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Hydroxyl accessibility and dimensional changes of Scots pine sapwood affected by alterations in the cell wall ultrastructure during heat-treatment

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Abstract

There is a complex link between the water sorption behavior and the presence of accessible hydroxyl groups in the wood cell wall, which can be altered by heat-treatment (HT). This study analyses the effect of changes in the cell wall ultrastructure caused by two HT techniques on the hydroxyl accessibility, water vapor sorption and dimensional changes of Scots pine (*Pinus sylvestris* L.) sapwood. HT of wood in pressurized hot water at 120-170 °C was applied to cause the preferential bond cleavage, whereas HT of wood in oven-dry state in superheated steam at 180-240 °C was performed to create additional covalent cross-links within the cell wall matrix. Removal of cell wall polymers by HT and water leaching reduced the oven-dry dimensions of wood and enhanced the cellulose aggregation during drying. Cellulose aggregation restricted the cell wall shrinkage in circumferential direction, resulting in inhomogeneous shrinkage of the cell wall with only little changes in lumen volume by HT. Cellulose aggregation also reduced the water-saturated dimensions, but a decrease in swelling was only achieved when additional cross-links were formed by HT in dry state. Additional cross-links in the cell wall matrix also resulted in an additional reduction in water sorption at 25 °C and 93 % RH. However, this was not caused by a further reduction in the hydroxyl accessibility. Instead, cross-linking was shown to reduce the amount of accessible OH groups that are simultaneously active in sorption, which was explained based on the concept of sorption of water dimers at hydroxyl group pairs at high RH levels.

Keywords: Elemental analysis; Hydrogen-deuterium exchange; Mercury intrusion porosimetry; Thermal degradation; Water interactions; Wood

1. Introduction

The capability of wood to adsorb and desorb water molecules in interaction with its surroundings causes dimensional changes and influences many physical properties of wood. This emphasizes the importance of studying wood-water interactions for a better understanding of the wood material behavior. By exposing wood to a stable temperature and relative humidity (RH), adsorption and desorption of water molecules reach an equilibrium state, eventually. This state can be quantified gravimetrically as the equilibrium moisture content (EMC) by relating the mass of adsorbed water to the dry mass of wood. It is commonly accepted that the water sorption of wood is linked to the presence of hydroxyl (OH-) groups as the main water-attractive sites. However, their accessibility and occupancy by adsorbed water molecules at different RH levels are still disputed [1–3].

It is known since long that the exposure of wood to elevated temperatures during heat-treatment (HT) reduces its uptake of water vapor from the surroundings, which results in an enhanced dimensional stability [4,5]. This property change is utilized in many industrial-scale HT processes in the temperature range between 160 and 240 °C to prolong the service life of solid wood in exterior applications [6]. The preferential degradation of amorphous cell wall polysaccharides by HT [7] is an obvious cause for the decrease in sorption, because the relative increase in lignin and crystalline cellulose decrease the number of accessible OH groups per gram dry mass (OH_{acc}) in the residue. While OH groups within the crystalline structure of cellulose are mostly inaccessible for water [8], their abundance is generally low in lignin. However, changes in chemical composition cannot fully explain the reduced sorption and swelling of heat-treated wood, thus additional factors must play a role [3,9].

HT of wood in dry state does not only cause the degradation of cell wall polymers, but also creates additional covalent bonds and cross-links within the cell wall matrix by inducing re-polymerization reactions that involve degradation products and remaining cell wall polymers [10–12]. While the thermal removal of cell wall polymers reduces the wood's dry dimensions, the covalent cross-links formed under dry conditions restrict the maximum capacity of the cell wall to incorporate water molecules, which is expressed as a decreased fiber saturation point [9] and a reduced volumetric swelling to the water-saturated state [5]. When suppressing the formation of cross-links in the cell wall

matrix by performing HT with wood in water-saturated state at mild temperatures ($< 180\text{ }^{\circ}\text{C}$) [12], a restriction in the capacity to incorporate water molecules is not achieved. In contrast, the preferential hydrolytic cleavage of covalent bonds within the cell wall matrix under these HT conditions increases the swelling [13] and the fiber saturation point of wood [14]. The structural changes of the cell wall matrix, by additional covalent cross-linking under dry heat conditions and preferential bond cleavage under wet heat conditions, also result in a change in the sorption behavior at 0-95 % RH, with a more efficient reduction in EMC by HT in dry state [13]. However, it remains a question how this sorption behavior is related to changes in OH_{acc} .

