Effect of compression combined with steam treatment on the porosity, chemical composition and cellulose crystalline structure of wood cell walls

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A B S T R A C T
The changes of porosity, chemical composition and cellulose crystalline structure of Spruce (Picea abies Karst.) wood cell walls due to compression combined with steam treatment (CS-treatment) were investigated by nitrogen adsorption, confocal Raman microscopy (CRM) and X-ray diffraction (XRD), respectively. A number of slit-shaped mesopores with a diameter of 3.7 nm was formed for the CS-treated wood, and more mesopores were found in the steam-treated wood. CRM results revealed cellulose structure was affected by treatment and β-aryl-ether links associated to guaiacyl units of lignin was depolymerized followed by re-condensation reactions. The crystallinity index (CI) and crystallite thickness (D 200) of cellulose for CS-treated wood were largely increased due to crystallization in the semicrystalline region. Higher degree of increase in both CI and D 200 was observed in both earlywood and latewood of steam-treated wood, ascribing to the greater amount of mesopores in steam-treated wood than CS-treated wood.

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1. Introduction

Wood is the most abundant natural and renewable materials on earth, and has a long history to be widely used for construction, furniture and packaging industry. However, the utilization of wood, especially the plantation wood, is restricted by its inherent limitations in its properties, such as poor dimensional stability and durability, low density and strength (Draž, Franz, Namyslo, & Kaufmann, 2015; Wei, Stark, & McDonald, 2015; Wikberg & Maunu, 2004). Considerable wood modification technologies were attempted in order to improve the performances of wood based materials. Great efforts are devoted to develop more environmentally benign and efficient modification methods (Fang, Mariotti, Cloutier, Koubaa, & Blanchet, 2012). Among them, compression combined with steam treatment (CS-treatment, or thermo-hydro-mechanical treatment) was able to improve the dimensional stability, durability as well as the mechanical strength of wood without deteriorating its advantages (Kutnar & Kamke, 2012). During CS-treatment, high temperature steam with more effective heat transfer than conventional heat treatment was applied to obtain a permanent fixation of compressive deformations. This can overcome the problem of typical compression treated wood that the compressive deformation is unstable and the structure can be easily recovered (completely/partially) after reheating and water/moisture exposure (Dwianto, Morooka, & Norimoto, 2000; Kekkonen, Ylisassi, & Telki, 2014). Hence, CS-treatment could transform wood into a new material with desired stability (Popescu, Lisa, Froidevaux, Navi, & Popescu, 2014).

Significant strides have been made in elucidating the changes in the macroscopic characteristics e.g., color and shape, dimensional stability and mechanical properties of wood materials after CS-treatment (Cai, Yang, Cai, & Sheldon, 2013; Inoue, Norimoto, Tanahashi, & Rowell, 1993; Lesar, Humar, Kamke, & Kutnar, 2013; Navi & Girardet, 2000). However, it was unclear about the mechanism caused by steam degradation and the rearrangement of bio-molecules under compressive condition. Changes in physical and chemical structure on a molecular level have not been fully characterized and understood. It is therefore desirable to monitor

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the decomposition of the polymer structure in the cell wall and gain knowledge of property changes in CS-treated wood.

In addition, wood is a cellular biomaterial with complex multi-component structure. The cell wall is primarily comprised of cellulose, hemicellulose and lignin. Cellulose molecules form microfibrils aggregates that are embedded in a soft matrix of hemicellulose and lignin (Salmen & Burger, 2009). The properties of wood are highly dependent on the chemical compositions, the orientation of cellulose microfibrils, and the molecular interactions of the cell wall polymer assembly (Burgert, 2006; Wei & McDonald, 2016).

