



Short Communication

A combined FT-IR microscopy and principal component analysis on softwood cell walls

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Abstract

A combined FT-IR microscopy and principle component analysis was used to investigate chemical variations between softwood species as well as types of wood cell walls; latewood tracheids, earlywood tracheids and earlywood ray parenchyma cells. The method allowed us to detect small spectral differences between cell types rather than species and to predict characteristic chemical components of each cell type. The method enabled information to be obtained which allowed a evaluation of the polysaccharide composition even in lignified woody plant cell walls.

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1. Introduction

The chemical nature of cell walls have a great influence on their mechanical function, therefore, a number of authors investigated the chemical composition and distribution of wood cell walls. In early studies of the polysaccharide composition of different wood cells, the samples were separated by screening and subjected to analysis of sugar residues using paper chromatography or gas chromatography (Hoffmann & Timell, 1972; Meier & Wilkie, 1959). These traditional methods can give information of great precision, but are not suited to the analyses of a large number of samples.

Spectroscopy analysis is extremely rapid and non-destructive. It has the ability to probe interactions between macromolecules. The use of microscopy in combination with spectroscopy enables regions as small as $50\ \mu\text{m} \times 50\ \mu\text{m}$ to be investigated. In recent years, because of improvements in instrumentation and computing, chemometrics, a combined FT-IR and statistical analysis, has become a powerful tool for biochemical analysis of plant cell walls. For instance, FT-IR microscopy was applied to cell walls of *Arabidopsis* and flax mutants (Chen et al., 1998). They applied a rapid screening method for a large number of phenotypes. Chemometrics was

applied to olive pulp cell walls after sequential extraction (Coimbra, Barros, Rutledge, & Delgado, 1998). This allowed polymers such as pectic polysaccharide rich in uronic acid, pectic polysaccharides rich in arabinose, arabinose-rich glycoproteins, xyloglucans, and glucuronoxylan to be distinguished.

In this study, a combined FT-IR and statistical analysis approach was applied to lignified softwood cell walls in order to test the potential of this method to give information on polysaccharide composition.

2. Materials and methods

2.1. Wood samples

Twenty-three samples (15 softwood species) were prepared: *Picea sitchensis*, *P. likiangasis*, *P. complanata*, *P. purpurea*, *P. glehni*, *P. jezoensis*, *Abies sachalinensis*, *A. firma*, *Chamaecyparis pisifera*, *Cryptomeria japonica*, *Cunninghamia konishiki*, *Pinus densiflora*, *Taiwania cryptomeria*, *Tsuga formosana* and *Thujopsis dolabrata*. Sections ($5\ \mu\text{m}$) were cut and dried between two slide glasses, then mounted on a BaF₂ window. Measurements were repeated three times for each cell type, i.e. latewood tracheids, earlywood tracheids and earlywood ray parenchyma cells. Spectra were obtained at a resolution of $8\ \text{cm}^{-1}$ and collected

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in transmission mode from 4000 to 700 cm^{-1} using a FT-IR (Shimadzu 8200, Japan) equipped with a microscopy accessory (Shimadzu AIM-8000, Japan).

2.2. Reference samples

In order to assign the absorbance of polysaccharides in a spectrum, we referred to the published data (Coimbra et al., 1998; Coimbra, Barros, Rutledge, & Delgadillo, 1999; Kačuráková, Capek, Sasinklová, Wellner, & Ebringerová, 2000), and we also prepared the following six samples for our own calibrations. Acetylglucomannan (Ohnishi, Watanabe, & Koshijima, 1992; Watanabe, Azuma, & Koshijima, 1987), which is a major hemicellulose of softwood, milled wood lignin and softwood xylan (prepared from *Chamaecyparis obtusa*) were used. Pectin from citrus (Wako Chem., Japan) and microcrystalline cellulose (Avicel, Asahi Kasei Co., Japan) were of commercial grade. Sample (1 mg) was mixed with 100 mg of KBr in a mortar and pressed into a transparent pellet. FT-IR spectra were obtained at a resolution of 4 cm^{-1} in transmission mode (Shimadzu 8200, Japan).