Answering this question requires the quantification of OH_{acc} in wood, which can be realized in hydrogen-deuterium exchange (HDX) experiments [15]. The technique is based on exposing wood to deuterated water (D_2O), during which wood OH groups that form H-bonds with (deuterated) water have their hydrogen exchanged by deuterium. The amount of exchanged OH groups equals OH_{acc} and can be detected either by spectroscopic methods [16,17] or gravimetrically as a mass increase by 1 g per mol exchanged hydrogen [3,14,18]. For unmodified wood, an OH_{acc} in the range between 7 and 11 mmol g^{-1} is usually determined by HDX [3,19–21]. This large variation may be explained by differences in chemical composition between wood species [22] and/or an effect of the wood's sorption history on the OH accessibility [21]. HDX experiments have been applied successfully to analyze the reduction in OH_{acc} of wood caused by drying [16,21], Kraft pulping [17], and chemical modification with carboxylic acid anhydrides [23,24].

The HDX technique has also been applied to heat-treated wood, but the results showed a poor correlation between EMC and OH_{acc} [3]. This outcome contradicts with the presumed role of OH groups in wood-water interactions [1,25] and requires a re-evaluation. Rautkari et al. [3] performed the HDX in repeated adsorption-desorption cycles with D_2O vapor (0-85% partial pressure) in a dynamic vapor sorption (DVS) apparatus. However, such repeated cycles may cause hornification-like effects that change the cell wall structure and OH accessibility, and can be replaced by a single, sufficiently long cycle [18]. Furthermore, a reversible reduction in water sorption and OH_{acc} can already be achieved by wood drying, irrespective of thermal degradation reactions [26,27]. Exposure to nearly saturated water

vapor can only partially remove such drying-effects, but full removal can be achieved by water-saturation [21]. These reversible effects in heat-treated wood must be considered in experimental setups when studying water interactions [28].

In this study, the effects of irreversible cell wall matrix changes caused by different HT techniques were studied after water leaching of the heat-treated wood. Based on a previous study [12], two HT techniques were applied to Scots pine sapwood, which both decreased the amorphous carbohydrate percentage in the residue, but differed in their impact on the cell wall matrix ultrastructure. HT in pressurized hot water at 120-170 °C facilitates the covalent bond cleavage, while HT in dry state in superheated steam at 180-240 °C favors the formation of additional covalent cross-links within the wood cell wall matrix [12]. Changes in the dimensions of the wood samples caused by HT were analyzed after oven drying and after water soaking, which was further supported by mercury (Hg) intrusion porosimetry measurements. EMC and OH_{acc} were quantified in a DVS apparatus using H₂O and D₂O vapor. Since the applied process conditions differed significantly between the HT techniques, changes in dry mass and the elemental chemical composition (O/C molar ratio) caused by HT are used as the bases for comparison.

2. Material and methods

2.1. Material

Scots pine sapwood (*Pinus sylvestris* L.) was cut to dimensions of 20×20×10 (R×T×L) mm³. The samples were clear of heartwood, knots and macroscopic defects. Fifteen replicates per sample group (treatment process and peak temperature) were used. All samples were oven-dried using a temperature sequence of 40, 60, 80 and finally 105 °C, with each temperature being held for 24 h, before the initial dry mass and dimensions were determined.

2.2. Heat treatments

Heat-treatments were performed as described previously [12], either as wet heat-treatment (HT_{wet}) or as dry heat-treatment (HT_{dry}). For HT_{dry}, oven-dry samples were heated in an oven at atmospheric

pressure with superheated steam to a peak temperature of 180, 195, 210, 225 or 240 °C, which was held for 180 minutes, before cooling the oven to room temperature. For HT_{wet}, the oven-dried samples were water-impregnated (50 mbar for 1 h) first, and then placed in small vessels with addition of deionized water (solid:liquid 1:20 g/g). The filled, closed vessels were heated in a slow rotating air bath digester to a peak temperature of 120, 130, 140, 150, 160 or 170 °C, which was held for 120 min before cooling for 30 min under continuous tap water flow.

2.3. Change in mass and dimensions

All samples, including the reference samples, were leached with deionized water after the treatment. Dry heat-treated samples and reference samples were vacuum-impregnated (50 mbar for 1 h) with deionized water before leaching. Within the course of two weeks, the water was changed daily to remove the remaining degradation products. The full water saturation during the leaching process is also required to remove reversible effects that influence the water sorption of heat-treated wood [28]. The samples were then dried carefully at ambient conditions for a minimum of one week and the temperature sequence described above was applied subsequently. The change in dry mass after treatment and water leaching was calculated as relative dry mass (M_{dry}/M_0) by relating the final dry mass to the initial dry mass.