In our previous studies (Guo, Song, Salmen, & Yin, 2015; Yin, Berglund, & Salmen, 2011), it was shown that CS-treatment and steam treatment led to the changes of chemical components, especially hemicelluloses, in wood cell walls. Specifically, a progressive degradation of the carbonyl groups in the glucuronic acid unit of xylan and the mannose units in the glucomannan would occur. This resulted in the decrease of moisture absorbing sites and the linkages among the polymers in the wood cell wall, which are wholly accounted for changes of the hygroscopicity and indentation modulus of the wood cell wall. However, the degradation pathways of cellulose and lignin have not been clarified. It is known that the origins of cellulose molecular chains, such as the crystalline, semicrystalline and amorphous regions, have various effects on the characteristics of wood (Inagaki, Siesler, Mitsui, & Tsuchikawa, 2010). In addition, the crystalline regions of cellulose exhibit higher density and lower chemical reactivity, and can determine the mechanical properties of plant cell wall (Wei, McDonald, & Stark, 2015; Wei & McDonald, 2016). Moreover, each glucopyranose ring of cellulose has three hydroxyl groups on average, implying cellulose is highly hydrophilic material (Kulasinski, Guyer, Keten, Derome, & Carmeliet, 2015; Wei, Liang, & McDonald, 2015). Lignin is biosynthesized from three basic monomeric units, syringyl, guaiacyl, and p-hydroxyphenyl alcohol. Lignin is a highly amorphous macromolecule that plays an important role of supporting material in wood cell wall (Song, Yin, Salmen, Xiao, & Jiang, 2014).

Therefore, gaining more knowledge about the structure of cellulose and lignin should provide insight into the mechanism of cell wall changes responding to CS-treatment. Furthermore, wood cell walls exhibit large mesopores because the spaces between the cellulose microfibrils could be partially filled by lignin, hemicellulose and extractive (Yin, Song, Lu, Zhao, & Yin, 2015). The structure of mesopores in wood cell wall could affect the sorption and transport of moisture. Any changes in the mesopore structure due to CS-treated modification could determine the properties of modified wood.

In this paper, the effects of CS-treatment on porosity, chemical composition and cellulose crystalline structure on a molecular level were studied in details. Nitrogen adsorption was studied for the mesopore structure upon treatments. Confocal Raman microscopy (CRM) was used to investigate the chemical composition distribution in wood cell wall including the secondary cell walls (S) and cell corners (CC). X-ray diffraction (XRD) was used to characterize the cellulose crystalline structure. Differences between the earlywood and latewood were explored as well. The results would promote the understanding of the effect and mechanism of degradation and rearrangement of chemical composition, especially cellulose, during CS-treatment.

2. Experimental

2.1. Materials

Small specimens (dimensions were 20 × 20 × 25 mm in the tangential (T), radial (R), and longitudinal (L) directions, respectively) cut from Spruce (Picea abies Karst.) sapwood section were subjected to CS-treatment in a laboratory-scale autoclave as described in our previous studies (Guo et al., 2015). To be more specific, the CS-treatment was conducted with a compression ratio of 50% in radial direction (the percentage of the decrease in thickness to the initial thickness of the specimen) at 110 °C for 6 min, followed by a steam process at 160 °C for 30 min. For comparison, specimens were treated with steam only without using compression treatment in the autoclave at 160 °C for 30 min. All the treated samples were put into a preheated autoclave, with pressurized steam being applied to regulate the corresponding prescribed temperature. The treated samples were cooled down to room temperature inside the autoclave and then conditioned to an equilibrium moisture content close to 12% by storing them in a conditioning room maintained at 20 °C and 65% relative humidity for >20 days.

Small wood pieces containing whole growth rings with an age of 30 years were cut from the surface of the treated samples and then separated into five samples, see Fig. S1 (Supplementary). Sample 1 (S1) was divided into earlywood and latewood with aid of a stereo-microscope (66E, Leica, Germany) and then cut into wood sticks (5 mm in longitudinal direction, 1 × 1 mm² in cross section) for nitrogen adsorption measurement. Sample 2 (S2) was sliced in radial directions (200 µm thickness) for examination with a field emission scanning electron microscope (FE-SEM). Sample 3 (S3) was sliced in radial directions (10 µm thickness) for atomic force microscope (AFM). Sample 4 (S4) was sliced in cross sections (10 µm thickness) for CRM analysis. Earlywood and latewood of Sample 5 (S5) were isolated in the same way as S1, and then were milled into powder for X-ray diffraction (XRD) measurement.