2.3. Spectral analysis

Area-normalized and smoothed spectra in the region of 1200–800 cm^{-1} after conversion to absorbance mode were subjected to secondary derivatization. From these spectra consisted of 209 objects (spectra) and 109 variables (wavenumber), PCA was performed with a software unscrambler (CAMO ASA, Norway).

3. Results and discussion

3.1. Grouping

A softwood comprises mainly of three types of cells; earlywood tracheids, latewood tracheids and ray cells as indicated in a typical wood section (Fig. 1). Area-normalized spectra in the region 1200–800 cm^{-1} obtained from wood samples are shown in Fig. 2a. The region at 1200–800 cm^{-1} , which is dominated by stretching vibrations of C–O, C–C, ring structures and deformation vibrations of CH_2 groups (Liang & Marchessault, 1959; Tsuboi, 1957), was found to be useful for the identification of polysaccharides (Kačuráková et al., 2000). The second derivative, which is known to be beneficial in highlighting the difference between spectra (Michell, 1990), was obtained from the area-normalized spectra and is shown in Fig. 2b. Four strong negative peaks were noticeable near 1164, 1110, 1060, 1033 cm^{-1} in the secondary derivative mode, but the differences between the spectra were small.

Principal component analysis (PCA) is one of the most common multivariate techniques. The purpose of this method is to decompose the data matrix and concentrate the source of variability in the data into the first few PCs.

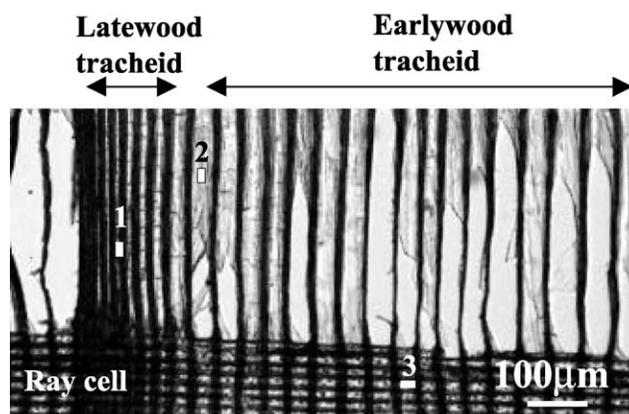


Fig. 1. A typical sample image of a wood section. The small white areas indicate the areas scanned by FT-IR. Notes: 1. Latewood tracheid. 2. Earlywood tracheid. 3. Ray parenchyma cell.

The scatter plots of PC1 (variability; 62%) \times PC2 (variability; 13%) and PC2 \times PC3 (variability; 9%) are shown in Figs. 3 and 4, respectively. Despite the difference between wood species, the plots gave information about groups of cell types. This may indicate that the difference between chemical composition is larger between cell types within species than between species. The scatter plot of PC1 against PC2 (Fig. 3) shows differentiation between earlywood tracheids and latewood tracheids. In short, earlywood samples clustered in the negative direction of PC1, while latewood samples were almost always in the positive direction of both PC1 and PC2. In contrast, the ray cells were scattered over the whole of PC1 range. In the plot of PC2 against PC3 (Fig. 4), the ray cells mainly appeared in the quadrant where both PC2 and PC3 were negative.

3.2. Interpretation of PC loadings

These groupings may be caused by differences in the chemical components of each cell type. We attempted to interpret each PC loading in terms of chemical composition.