Change in dimensions was calculated as described by Obataya and Higashihara [29], except that the cross-sectional area was measured instead of the volume. Relative dry (A_{dry}/A_0) and relative wet dimensions (A_{wet}/A_0) were calculated by relating the cross-sectional area of each sample in oven-dry (A_{dry}) or in water saturated state (A_{wet}) after HT and water leaching to the initial dry cross-sectional area (A_0). Relative swelling was computed by subtracting the relative dry dimensions from the relative wet dimensions. Relative wet dimensions and relative swelling were measured again after drying and re-soaking the sample by vacuum-impregnation with deionized water, which was followed by water soaking for 24 h.

2.4. Elemental analysis

Ten out of fifteen replicates were milled and mixed in a Wiley mill to pass through a 30 mesh screen. The particles were sieved and only the fraction between 0.2 and 0.63 mm was used. The particles were dried at 103 °C for 12 h and stored in a desiccator over silica gel until the analysis. The carbon, hydrogen and nitrogen contents (CHN%) were determined on a 2400 Series II CHNS/O Elemental Analyzer (Perkin Elmer Inc., USA) using 2 ± 0.5 mg of dry particles per measurement. Acetanilide (C 71.09 %, H 6.71 %, N 10.36 %, O 11.84 %) was used for calibration. The oxygen content was calculated by subtracting CHN% from 100%. The measurements were performed in triplicate.

2.5. Mercury intrusion porosimetry

The porosity of untreated and heat-treated samples were analyzed according to the procedure described by Li et al. [30], using an AutoPore IV 9500 V1.07 instrument (Micromeritics Instrument Corporation, USA). Samples that originated from the same slat before HT were used and oven-dried test pieces with dimensions of $6 \times 6 \times 5$ (R×T×L) mm³ and mass of about 0.3 g were prepared from these samples. Prior to the Hg intrusion, the bulk density (ρ_B , in g cm⁻³) was determined. Hg intrusion was performed using a pressure between 0 and 414 MPa with an equilibrium time of 10 s at the initial and the final pressure level. The pore volume (n, in %) was calculated as the pore volume of intruded mercury related to the total volume of the respective test piece. The cell wall density (ρ_C , in g cm⁻³) was determined using equ. 1:

$$\rho_C = \rho_B / [1 - (n/100)] \quad (\text{equ. 1})$$

2.6. Hydrogen-deuterium exchange

Accessible OH-groups were quantified by measuring the mass increase after deuterium exchange in a DVS apparatus (DVS Advantage ET, Surface Measurement Systems, London, UK) with a measuring accuracy of 0.1 µg and a gas flow of 200 cm³ min⁻¹ at 25 °C during the measurement. Samples that were cut from the same slat before HT were used. Approx. 15.5 (±0.3) mg of material was taken from the middle of each sample using a razor blade. This material was subjected to the following sequence: (a) a drying step under dry N₂ flow (0% RH) until the mass change per minute was less than 0.0005 % min⁻¹

¹ over a 10 min period; (b) a partial pressure of 93 % using a mixture of dry N₂ and deionized water for 12 h; (c) a drying step as described above; (d) a target partial pressure of 93 % using a mixture of dry N₂ and deuterium oxide; and finally (e) another drying step as described above. An example of the change in mass and partial pressure in dependence on the time in the different steps is shown in **Figure 1**. All measurements were performed in duplicate and average values are presented. The EMC at 25 °C and 93 % RH (in mmol g⁻¹) was determined according to equ. 2.

$$\text{EMC} = 1000 \times (m_b - m_c) / (m_c \times M_{\text{water}}), \quad (\text{equ. 2})$$

where m_b and m_c is the mass at the end of step b and c, respectively, and M_{water} is the molar mass of water (18.01528 g mol⁻¹). OH accessibility (OH_{acc} , in mmol g⁻¹) was calculated as described by Pönni et al. [18], using the mass at the end of step c and e as initial and final mass, respectively.

Figure 1: Exemplary change in sample mass (in mg) in dependence on the time (in hours) during a hydrogen-deuterium exchange experiment. The dashed line highlights the dry sample mass determined in step (c). The increase in dry mass by the hydrogen-deuterium exchange is shown in the inset. A description of the different partial pressure steps (a-e) can be found in the text.

Additional measurements were conducted to verify the accuracy of the above HDX sequence. By using deionized water instead of deuterium oxide (D₂O) in step (d), the decrease in EMC caused by the intermediate drying step (c) was determined to less than 0.13 mmol g⁻¹ for unmodified Scots pine sapwood. Furthermore, OH_{acc} of selected samples was additionally determined using the HDX sequence described by Kymäläinen et al. [19], except that the D₂O vapor exposure was prolonged to

12 h. This HDX sequence measures the initial and final dry mass after drying under dry N₂ flow at 60 °C for 6 h.