2.2. Nitrogen adsorption

The samples were supercritically dried using a critical point drier (EM CPD300, Leica, Germany). More specifically, wood sticks were firstly dehydrated using a series of ethanol with different concentration, 80%, 90%, 95%, and 100%. The dehydrated samples were introduced into the drier apparatus, which was filled with liquid carbon dioxide. During supercritical drying, the liquid carbon dioxide was transformed into a supercritical fluid with a null surface tension. When the temperature was increased beyond its critical point (304.25 K at 7.39 MPa), the valve of the drier was gradually opened to allow gas to escape. In this way, shrinkage due to capillary pressure was avoided.

Nitrogen adsorption test was carried out using a surface area and pore-size analyzer (Autosorb iQ, Quantachrome, USA) at 77 K. Before the adsorption measurements, samples (1.0 g–1.5 g) were degassed at 80 °C for 10 h under a high vacuum (<10⁻⁵ Pa) to remove any moisture or adsorbed contaminants from sample surfaces. By means of the nitrogen adsorption isotherms, pore volume was derived from the amount of adsorbed nitrogen at a relative pressure close to unity, assuming the pores were filled with liquid adsorbate. Shapes of pores were calculated using the hysteresis loops. The total pore volume (V tot) was calculated from the volume of the liquid nitrogen, maintained at the relative pressure of 0.99. The Brunauer-Emmett-Teller (BET) surface area (S BET) of the samples was determined using an accelerated surface area (Brunauer, Emmett, & Teller, 1938). The pore-size distribution was calculated using the Barrett-Joyner-Halenda (BJH) method (Barrett, Joyner, & Halenda, 1951).

2.3. Field emission scanning electron microscope (FE-SEM)

The morphology of samples was examined by FE-SEM (JSM-6301F, JEOL, Japan) with a secondary electron detector operating at 5 kV–10 kV. Radial section of wood samples were cut and super-
critically dried. Surfaces were coated with a thin layer of platinum using a sputter coater (JSM-5500LV).

2.4. Atomic force microscope (AFM)

AFM (Dimention Icon, Bruker, Germany) was used to characterize the surfaces of treated samples with a NanoScope V controller. Radial sections of wood samples were cut and supercritically dried. A standard 5 μm scanner was used with tapping mode etched silicon probes (TESP; Veeco NanoProve, USA). In addition, a customized Nikon optical microscope with a deep focus was used to aid the positioning of the AFM tip to the desired location on the cell wall. An autotuning resonance frequency was in the range of 250 kHz–300 kHz and a scan rate of 0.5 Hz–2.0 Hz was used.

2.5. Confocal Raman microscopy (CRM)

For sample preparation, 10 μm thickness cross sections were cut from the samples using a rotary microtome (RM 2255, Leica, Germany). The cross sections were placed on glass slides and wetted with deuterium oxide. To avoid evaporation and drying out during measurements, the sections were covered with glass coverslips (0.17 mm thickness) and sealed with nail polish, as described in previous studies (Li, Sun, Zhou, Peng, & Sun, 2015; Wang, Keplinger, Gierlinger, & Burgert, 2014).

The Raman spectra were acquired with a LabRam Xplora confocal Raman microscope (Horiba JobinYvon, Longjumeau, France) equipped with a confocal microscope (BX51, Olympus, Japan) and a motorized scan stage. To achieve high spatial resolution, an MPPlan 100× oil immersion microscope objective (numerical aperture (NA)=1.4, Olympus) and a laser in the visible wavelength range (λ=532 nm) were used. The linearly polarized laser light was focused with diffraction limited spot size of 0.61A/NA onto samples and the Raman light was detected with an air cooled back-illuminated CCD behind the spectograph. The laser power on the samples was ~8 mW. Labspec 5 software was used for image processing and spectra analyses. The wavenumber ranged from 3000 cm⁻¹ to 10000 cm⁻¹ with a confocal aperture of 100 μm and a slit width of 100 μm. The reported depth resolution based on the silicon (standard) phonon band at 520 cm⁻¹ was 2 μm. Chemical images were generated using a sumfilter by integrating over defined wavenumber regions in the spectra. The filter calculated the intensities within the chosen borders and the background was subtracted by taking the baseline from the first to second border. Samples in Spruce earlywood and latewood regions were selected for Raman mapping at 0.7 μm steps and an integration time of 2 s. The overview chemical images allowed to separate cell wall layers (S and CC) with different chemical compositions, and to mark distinct cell wall regions for constructing average spectra.

