

The orientation of different absorption peaks were given based on the relative absorbance, RA, calculated as Eq. 1;

\[
RA = \frac{(I_p-I_{min})}{(I_{max}-I_{min})}
\]

where \(I_p\) is the intensity of the absorbed IR radiation at a given angle of polarisation, \(I_{max}\) is the maximum intensity in the polarisation interval (0-90\degree) and \(I_{min}\) is the minimum intensity in the polarisation interval (0-90\degree). To further examine the orientation dependence of the polymers vector diagrams of the absolute absorbances were displayed.

Results and discussion

A number of different wood tissues were examined with regard to the orientation of lignin and of the carbohydrates, primarily the cellulose.

Norwegian spruce

Fig. 1 shows a light microscope picture of a radially cut earlywood section together with an FTIR image of the total absorbance of this section. Fibre wall areas and middle lamella areas may here be distinguished.

In Fig. 2 the relative absorbance and the absorbance difference (0 - 90\degree) for lignin and cellulose across the wood section is shown. The cellulose signals showed a clear orientation with only exceptional points of no orientation (absorbance difference =1). For lignin, the orientation degree was much lower with more frequent areas of no or very low degree of orientation.
Fig. 1 – Microscopic picture (bottom) of a radially cut cross section through the earlywood of an annual ring of spruce wood. Fibres are oriented top to bottom of the figure. The top figure displays the FTIR imaging picture of the total absorbance of the same region.

![Microscopic picture](image)

Fig. 2 – Specific absorbance, related to the total absorbance in each point, and absorbance difference (0 to 90°) of lignin and cellulose across a radial section of spruce wood.

![Graph](image)

Fig. 3 shows the relative absorbance for lignin and cellulose from an area of the double cell wall, here as a function of the polarisation angle. It is obvious that the cellulose showed a strong indication of an orientation of the microfibrils at an angle close to zero, i.e. corresponding to the longitudinal fibre axis. For the lignin the orientation was less precise although a preference for an orientation of the phenylpropane units in the longitudinal direction of the fibre, i.e. in the direction of the cellulose microfibrils seems clear.
If instead observing the orientation dependence of the total absorption, as in Fig. 4, it is clear that, for both the lignin and cellulose peak, there was a large absorption also at 90° polarisation. The cellulose shows a much higher degree of orientation than that of the lignin. A component of oriented lignin is however quite clear from the comparison with the orientation distribution for a fully isotropic material; the dotted line. One must also here keep in mind that IR spectra often contain overlapping peaks, or peaks that are not fully resolved. Thus, the lignin absorption peak at 1500 cm\(^{-1}\) is to some extent influenced by the orthogonally oriented CH\(_2\) vibration of xylan\([16]\) at 1460 cm\(^{-1}\). As the xylan is oriented along with the cellulose the 90° oriented CH\(_2\) peak at 1460 cm\(^{-1}\) will be low at 0° and high at 90° counteracting the orientation signal seen for the lignin at 1500 cm\(^{-1}\). For the cellulose, all peaks are heavily overlapping. To illustrate the relations one may look at the cellulose C-OH bending vibration at 1425 cm\(^{-1}\) which shows a distinct peak at all polarisation angles. In Fig. 5 the orientation dependence is compared for the total absorption (peak to baseline) of the 1425 cm\(^{-1}\) vibration with that of the 1425 cm\(^{-1}\) peak itself (taken from a line connecting the valleys of each side of the peak at 1445 cm\(^{-1}\) and 1400 cm\(^{-1}\) respectively). Obviously such an estimation of the contribution of the absorption at 1425 cm\(^{-1}\) is somewhat underestimating the actual contribution from the C-OH bending as a large portion of the absorption signal is neglected (the difference between the signal of total absorption and peak absorption in Fig. 5) as some of this absorption must be attributed to the 1425 cm\(^{-1}\) vibration. From the behaviour of the peak absorption in Fig. 5 it is though clear that a very distinct orientation of the cellulose microfibril around 0° to 5° may be estimated, the angle of the maximal vector length.

![Graph showing orientation distribution of absorbance for lignin and cellulose in a double cell wall area of spruce wood.](image)

**Fig. 3** – Orientation distribution of absorbance for lignin and cellulose in a double cell wall area of spruce wood. Peak values for different polarisations were normalized to the interval from 0 to 1.
Fig. 4 – Polar diagram (0 to 90°) of the absolute absorbance of lignin and cellulose for a double cell wall area of spruce wood. 0° polarisation is in the longitudinal fibre direction. The dotted line represents non-oriented substances.