3. Results and discussions

3.1. Changes in mass and O/C molar ratio

Both HT techniques caused considerable loss in dry mass, which was determined as a decrease in M_{dry}/M_0 with increasing peak temperature during HT. Auto-hydrolysis of wood during HT_{wet} removed cell wall polymers, i.e. hemicelluloses, effectively, resulting in a lower residual dry mass after HT_{wet} at 170 °C ($M_{\text{dry}}/M_0 = 0.70$) than after HT_{dry} at 240 °C ($M_{\text{dry}}/M_0 = 0.77$). The high effectiveness of wood auto-hydrolysis is caused by the catalytic effect of hydronium ions generated by water auto-ionization [31] and by the formation of acetic acid from the acetyl groups in wood hemicelluloses [32]. However, Altgen et al. [12] evidenced that, although both HT techniques cause the preferential de-polymerization of amorphous carbohydrates, dry mass changes are insensitive for differences in the degradation reactions in wood between the HT techniques. HT_{dry} favors re-polymerization reactions within the cell wall matrix, because the high treatment temperatures and the absence of water enables a more facile dehydration of sugars to furan-type derivatives that may react with the lignin [12,33]. The loss in hemicelluloses by HT_{dry} is, thus, underestimated by gravimetric measurements [12]. In contrast, HT_{wet} causes additional de-polymerization of lignin by cleavage of ether bonds [34], which results in the overestimation of the hemicelluloses loss based on changes in dry mass [12].

In contrast to gravimetric measurements, elemental analysis is sensitive to thermal degradation reactions within the wood residue that do not cause a significant loss in dry mass [35]. As illustrated in **Figure 2**, differences in the O/C ratio as a function of M_{dry}/M_0 are evident between the two HT techniques. The measured loss in O/C ratio of both HT techniques principally follows the theoretical loss in O/C by the removal of compounds with the formula C_nH_{2n}O_n (e.g. hexoses n=6, pentoses n=5, acetic acid n=2, formaldehyde n=1). This high loss in sugars is not only caused by the thermal decomposition during HT, but also by removing water-soluble degradation products from the wood

during the subsequent water leaching. Nonetheless, there are deviations from this theoretical loss depending on the HT technique.

Figure 2: O/C molar ratio ($N=3$) in dependence on the relative dry mass (M_{dry}/M_0 ; $N=15$; $\pm$ standard deviation). The theoretical loss in O/C based on the assumption that mass loss is solely caused by removal of compounds with the chemical formula $C_nH_{2n}O_n$ (dashed line) or by the removal of H_2O (dotted line) is illustrated.

Loss in O/C ratio of dry heat-treated samples is larger than the theoretical loss by $C_nH_{2n}O_n$ removal. This is caused by the dehydration of hemicelluloses to furan-type derivatives and their fixation in the wood structure, i.e. by their involvement in re-polymerization reactions with the lignin [33,36]. This reaction pathway only removes water from wood, which results in a much larger reduction in O/C ratio as a function of M_{dry}/M_0 than the removal of $C_nH_{2n}O_n$. Re-polymerization of furan-type derivatives with remaining cell wall polymers is one cause for the formation of cross-links within the cell wall matrix [36]. In contrast, the decrease in O/C ratio of the wet heat-treated samples is lower than expected when considering the pure loss in $C_nH_{2n}O_n$. Dehydration of sugars is less facile in aqueous treatments, presumably because water molecules compete with OH groups on sugars for available protons, thereby hindering the protonation of sugars that initiates their dehydration to furan-type derivatives [37,38]. During HT_{wet} , sugars may also be removed from the wood by diffusion in the process water, before being dehydrated. Thereby, furan-type derivatives are formed in the process water at some distance from the cell wall and are removed with the process water or during water-leaching. Furthermore, the preferential bond cleavage in the cell wall matrix during HT_{wet} by wood auto-hydrolysis may partially

counteract the loss in O/C ratio by $C_nH_{2n}O_n$ loss due to the consumption of water during hydrolysis. This can be further supported by the additional loss in carbon-rich lignin through the cleavage of ether bonds in lignin during HT_{wet} [31,34,39].