Lignin and carbohydrates can be imaged simultaneously by selecting the characteristic Raman bands (Supplementary Table S1). Cellulose and hemicellulose are carbohydrate polymers that possess similar chemical bonds, resulting in the signals of hemicelluloses would be obscured by those of cellulose (Agarwal & Ralph, 1997). Therefore, only Raman images and spectra of cellulose and lignin of earlywood and latewood in untreated, CS-treated and steam-treated wood cell walls were analyzed in this study. Three vibration bands of cellulose, the C=O–C′ linkages stretching at 1096 cm⁻¹, the C=C and C=O stretching plus the HCC and HCO bending at 1149 cm⁻¹, the C=H and C=H₂ stretching at 2897 cm⁻¹, were monitored (Agarwal, 2006; Agarwal, Reiner, & Ralph, 2010; Li et al., 2015; Wang et al., 2014). The characteristic band of lignin was the aromatic skeletal vibrations close to 1600 cm⁻¹, which was accompanied by a shoulder at 1650 cm⁻¹ assigned to the conjugation to C=CC of conifer alcohol and a C=O stretch of coniferaldehyde. Furthermore, the lignin signals of guaiacyl ring showed at 1271 cm⁻¹ and aliphatic O–H bending was at 1333 cm⁻¹ (Agarwal & Atalla, 1986; Agarwal, 2006; Agarwal, McSweeney, & Ralph, 2011; Gierlinger, Keplinger, & Harrington, 2012).

2.6. X-ray diffraction (XRD)

The XRD patterns of samples were measured using the XRD (D/max 2200, Rigaku, Japan), with Ni-filtered CuKα radiation (λ=0.154 nm) at 40 kV and 30 mA. Scattered radiation was detected in the range of 2θ=5–40° at a scan rate of 2°/min. The background was subtracted and peaks were resolved using PeakFit (Sea-Solve Software Inc., Richmond, CA). The crystallinity index (Crl) was determined using the peak area method as follows (Hult, Iversen, & Sugiyama, 2003):

\[
Crl = \frac{A_{1–10} + A_{110} + A_{012} + A_{200} + A_{004}}{A_{1–10} + A_{110} + A_{012} + A_{200} + A_{004} + A_{am}} \times 100\%
\]

where \(A_{am}\) represents the peak areas of crystallographic reflections in wood denoted by the Miller indices (hkl). Crl was calculated from areas \(A_{am}\) of the reflections (1–10), (110), (012), (200) and (004) to the total area of both the crystalline and amorphous contributions (\(A_{am}\)).

The crystal size dimension \(D_{am}\) was evaluated by Scherrer’s equation (Wei, McDonald et al., 2015):

\[
D_{am} = \frac{K \lambda}{\beta_{1/2} \cos \theta}
\]

where \(K\) is the Scherrer constant (K=0.9), \(\lambda\) is the wavelength of the X-ray, \(\beta_{1/2}\) is the peak full width at half of maxima intensity (fwhm = 2°) obtained by IGOR Pro, when peak fitting was conducted with Gaussian function, and \(\theta\) is the diffraction angle.

2.7. Data statistical analysis

The Analysis of Variance (ANOVA) was done on the data using SAS program (SAS Institute 9.0, USA). Least-significant-difference tests were used to test the significant difference at 95% confidence interval of probability. Average was calculated from three replicates.

3. Results and discussion

3.1. Nitrogen adsorption

The nitrogen adsorption test allows the measurement of pore size of materials smaller than 50 nm without changing the original features of the materials (Clair, Gril, Di Renzo, Yamamoto, & Quignard, 2008; Chang et al., 2009). The nitrogen adsorption-desorption isotherms of both earlywood and latewood in the untreated, CS-treated and steam-treated wood cell walls are shown in Fig. 1a and b. The isotherm of earlywood in the untreated wood was intermediate between type II and type IV, according to the international union of pure and applied chemistry (IUPAC) classification (Sing et al., 1985). It presented the hysteresis loop that was typical of mesopore adsorbents. The shape of the hysteresis loop indicated that the mesopores belonged to type H3 with slit-shaped pores, which is consistent with previous findings (Clair et al., 2008; Chang et al., 2009). Earlywood after treatment adsorbed a larger amount of nitrogen, especially for the steam-treated wood. A similar trend was observed in the latewood.