The loading of PC1 (Fig. 2c) had a strong positive correlation at 1164 (1-1), 1110 (1-2), 1060 (1-3), 1033 (1-4) cm^{-1} . Similar peaks are present in standard samples shown in Fig. 5a (microcrystalline cellulose: 1164–1160(a1), 1114–1110(a2), 1060(a3) and 1033(a4) cm^{-1}) and Fig. 5b (acetylglucomannan: 1060(b2) and 1030(b3) cm^{-1}). Glucomannan from softwoods is an essentially linear molecule. It has a backbone chain consisted of D-glucopyranose and D-mannopyranose residues linked together by β (1-4)-linkages. Thus the backbone resembles that of cellulose, but differs from it both in terms of the presence of mannosyl residues and in its shorter length. This structural similarity as well as the simultaneous response in the dynamic mechanical FT-IR analysis (Åkerholm & Salmén, 2001), indicate an intimate association between

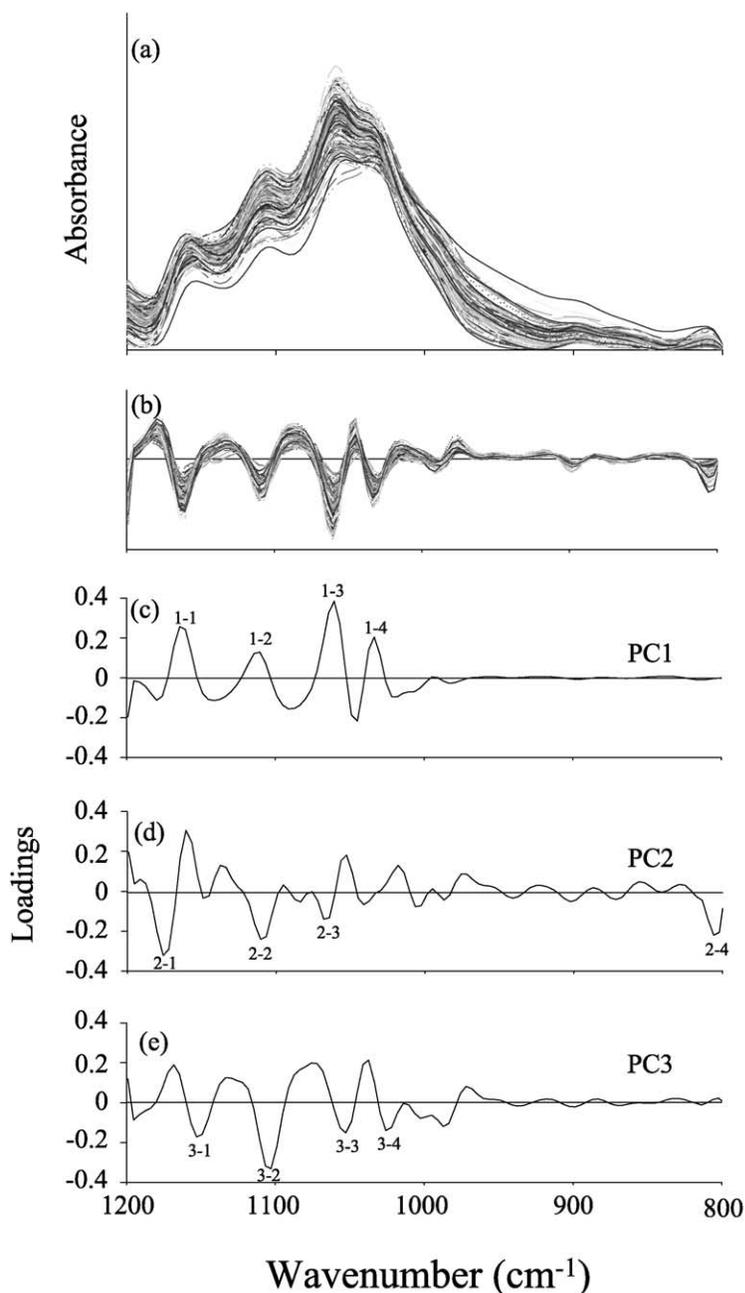


Fig. 2. (a) FT-IR spectra; (b) secondary derivative peaks; (c) score loadings of PC1. The bands labeled 1-1 to 1-4 correspond to wavenumbers near 1164, 1110, 1060 and 1033 cm^{-1} . (d) Score loadings of PC2. The bands labeled 2-1 to 2-4 correspond to wavenumbers near 1170, 1110–1106, 1068–1064 and 806–802 cm^{-1} . (e) Score loadings of PC3. The bands labeled 3-1 to 3-4 correspond to wavenumbers near 1152, 1102, 1052 and 1025 cm^{-1} .