3.2. Dimensional changes

Heat-induced dimensional changes of Scots pine wood in longitudinal direction are usually lower than 0.12 % even at mass loss levels exceeding 20 % [40]. Thus, the loss in wood volume is closely reflected by the loss in cross-sectional area that was measured in the present study. After HT, the relative dry dimensions (A_{dry}/A_0) decreased as a linear function of M_{dry}/M_0 , independent of the HT technique (**Figure 3**). Thus, the loss in dry dimensions was not affected by the differences in chemical reactions described above, but by the quantity of removed cell wall material. The A_{dry}/A_0 as a function of M_{dry}/M_0 does not follow a slope of 1 and only about 40 % of the removed dry mass resulted in a decrease in dry volume. However, a slope of 1 would be expected if the cell wall shrunk homogeneously in transverse direction and if the cell wall density remained constant.

Figure 3: Relative dry dimensions (A_{dry}/A_0), in dependence on the relative dry mass (M_{dry}/M_0). Calculated values represent the theoretical relative dry volume that is derived from the Hg intrusion data and equ. 3, with a linear regression curve (dotted line). (N=15; $\pm$ standard deviation)

The change in dry dimensions was further analyzed by means of Hg intrusion porosimetry for selected samples (**Table 1**). The method is only sensitive to pores that can be accessed by Hg, thus pores smaller

than 1.8 nm are not detected [41]. Furthermore, it has been suggested previously that the large pressure applied during Hg intrusion compresses micro-cavities in wood, especially after weakening the cell wall by HT [42]. Nonetheless, the cell wall density of 1.46 g cm^{-3} that was determined for the reference sample is in good agreement with the commonly assumed cell wall density of wood around 1.5 g cm^{-3} . Both HT techniques decreased the cell wall density with increasing treatment temperatures, reaching a minimum of 1.28 g cm^{-3} (HT_{wet} at 170 °C). This indicates a lack of cell wall shrinkage after thermal removal of cell wall polymers, which leaves micro-cavities in the cell wall that cannot be assessed by the mercury and reduces the loss in A_{dry}/A_0 . However, as illustrated by Obataya and Higashihara [29] the loss in cell wall density in the range between 1.3 and 1.5 has only a small impact on the change in volume. Therefore, the deviation from a slope of 1 for A_{dry}/A_0 as a function of M_{dry}/M_0 must be caused by an additional restrictive mechanism.

Table 1: Cell wall density, bulk density and porosity measured by Hg intrusion porosimetry.

T_{max} (°C)	Cell wall density (g cm⁻³)	Bulk density (g cm⁻³)	Porosity (%)
Reference	1.46	0.56	61.7
HT _{dry}			
210	1.36	0.55	59.3
240	1.34	0.58	56.8
HT _{wet}			
140	1.39	0.62	55.1
170	1.28	0.52	59.6

On the level of a single cell, the transverse lumen area would shrink to an equal extent as the cell wall area, if cell wall shrinkage occurred in a homogeneous manner. However, Hg intrusion measurements evidence that both HT techniques only caused a minor decrease in porosity compared to the reference (**Table 1**), indicating that the lumen volume did remain nearly constant and that the cell wall did not shrink homogeneously. The Hg intrusion results were also used to calculate the theoretical relative dry volume (V_{dry}/V_0) based on equ. 3, which was already applied by Obataya and Higashihara [29].

$$V_{\text{dry}}/V_0 = 1 - (M_{\text{dry}}/M_0)(\rho_B/\rho_C), \quad (\text{equ. 3})$$

This equation assumes that the lumen volume remains constant and that the loss in macro-scale volume by thermal removal of cell wall polymers is solely based on cell wall shrinkage in cross-lamellar

direction. As can be seen in **Figure 3**, the theoretical change in volume follows the measured A_{dry}/A_0 very closely. This indicates a restriction for heat-induced shrinkage in circumferential direction. The proposed shrinkage pattern by HT and subsequent water leaching is illustrated in **Figure 4**, where (B) and (C) were calculated to contain the same cell wall mass, 80% of the reference (A), with less shrinkage in (C) as one can observe by comparison of the size of unit parallelograms.

Figure 4: Dry cell wall shrinkage patterns by HT, visualized by an idealized hexagonal cell wall geometry with constant cell wall density. The parallelograms represent the repetition units of the 2D-lattice. The principal directions of shrinkage of the cell wall are indicated by the l -vector (circumferential direction) and n -vector (cross-lamellar direction). (A) reference; (B) 20% dry mass loss with isotropic free shrinkage; (C) 20% dry mass loss without lumen shrinkage.