Examples of pore volume distributions against pore diameter for untreated, CS-treated and steam-treated wood cell walls are plotted in Fig. 1c and d. All the samples presented broad pore size distributions, and the peak between 3 nm and 4 nm was sharp. This indicated that many slit-shaped mesopores with a diameter of 3.7 nm existed in the samples. However, the intensities
of peak in the curves were in the order of steam-treated > CS-treated > untreated wood. This suggested that there was a greater amount of mesopores in the treated samples than the untreated sample, as indicated by the SEM (Fig. 1e) and AFM (Fig. 1f) micrographs of the radial sections of cell walls in the latewood of CS-treated wood. Moreover, the amount of mesopores in the CS-treated samples was less than those in the steam-treated samples.

Total pore volumes \( V_{\text{total}} \) and BET specific surface areas \( S_{\text{BET}} \) of wood cell walls were changed significantly after both CS-treatment and steam treatment. The \( V_{\text{total}} \) of the earlywood and latewood of treated wood were greater than those of untreated wood (Table 1). The \( V_{\text{total}} \) values of the native earlywood and latewood were 0.0046 cm\(^3\)/g and 0.0026 cm\(^3\)/g, respectively. \( V_{\text{total}} \) values of the earlywood were increased by 17\% for the CS-treated wood and 41\% for the steam-treated wood. For the latewood, larger increments were obtained due to treatment, for example, after steam treatment \( V_{\text{total}} \) of the latewood was increased by 85\%. The influences of treatment on \( S_{\text{BET}} \) had the similar trend to \( V_{\text{total}} \). For earlywood, it displayed an increase of 35\% and 72\% for CS-treated wood and steam-treated wood, respectively, while the \( S_{\text{BET}} \) of latewood showed an increase of 53\% for the CS-treated wood and 122\% for the steam-treated wood, respectively. More mesopores are formed after CS-treatment and steam treatment, which could result in the permeability and capillary condensed water uptake in wood cell walls.

3.2. Confocal Raman microscopy

3.2.1. Changes in cellulose distribution

Raman imaging was conducted to reveal the cell wall chemistry and structural details with a resolution at the micro-level. False color images were generated by integrating over the intensity of defined Raman spectra. The spatial distribution of cellulose was visualized, based on the peak at 2897 cm\(^{-1}\), due to the stretching of the C–H and C–H\(_2\) (Fig. 2). For the untreated wood, the highest concentration of cellulose was observed in the S regions and the lowest level in the CC regions (Fig. 2a and d). This is consistent with the wood cell wall structure that had been previously reported (Schmidt et al., 2009; Whiting & Goring, 1983; Wang et al., 2014). The distribution of cellulose in the CS-treated (Fig. 2b and e) and the steam-treated wood (Fig. 2c and f) showed a similar pattern to that in the untreated wood. However, the intensity of the cellulose signal in earlywood and latewood for treated wood decreased slightly in the S and CC regions, which was further confirmed by Raman spectra (Fig. 3).