Fig. 5 – Polar diagram (0 to 90°) of the absolute absorbance as well as the peak absorption for the cellulose C-OH bending peak at 1425 cm⁻¹ for the secondary wall of a TMP fibre. 0° polarisation is in the longitudinal fibre direction. The dotted line represents non-oriented substances.
In Fig. 6 the orientation dependence for the polymers in the compound middle lamella region is shown. Due to the size of the pixel resolution of the imaging FTIR of 6.25 µm x 6.25 µm there is an overlap to the primary, S₁ and S₂ walls. It is here clear that the lignin in the middle lamella region shows a complete lack of orientation (the orientation distribution vector is the same in all directions; the dotted line), i.e. the lignin is isotropic. For the cellulose two different orientations are seen, one at about 90° and one being 0°. It could here be speculated that the 90° oriented components could come from microfibrils in the S₁ layer, while the 0° contribution would come from a part of the S₂ wall being incorporated in the view.

Serbian spruce
In Fig. 7 the orientation distribution is illustrated for fibres from a branch of Serbian spruce. Evidently there is here a clear component of the lignin that is oriented more or less in the direction of the fibre axis and the cellulose microfibrils. It is at the same time clear that some parts of the lignin are less organised displaying a more isotropic behaviour.

Hybrid aspen
The orientation distribution of lignin and cellulose in a hybrid aspen is displayed in Fig. 8. In this case the area examined is composed of both the cell wall areas as well as the middle lamella region connecting the fibres. Although a component of lignin orientation in the direction of the cellulose direction and the fibre direction is visible, it is clear that the isotropic component is much larger than for the other fibre samples examined. This fact could well be based on the fact that the lignin signal is a mix of an totally isotropic middle lamella region and the secondary wall region displaying some degree of orientation of the lignin.

![Absorbance 0°](image.png)
It seems clear from the results here obtained that some parts of the lignin in the secondary cell wall structure of all fibre types examined shows an orientation distribution. This part of the lignin seems to have an orientation in the direction of the cellulose microfibrils and the fibre axis. The structure of the cellulose microfibril/hemicellulose aggregates serving as the template when lignin is deposited are in the secondary wall not fully straight but possesses some degree of undulation. This could probably also account for some of the variation in orientation seen for the cellulose component. The undulating structure results in that pores are created with an aspect ratio larger than one in the direction of the cellulose microfibrils, i.e. the pores are longer in the direction of the fibre axis, see Fig. 10. Due to the limited size of these pores it is to be expected that when lignin polymerises in the spaces between the
carbohydrate aggregates an orientation in the longitudinal direction of the pores will be favoured due to the spatial limitations. Strong non-covalent attractive interactions between the phenyl rings and the OH-groups of the carbohydrates may also be expected to contribute to an orientation in the direction of the cellulose microfibrils. Molecular dynamics modelling have also indicated a structured orientation of lignin model oligomers in parallel to carbohydrate surfaces.\cite{18}

For the lignin polymerised in the middle lamella region such constrains do not exist why a more random structure may be created. The lignin macromolecule is highly flexible, probably due to the large amount of ester bonds and can easily assume many different stable or meta-stable conformations, resulting in reversible dramatic changes in molecular size and volume. \cite{19} Thus the non-oriented structure of the lignin indicted from Fig. 6 seems as a confirmation of such an random lignin structure.

**Conclusions**

It is here shown that the lignin in both the tree trunk and in its branches for both hardwood and softwood species are to some extent structurally ordered with a preferred directionality following that of the cellulose microfibrils and the direction of the fibres. It is believed that this structuring of the lignin might be the result of the structural constrains within the cell wall as occurring due to the previously deposited cellulose/hemicellulose organisation of a strongly oriented assembly.

Contrary it is here indicated that the lignin deposited within the middle lamella region display a totally isotropic, i.e. non-oriented structure. The differences in the organisation of these two types of lignin should clearly have an impact on both mechanical as well as chemical properties of the different cell wall structures.

**Acknowledgements**

This work was funded in part by Biomime, the Swedish Centre for Biomimetic Fibre Engineering (a collaboration between the Schools of Biotechnology and Chemical Science and Engineering at The Royal Institute of Technology (KTH), the Umeå Plant Science Centre (UPSC) and Innventia) and in part by the Wallenberg Wood Science Center (WWSC) of KTH and Chalmers University. Grant 173017 of the Ministry of Science and Technology of the Republic of Serbia also supported this study.

Fig. 9 – Polar diagram (0 to 90°) of the absolute absorbance of lignin and cellulose from a branch fibre of maple. 0° polarisation is in the longitudinal fibre direction. The dotted line represents non-oriented substances.
Fig. 10 – Schematic structure of the secondary cell wall of (softwood illustrated) tracheids with the undulating cellulose/hemicellulose aggregate structure oriented in the fibre direction. The spaces left over after this initial deposition will structure the organisation of the lignin molecules deposited afterwards.

References

14. Liang, C. Y.; Marchessault, R. H. Infrared spectra of crystalline polysaccharides. II. Nativ
celluloses in the region from 640 to 1700 cm\(^{-1}\). *J. Polym. Sci.* 1959 39: 269-278.