In dry heat-treated wood, a cause for such a restriction in circumferential shrinkage might be the additional covalent bonds and cross-links that are formed in the cell wall matrix [12]. However, such additional covalent bond formation in the cell wall matrix should be absent in wet heat-treated wood, because the hydrolytic cleavage of bonds dominates during HT_{wet} [12]. Therefore, the restriction in

shrinkage might be caused by the stiff cellulose microfibrils instead. Thermal degradation during HT and subsequent water leaching removes cell wall matrix polymers that act as spacers between the cellulose microfibrils to facilitate cellulose aggregation and hydrogen bonding between individual cellulose molecules [43,44]. During drying of the wood after HT and water leaching, cellulose aggregation and hydrogen bonding may occur before the cell structure reaches a conformation that occupies minimum space. Cellulose aggregation stiffens the cell wall and prevents the shear displacement of cellulose microfibrils. Thereby, the almost longitudinal orientation of the cellulose microfibrils in the large S2 layer of the cell wall hinders longitudinal shrinkage, while the large cellulose microfibril angle in the S1 and S3 layer hinders circumferential shrinkage.

Cellulose aggregation and hydrogen bonding between cellulose molecules also affected the water-saturated dimensions of wet heat-treated wood (**Figure 5 A**). When measured during the water-leaching after the HT process, A_{wet}/A_0 did not decrease for wet heat-treated samples, indicating that water molecules filled the space that was created by the removal of cell wall matrix polymers to prevent a contraction of the wood during HT_{wet} . A_{wet}/A_0 even increased slightly up to 0.9 M_{dry}/M_0 and decreased to the reference value for lower M_{dry}/M_0 values. Presumably, the preferential bond cleavage during HT_{wet} enhanced the capacity of the cell wall matrix to incorporate water molecules. However, when re-soaking the wet heat-treated samples after drying, A_{wet}/A_0 decreased for M_{dry}/M_0 values below 0.9. Similar to the reduction in water retention value by the drying of wood pulp [45], drying of wet heat-treated samples caused a stiffening of the wood structure to restrict water incorporation and swelling during re-soaking. Since the differences in A_{wet}/A_0 between the measurements before and after drying increase with decreasing M_{dry}/M_0 , we conclude that this stiffening effect is related to cellulose aggregation after the removal of cell wall matrix polymers.

Figure 5: Relative wet dimensions (A_{wet}/A_0 ; A) and relative swelling (B) in dependence on the relative dry mass (M_{dry}/M_0). Closed and open symbols refer to the determination of water-saturated dimensions before and after oven drying, respectively. (N=15; $\pm$ standard deviation)

For dry heat-treated samples, A_{wet}/A_0 decreased as a linear function of M_{dry}/M_0 . HT_{dry} creates additional cross-links within the cell wall matrix while the wood is in shrunken (dry) state, which restrict the swelling of the wood by water soaking after HT [9,10,12,13]. HT_{dry} might also facilitate cellulose aggregation by removal of cell wall matrix polymers, as evidenced by X-ray scattering studies [46]. However, without water occupying space in the cell wall, cellulose aggregation already occurs during the treatment, resulting in no differences between the measurement of A_{wet}/A_0 before and after drying of dry heat-treated wood. Unfortunately, the effect of cross-linking reactions within the cell wall matrix

and the effect of cellulose aggregation on the reduction in A_{wet}/A_0 cannot be differentiated based on the present data.

The relative swelling, shown in **Figure 5 B**, is depending on the ratio of dimensional changes in dry and water-saturated state. After HT_{dry} , the relative swelling decreases as a linear function of M_{dry}/M_0 , evidencing that the decrease in wet dimensions exceeds the decrease in dry dimensions. In contrast, the relative swelling increased after HT_{wet} , evidencing that a restriction for swelling is not created by HT_{wet} . A decrease in relative swelling to the values of the reference samples was only achieved after drying when high quantities of cell wall matrix polymers were removed to facilitate cellulose aggregation.

3.3. Hydrogen-deuterium exchange

Residual amounts of adsorbed water molecules after the drying steps of the HDX sequence might cause a misestimation of OH_{acc} . This is because residual water molecules would add to the dry mass of the wood and because D_2O molecules may exchange the residual H_2O molecules during HDX, thereby adding 2 mol exchanged hydrogen per 1 mol residual water. Therefore, additional HDX measurements were performed with another drying protocol that uses the pre-heater of the DVS apparatus to determine the sample dry mass after exposure to elevated temperatures. As can be seen in **Table 2**, nearly identical OH_{acc} values were reached with both drying protocols. Therefore, we conclude that residual water molecules after the drying step did not bias our HDX measurements.

Table 2: Average OH_{acc} values (in mmol g^{-1} ; $N=2$) determined gravimetrically in the DVS apparatus using two different drying protocols.