In order to determine the subtle dynamic changes in the cellulose from different morphological regions related to different treatments, a set of average Raman spectra that were extracted from different wood sections were analyzed shown in Fig. 3. For untreated wood, the peak at 2897 cm\(^{-1}\) was more prominent, when compared to that at 1149 cm\(^{-1}\) and 1096 cm\(^{-1}\) in the spectra of S
regions. In the spectra of the CC regions, the peak at 1149 cm⁻¹ was more pronounced than that in the spectra of the S regions. The relative intensities of the peak at 2897 cm⁻¹ and 1149 cm⁻¹ decreased for both S and CC regions of earlywood and latewood after CS-treatment and steam treatment as compared to untreated wood. This indicated cellulose structure was affected during treatment. This might be attributed to two main factors: (a) partly degradation of cellulose in the S and CC regions during CS-treatment and steam treatment. The steaming caused a high level of degradation in the hemicellulose in the CS-treated wood and steam-treated wood, releasing the acetic acid and formic acid (Zhang, Ma, Ji, & Xu, 2012). Low pH and high temperature would facilitate the acidic hydrolysis of polysaccharides (Fengel & Wegener, 1984; Weiland & Guyonnet, 2003). The depolymerization reaction occurred in the amorphous region through β-(1,4)-bonds breaking between the saccharide units of cellulose and hemicellulose. (b) Cellulose fibril reorienting. The microfibril helix could be deformed perpendicularly to its axis during treatments. It is well known that the 2897 cm⁻¹ band is sensitive to the orientation of the fibrils, and the intensity is highest when the laser electric vector is perpendicular to the cellulose chain direction, so that any changes in this geometry would cause peak intensity changes. In addition, it is worth noting that the swelling of cell wall could occur during CRM examination. This could be one of the factors causing the decline of peaks at 2897 cm⁻¹ and 1149 cm⁻¹. However, both untreated and treated samples were wetted with deuterium oxide, and thus cell wall swelling is assumed to be not important for the purpose to compare the changes due to treatment.

Moreover, a greater reduction in the relative intensities of the peak at 2897 cm⁻¹ and 1149 cm⁻¹ were observed in the earlywood and latewood after steam-treated wood when compared to the CS-treated wood, probably due to a larger mesopores leading to better permeability of steam. Hence, cellulose structure was degraded or deformed more seriously in steam-treated wood.

### 3.2.2. Changes in lignin distribution

The spatial distribution of lignin was visualized, based on the peak at 1600 cm⁻¹ ascribed to the symmetric stretching of the C=O bonds in the aromatic ring (Fig. 4). In comparison with the distribution of the cellulose, the lignin distribution in the untreated wood showed an opposite pattern with the highest level of lignin concentration occurred in the CC regions and the lowest level was found in the S regions (Fig. 4a and d). Similar findings were reported by previous studies (Ji, Ma, & Xu, 2014; Wang et al., 2014). After treatment, the lignin distribution was changed significantly.
around wood secondary

Fig. 168 distinct indicating ties the wood; untreated, CS-treated and steam-treated wood. This indicated that losses of C=O and C=C groups linked to the aromatic skeleton of guaiacyl units probably occurred. On the contrary, the relative intensities of the absorption peak at 1333 cm⁻¹ ascribed to aliphatic O–H bending was significantly increased, suggesting that the thermal depolymerization of lignin was associated with the recondensation reactions during CS-treatment and steam treatment. The β-aryl-ether linkages of lignin were partially depolymerized in response to steaming, resulting in the formation of carbonium ions (Rousset, Lapierre, Pollet, Quirino, & Perre, 2009; Weiland & Guyonnet, 2003; Windeisen, Stroble, & Wegener, 2007). The presence of carbonium ions induced further condensation reactions under the catalysis of the high temperature and the presence of acetic acid. This confirmed our previous assumptions that cross-linking had been formed among the aromatic units in the lignin during steam-treated wood (Yin et al., 2011) and CS-treated wood (Guo et al., 2015).

The intensities of strong aryl ring stretching at 1600 cm⁻¹ increased in the CC regions but decreased in the S regions of wood cell wall after CS-treatment, which was in accordance with the description of the lignin distribution images (Fig. 4). This might be derived from the different lignin units from different morphological regions. As previously reported, lignin in the S regions was more of a linear type with less branching which could give a looser structure (Donaldson, 2001; Wang et al., 2012). The matrix substance were softened by the high temperature steam and become mobile (Kiemle et al., 2014). Therefore, it was easier for lignin units to be transited in the S regions under compression combined with steam treatment.

3.3. Cellulose crystalline structure

Crystalline structure of cellulose after treatments was studied by XRD. XRD diffractograms of both earlywood and latewood in the untreated, CS-treated and steam-treated wood are plotted in Fig. 5. The untreated wood displayed a typical native cellulose Iα pattern.