cellulose and glucomannan in softwood. In addition, the strong band derived from OH group in glucose is reported to appear at 1035 cm^{-1} (Kačuráková et al., 2000), which is present in PC1 loading as well as cellulose and glucomannan standards. In short, PC1 has a positive relationship with cellulose. The involvement of glucomannan is still speculative but its intimate association with cellulose suggests cooperative influence to the IR spectrum.

The plot of PC2 against PC3 in Fig. 4 also revealed groupings but the interpretation of the loadings was not

straightforward. In this scatter plot, the difference between tracheids and ray cells was pronounced. The cluster of ray cells appeared in the quadrant, where both PC2 and PC3 are negative. The significant bands of negative loading in PC2 were at 1170 (2-1: this band does not exist in the samples and has, thus been omitted), 1110–1106 (2-2), 1068–1064 (2-3) and 806–802 (2-4) cm^{-1} as shown in Fig. 2d. A notable band is 1068–1064 (2-3), which is slightly shifted from 1060 (1-3) cm^{-1} of cellulose. The bands at 1065 (2-3) (Coimbra et al., 1999) and 1068 cm^{-1} (Kačuráková et al., 2000) were assigned to arabinose, and therefore

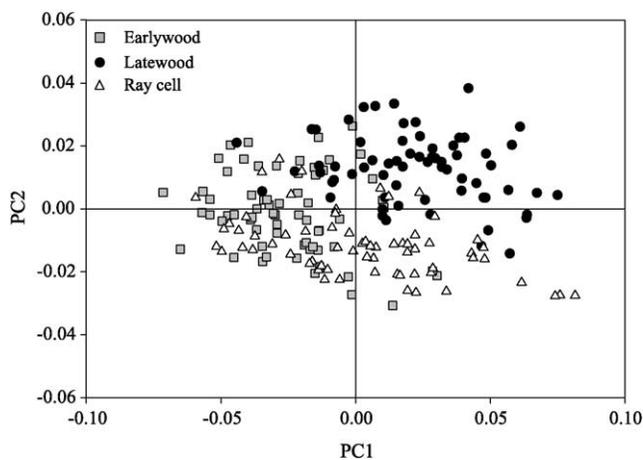


Fig. 3. PCA score plots (PC1 \times PC2) for wood cells.

the substance having arabinose units would have a negative influence on the PC2 values.

The strong negative loadings of PC3 are the bands at 1152 (3-1), 1102 (3-2), 1052 (3-3) and 1025 (3-4) cm^{-1} (Fig. 2e). Some of these bands are noticeable in the pectin standard spectrum, which has characteristic bands at 1153–1145 (c1) and 1049 (c4) cm^{-1} as in Fig. 5c. Moreover, the bands at 1150 and 1104 cm^{-1} were reported to be the main regions of absorbance for uronic acid (Coimbra et al., 1998, 1999). In short, negative values of PC2 and PC3 seemed to represent substances including arabinose units as well as pectic polysaccharide. However, attributing the influence factors to the minor cell wall components is not straightforward and the statement here is rational but speculative unless further chemical evidence is available.