Sample	N_2 flow at 25 °C until <0.0005 dmdt	N_2 flow at 60 °C for 6 h
Reference	10.28	10.27
HT_{dry} at 225 °C	9.21	9.17
HT_{wet} at 160 °C	8.90	8.99

Since there is a difference in chemical composition at a given mass change between HT_{wet} and HT_{dry} [12], M_{dry}/M_0 is not suitable as a basis for comparing the HDX results of wet and dry heat-treated samples. M_{dry}/M_0 does not allow the separation of effects related to differences in chemical composition from differences in the cell wall matrix ultra-structure. Instead, the O/C ratio was used as the basis for comparison and EMC as well as OH_{acc} (both in mmol g^{-1}) were normalized by the respective carbon

content (in mmol g⁻¹). Following the explanations of Willems [2], the O/C ratio is regarded as an estimation for the total number of polar sites per carbon in wood.

Figure 6: EMC per carbon ratio (A) and OH_{acc} per carbon ratio (B) in dependence on the O/C ratio. The dotted line in (B) represents a linear regression curve.

The decrease in EMC/C ratio as a function of the O/C ratio was non-linear, especially in case of the dry heat-treated wood, with a strong decrease at high O/C ratios and a lower decrease for low O/C ratios (**Figure 6 A**). Such non-linear decrease is also known from the correlation between EMC and mass loss during HT [47]. HT of wood in dry state was more efficient in reducing the EMC than HT of wood in wet state. Differences in EMC/C ratio between the HT techniques increased with decreasing O/C ratio. A higher effectiveness in reducing the water sorption by HT of wood in dry state has been observed

previously [13,48,49], and was explained by the more facile cross-linking of the cell wall matrix in dry heat-conditions [13]. However, in contrast to EMC/C, the reduction in $\text{OH}_{\text{acc}}/\text{C}$ ratio can be described as a linear function of the O/C ratio, irrespective of the HT technique applied (**Figure 6 B**). Thus, OH_{acc} is determined by the overall loss in polar groups in wood, which is estimated by the O/C ratio. Using a linear regression curve, a slope of 0.55 is determined, which increases to 0.61 when removing the data point with the lowest O/C ratio (HT_{dry} at 240 °C) as an outlier. Therefore, only about 60 % of the thermally removed oxygen species resulted in a loss in OH_{acc} , which indicates that: (a) some of the thermally removed OH groups were inaccessible and/or (b) some of the thermally removed oxygen species originated from moieties other than OH groups. We note that an extensive removal of sugars, as suggested from the thermal mass change versus O/C ratio (**Figure 2**), is consistent with a slope between 0.5 and 0.6 mol OH_{acc} per removed mole of O, considering 2 OH-groups per pentosan unit ($\text{C}_5\text{H}_8\text{O}_4$) and 3 OH-groups per hexosan unit ($\text{C}_6\text{H}_{10}\text{O}_5$), respectively.

Figure 7: EMC at 25°C and 93 % RH (mmol g⁻¹) in dependence on the OH accessibility (mmol g⁻¹). The dotted lines represent linear regression curves for wet and dry heat-treated samples with an R^2 of 0.99. The dashed lines highlight the loss of one and two adsorbed water molecules at equilibrium state per removed accessible OH group, respectively.

The uniform, O/C-dependent decrease in OH_{acc} evidences that differences in the cell wall matrix ultrastructure between wet and dry heat-treated samples that caused large differences in the water-saturated dimensions and relative swelling, did not affect the OH_{acc} in wood. Thus, the formation of

covalent cross-links during HT_{dry} , which has been suggested to further reduce the sorption of heat-treated wood [10,13], does not function by limiting the access of remaining OH groups for water molecules, but by decreasing the number of accessible OH groups that are simultaneously active in sorption [50]. This is confirmed by **Figure 7**, which shows that the decrease in EMC in dependence on OH_{acc} can be well described by linear functions, with a much larger regression slope for dry than for wet heat-treated samples.

Figure 8: Schematic illustration of the proposed occupancy of accessible OH groups by water molecules at ca. 95 % RH. (A) Sorption of water dimers at accessible OH group pairs to an occupancy of 1 in unmodified wood; (B) thermal removal of 2 accessible OH groups removes 4 water molecules and leaves 2 accessible OH group singles; (C) mechanical relaxation restores 1 OH group pair from the OH group singles, resulting in only 2 removed water molecules.