Fig. 4. Raman images showing the distribution of lignin in earlywood (a–c) and latewood (d–f) in untreated (a and d), CS-treated (b and e), and steam-treated (c and f) wood around the peak at 1600 cm⁻¹ (1690–1560 cm⁻¹). S = the secondary cell wall; CC = the cell corner.
which had a parallel up arrangement of cellulose chains. The typical peaks were observed at 14.8°, 16.5°, and 22.14°, corresponding to (1–10), (110) and (200) planes (Guo, Guo, Wang, & Yin, 2016; Wei, McDonald et al., 2015). The diffraction peaks of the (1–10) and (110) planes merged together. Consistent to this, the unit cell of native cellulose was found to be monoclinic, that the cellulose fiber axis was parallel to the c-axis, while the cellulose chains were hydrogen bonded into planar sheets along the b-axis. These sheets then assembled into stacks along the a-axis with van der Waals interactions between the layers (Peura et al., 2006). After treatment, there was a significant increase in the intensity of the (200) reflection, especially in the steam-treated wood. Interestingly, the (004) reflection experienced no change in the earlywood and latewood of the CS-treated wood and steam-treated wood, suggesting that the cellulose molecular chains remained largely unaffected by the high temperature steam and compression.

The CrI was calculated as the ratio of the area arising from the crystalline phase to the total area. The CrI of both earlywood and latewood after CS-treatment and steam treatment were larger than those of the untreated wood. In addition, the steam-treated wood had the largest CrI (Table 2). The CrI of the earlywood for untreated wood was calculated to be 25.8%. The values of the CrI increased to 30.3% for the CS-treated wood and 49.2% for the steam-treated wood, indicating an increase of 18% and 91%, respectively. A similar phenomenon was obtained with the latewood, showing a growth of 9% for the CS-treated wood and 75% for the steam-treated wood, when compared to the untreated latewood. ANOVA analysis was further used to investigate the statistical significance for the CrI derived from CS-treatment and steam treatment. It was indicated that both steam treatment and CS-treatment had a significant influence on the CrI at a significance level of 5%, as showed by the different symbol letter towards CrI among woods in Table 2.

The average crystallite thickness (D200) of the untreated wood was 2.73 nm for the earlywood and 2.51 nm for the latewood. However, the values of the CS-treated wood increased to 2.89 nm for the earlywood and 2.87 nm for the latewood, indicating an increase of 6% and 14%, respectively. The values of the steam-treated wood increased to 3.03 nm for the earlywood and 2.96 nm for the latewood, indicating an increase of 11% and 18%, respectively. In accordance with this, ANOVA analysis also showed that both CS-treatment and steam treatment resulted in a significant increase of the cellulose crystallite dimensions.

It was clear seen that both CrI and D200 were increased after CS-treatment and steam treatment. This agreed with earlier results for CS-treated wood (Itō, Tanahashi, Shigematsu, & Shinoda, 1998; Guo et al., 2015) and hydrothermal treated wood (Bhuian, Hirai, & Sobue, 2000). The increase of CrI was probably due to the fact that cellulose became more crystalline when wood was subjected to CS-treatment and steam treatment. This was further supported by the indication that the native state of wood-cellulose was not-crystalline (Agarwal, Ralph, Reiner, & Baez, 2016) and the increase of crystallinity index after CS-treatment was mainly due to the increase or reordering in the crystalline region of cellulose using Wide-angle X-ray scattering (WAXS) method, but not from the degradation of amorphous domains (Guo, Rennhofer, Yin, & Lichtenegger, 2016). Cellulose macromolecules stored vivo stress due to their semicrystalline nature (Kataoka & Kondo, 1998). The matrix substance was softened by the high temperature steam and became mobile during the CS-treatment, which loosened the inner stress in the crystalline region of the cellulose (Kiemle et al., 2014; Tanahashi, Goto, Horii, Hirai, & Higuchi, 1989). Under such conditions, the cellulose crystallite extended in the crystalline region and crystallization occurred in the semicrystalline region. Moreover, a greater rise in both CrI and D200 were observed in the earlywood and latewood after steam-treated wood, when compared to the CS-treated wood, probably due to a larger mesopores leading to the greater inner stress relaxation during the steam treatment.