To summarize, a combined FT-IR and PCA analysis is a powerful method for obtaining information for a quick evaluation of the polysaccharide composition even from lignified woody plant cell walls. Interestingly the grouping by cell wall composition was not based on wood species but cell types. The higher proportion of cellulose in earlywood tracheid than in late wood was suggested from paper

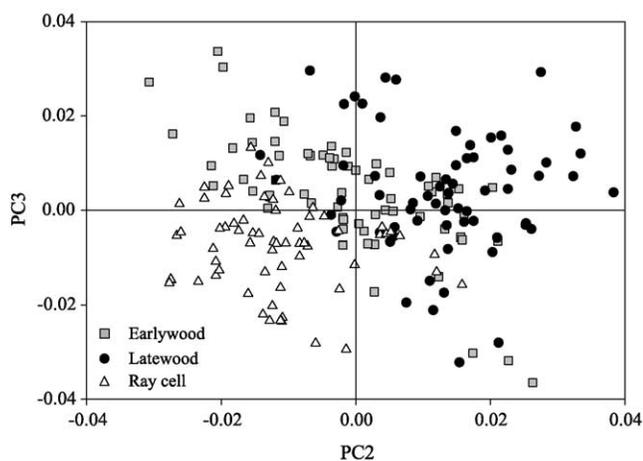


Fig. 4. PCA score plots (PC2 \times PC3) for wood cells.

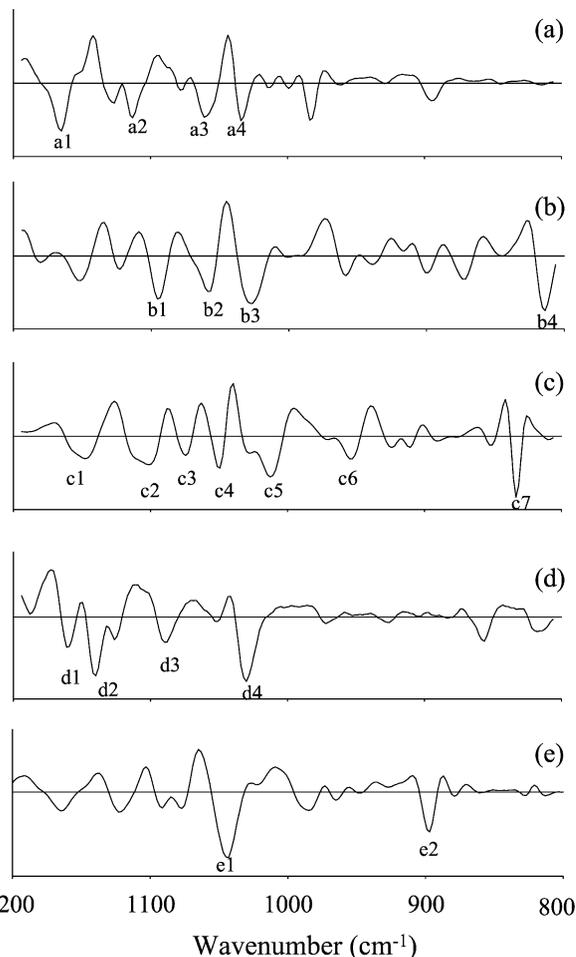


Fig. 5. Second derivative spectra from standard samples. (a) Microcrystalline cellulose. The bands labeled a1–a4 correspond to wavenumbers near 1164–1160, 1114–1110, 1060 and 1033 cm^{-1} . (b) Acetylglucosaminan. The bands labeled b1–b4 correspond to wavenumbers near 1094, 1060, 1030–1026 and 814 cm^{-1} . (c) Pectin. The bands labeled c1–c7 correspond to wavenumbers near 1153–1145, 1108–1097, 1074, 1049, 1010, 953 and 833 cm^{-1} . (d) MWL. The bands labeled d1–d4 correspond to wavenumbers near 1161, 1140, 1088 and 1030 cm^{-1} . (e) Softwood xylan. The bands labeled e1–e2 correspond to wavenumbers near 1043 and 897 cm^{-1} .

chromatograms (Larson, 1966), while the cellulose content in ray cells were more variable and it is probable that they contain more pectic materials. This combined method may be applicable to other sets of samples, for example, samples of different physical properties or different parts of a tree having different functions.

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