By dividing the EMC at 25 °C and 93 % RH by OH_{acc} an occupancy of 1.01 water molecules per accessible hydroxyl group is determined for unmodified Scots pine sapwood, which is close to the occupancy of 1.1 at 20 °C and 92 % RH that was calculated by Berthold et al. [51] for lignocellulosic materials. Recently, Willems [50] suggested that the accessible OH groups form pairs that act as sorption sites for water molecules with strongly bound water monomers at low RH regimes and loosely bound water dimers at high RH regimes, to reach an OH_{acc} occupancy of 1 around 95 % RH. This full occupancy of accessible OH group pairs by water dimers at around 95 % RH is illustrated schematically in **Figure 8 A**. Following the theoretical considerations of Willems [50], the thermal removal of

accessible OH groups destructs sorption sites, thereby creating OH group singles that do not contribute to water sorption at the thermodynamic equilibrium (**Figure 8 B**). However, these OH group singles are still accessible for (deuterated) water and, thus, measured by HDX. This is evidenced by HDX experiments on acetylated wood showing that OH_{acc} is approximately reduced by the number of acetyl-substituted OH groups and not by twice their number [24]. HDX of OH group singles may be explained by the dynamic change of OH group pairs with nearby OH group singles, enabling all accessible OH groups to be part of an OH group pair that binds (deuterated) water, eventually. Furthermore, (deuterated) water may also adsorb to OH group singles momentarily to initiate HDX, even if this is not the most energetically favorable constellation.

When the concept of water dimer sorption at OH group pairs is assumed, one may expect that the EMC of heat-treated wood is reduced by 2 mol water per mol thermally removed accessible OH groups. However, destructed sorption sites may be restored by reorganization of the cell wall matrix via mechanical relaxation to form new OH group pairs from remaining accessible OH-groups singles [50], as illustrated in **Figure 8 C**. Indeed, the decrease in EMC in dependence on OH_{acc} (**Figure 7**) occurs at a much lower slope than 2 mmol g⁻¹ EMC per mmol g⁻¹ OH_{acc} for wet heat-treated samples. Since HT_{wet} rather causes covalent bond cleavage than the formation of cross-links [12], the polymer mobility of the cell wall matrix in wet heat-treated wood is sufficiently high to enable mechanical relaxation and restoration of destructed sorption sites. Nevertheless, a full restoration to a reduction of only 1 mmol g⁻¹ EMC per mmol g⁻¹ OH_{acc} is not achieved, and this deviation from a slope of 1 increases with decreasing OH_{acc} and increasing treatment temperature. Presumably, the decrease in the amount of amorphous matrix polymers along with a stiffening effect caused by cellulose aggregation decrease the possibility for accessible OH group singles to find counterparts and restore sorption sites.

In dry heat-treated wood, the formation of covalent bonds and cross-links may significantly decrease the cell wall matrix polymer mobility to prevent the restoration of sorption sites by mechanical relaxation (**Figure 8 B**). In line with this theoretical consideration, the decrease in EMC in dependence on OH_{acc} is much larger than for wet heat-treated wood. However, HT_{dry} even resulted in a decrease larger than 2 mmol g⁻¹ EMC per mmol g⁻¹ OH_{acc} , which suggests the occurrence of an additional

mechanism beyond the thermal removal of OH_{acc} . Annealing of amorphous polymers at process conditions that exceed their softening temperature during HT has been suggested to contribute to the reduction in sorption that is directly related to thermal degradation reactions [26,27]. Such an annealing effect might act by destructing sorption sites to create accessible OH group singles without reducing OH_{acc} [50]. Although the annealing effect in heat-treated wood is known to be at least partially reversible by water soaking at room temperature [26,27], the reduced polymer mobility of the cell wall matrix in dry heat-treated wood might hinder its full reversibility. This would additionally lower the EMC, without affecting OH_{acc} .

4. Conclusions

Changes in dry dimensions and Hg intrusion porosimetry measurements after HT and water leaching suggest a shrinkage of the cell wall without cell lumen change by thermal removal of cell wall polymers. This was explained by enhanced cellulose aggregation that restricts cell wall shrinkage in circumferential direction during drying. Cellulose aggregation also caused a reduction of the dimensions in water-saturated state, but this reduction was much larger when covalent cross-links were formed in the cell wall matrix during the HT of wood in dry state. Such enhanced cross-linking also caused an additional reduction in EMC at 25 °C and 93 % RH for dry heat-treated wood, but did not affect hydroxyl accessibility. The latter was only reduced by the thermal removal of polar sites in wood, independent of changes in the ultrastructure. We suggest that the reduction in EMC by cross-linking functions by reducing the amount of accessible OH groups that are simultaneously active in sorption. This may be explained based on the concept of sorption of water dimers at OH group pairs at high RH regimes, wherein crosslinks prevent the formation of new water sorption sites by rearrangement of isolated OH-groups after HT into OH-group pairs.

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6. References

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