### 3.4 Cell wall structure in response to CS-treatment and steam treatment

The cellulose molecules form microfibrils aggregation that are embedded in matrix of hemicellulose and lignin (Salmén & Burgert, 2009), constituting the solid fraction of wood cell wall (Fig. 6A). Although a high intermixing of the matrix wood polymer among the cellulose, the wood cell wall exhibits a porosity of molecular scale dimensions, due to the partial filling of spaces among the cellulose microfibrils with lignin, hemicellulose and extractive (Yin et al., 2015). It has been speculated that the physical, chemical and mechanical properties of wood are governed by the complex arrangement of the solid fraction and porosity of wood cell walls. Recently, the changes in hygroscopicity and micromechanical properties after CS-treatment and steam treatment were investigated in our previous studies (Yin et al., 2011; Guo et al., 2015). It was revealed that hygroscopicity significantly decreased with both CS-treatment and steam treatment, while the micromechanical properties decreased for the steam treatment and increased for the CS-treatment. In this study, direct experimental evidence was replenished when changes were observed in the porosity, chemical composition and cellulose crystalline structure of CS-treated and steam-treated wood cell walls.

The hygroscopicity in wood cell walls could be correlated with absorbed water and capillary condensed water. However, it should be noted that both CS-treatment and steam treatment showed a greater proportion of mesopores in wood cell walls. The presence of these mesopores promoted capillary condensed water.
uptake, which would result in increased hygroscopicity. However, the hygroscopicity of both CS-treatment and steam treatment decreased as our previous studies. Therefore, the effect of capillary condensed water on the hygroscopicity was ignored. As illustrated diagrammatically in Fig. 6C, steam can depolymerize lignin by hydrolyzing the β-aryl-ether links associated to the guaiacyl units, and then carbonium ions are generated. The presence of carbonium ions induced further re-condensation reactions, resulting in a significant decrease in the C=C and the C=O linked to the lignin aromatic skeleton. Moreover, the depolymerization reaction occurred in the amorphous regions through β-(1,4)-bonds breakage between the saccharide units of cellulose and hemicellulose, resulting in a reduction in the availability of the overall carbohydrate –OH groups. Together with our previous findings by FT-IR analysis (Yin et al., 2011; Guo et al., 2015), the clear progression of C=O, assigned to the O–C=O in the glucuronic acid unit of xylan, and a loss of mannose units in the glucromannan backbone indicated that C=O were lost in the hemicellulose. Therefore, it was assumed that the reduction in hygroscopicity after the CS-treatment and steam treatment was highly related to the decrease of accessible sorption groups, e.g., hydroxyl and carbonyl groups, and thus provided the fixation of the compression combined with steam treatment with limited structure deformation recovery.

The strength and rigidity of the cell wall are probably related to the crystallinity index of cellulose, the cross-linking of lignin and the cell wall density (Guo et al., 2015). The increase in the relative crystalline cellulose content (Fig. 6B) and the cross-linking of lignin after CS-treatment and steam treatment would lead to an improvement in mechanical properties. However, a larger number of mesopores was formed due to the degradation of amorphous polysaccharides, leading a lower cell wall density during steam treatment. Consequently, a decrease in the micromechanical properties was observed with steam-treated wood when compared to the untreated wood. In contrast, the applied compression forces resulted in a stiffer and stronger structure probably as a result of the higher cell wall density.

4. Conclusions

Compression combined with steam treatment (CS-treatment) caused obvious changes on the porosity, chemical composition and cellulose crystalline structure of wood cell walls. A large number of slit-shaped mesopores with a diameter of 3.7 nm was formed in the CS-treated wood cell walls, and more mesopores were found in the cell walls of the steam-treated wood. The cellulose structure was affected and β-aryl-ether links associated to guaiacyl units of lignin was depolymerized followed by re-condensation reactions. The chemical reaction led to the decreases of hydroxyl and carbonyl groups in the treated wood cell walls, and hence less moisture sorbing sites were available. This provided better fixation of the compression combined steam treatment. The CrI and D_{200} of cellulose in CS-treated wood cell wall were largely increased due to crystallization that occurred in the semicrystalline region, but cellulose molecules chains in the crystalline region remained unaffected by CS-treatment. Furthermore, the larger increase in the CrI and the D_{200} were observed in the earlywood and latewood for steam-treated wood, which was probably contributed to the greater amount of mesopores as compared to CS-treated wood.


