Hyperspectral imaging and chemometrics to investigate the chemical wood modification

Muhammad Awais
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Wood possesses an anisotropic hierarchical structure that causes a heterogeneous distribution of chemical reagents in modified wood at different spatial scales. Due to the heterogeneity in chemical distribution, localised regions of wood may remain susceptible to moisture uptake, dimensional instability, and fungal decay. The identification of regions with insufficient chemical uptake is necessary to develop efficient treatment processes, but standard gravimetric methods are insensitive to the location of chemical reagents within the wood. The primary objective of this thesis was to analyse the suitability of spectroscopic-based imaging methods to reveal the distribution of chemical reagents in modified wood at different length scales. The studies focused on the chemical modification of wood with acetic anhydride, paraformaldehyde, and thermosetting resins. The added chemical reagents are known to either react with cell wall polymers to create covalent bonds or to polymerize macromolecules within the cell wall space.

To analyze the chemical changes caused by the modification agents on different spatial scales, the studies combined two chemical imaging techniques that differ in their lateral resolution to identify the process-dependent heterogeneity in modified wood. Near-infrared (NIR) hyperspectral imaging identified and quantified the distribution of chemical reagents and the corresponding moisture content at a macroscopic scale of a few millimeters. Chemometric analysis not only revealed the sample-to-sample variations in chemical uptake and the associated moisture content but also highlighted the localised variations, most notably earlywood and latewood differences. Confocal Raman imaging validated the differences between earlywood and latewood on the cellular level and visualised chemical differences between cell wall regions. Following this, the moisture uptake and the consequent swelling of the modified samples were determined by the dynamic measurements of mass and dimensions within the hygroscopic range. The results indicated the effectiveness of chemical modifications in reducing the moisture content of untreated wood.

Overall, the results in this thesis demonstrated the ability of chemical imaging techniques to localise chemical reagents in small woodblocks and larger board sections. The findings provide a step forward in understanding the chemical changes caused by wood modification in different hierarchical structures in wood on different length scales. In the future, the methods may be used to characterise other treatments and processes that affect the wood composition.
Acknowledgements

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lost during this PhD journey (Ammi Gee, I have fulfilled your wish for me to obtain a PhD, and I hope you are proud of me); Muhammd Shafique, Kaynat Shah, Anas Hameed and Arooj Kanwal, for their immense support not only throughout the research period but also in every aspect of my life.

Espoo, 31 May 2023
Muhammad Awais
Dedicated to my mother Shahnaz Shafique (01.01.1957–16.12.2020)
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Reference
List of Abbreviations and Symbols

(CH₂O)ₙ  paraformaldehyde
A      Absorbance
Ao     Cross-sectional area at 0% relative humidity
Aₜ     Cross-sectional area at a given time
CCD    Charge-coupled device
D      Values of dark reflectance image
D₂O    Deuterium oxide
DoE    Design of experiments
DVS    Dynamic vapour sorption
E      Residual matrix
EMC    Equilibrium moisture content
FOV    Field of view
FTIR   Fourier transform infrared spectroscopy
I      Relative reflectance
Io     Measured reflectance
L      Longitudinal
LV     Latent variables
MC     Moisture content
mₐ     initial dry mass in sorption measurements
M_D₂O  Molar mass of D₂O
MF     Melamine formaldehyde
m_f    final dry mass in sorption measurements
m_i    initial dry mass with aluminium tape
m_m    final dry mass
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_0$</td>
<td>initial dry mass</td>
</tr>
<tr>
<td>$m_{\text{RH}}$</td>
<td>mass after conditioning at a specific relative humidity</td>
</tr>
<tr>
<td>MSI</td>
<td>Multispectral imaging</td>
</tr>
<tr>
<td>NA</td>
<td>Numerical aperture</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NIR</td>
<td>Near-infrared</td>
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<tr>
<td>P</td>
<td>Loading vectors</td>
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<tr>
<td>PCA</td>
<td>Principal component analysis</td>
</tr>
<tr>
<td>PCs</td>
<td>Principal components</td>
</tr>
<tr>
<td>pF</td>
<td>paraformaldehyde</td>
</tr>
<tr>
<td>PF</td>
<td>Phenol formaldehyde</td>
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<tr>
<td>PLSR</td>
<td>Partial least squares regression</td>
</tr>
<tr>
<td>R</td>
<td>Radial</td>
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<tr>
<td>RH</td>
<td>Relative humidity</td>
</tr>
<tr>
<td>RMSEC</td>
<td>Root mean squared error of calibration</td>
</tr>
<tr>
<td>RMSECV</td>
<td>Root mean squared error of cross-validation</td>
</tr>
<tr>
<td>RMSEP</td>
<td>Root mean squared error of prediction</td>
</tr>
<tr>
<td>RMSEP_{img}</td>
<td>Root mean squared error prediction of the pixel population</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>$S_A$</td>
<td>Dimensional changes</td>
</tr>
<tr>
<td>sccm</td>
<td>Standard cubic centimetres per minute</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SNV</td>
<td>Standard Normal Variate scaling</td>
</tr>
<tr>
<td>SWIR</td>
<td>Short wave near-infrared</td>
</tr>
<tr>
<td>$T_p$</td>
<td>Scores</td>
</tr>
<tr>
<td>T</td>
<td>Tangential</td>
</tr>
<tr>
<td>W</td>
<td>Values of white reflectance image</td>
</tr>
<tr>
<td>WPG</td>
<td>Weight percentage gain</td>
</tr>
<tr>
<td>ZnCl$_2$</td>
<td>Zinc chloride</td>
</tr>
</tbody>
</table>
List of Publications

This doctoral dissertation comprises a summary and the following publications, referenced in the text by their numerals.

I. **Muhammad, Awais;** Altgen, Michael; Mäkelä, Mikko; Altgen, Daniela; Rautkari, Lauri. 2020. Hyperspectral near-infrared image assessment of surface acetylated solid wood. ACS Applied Bio Materials, 3(8), 5223-5232. 10.1021/acsabm.0c00626.


III. **Muhammad, Awais;** Altgen, Michael; Belt, Tiina; Teräväinen, Venla; Mäkelä, Mikko; Altgen, Daniela; Nopens, Martin; Rautkari, Lauri. 2022. Wood-water relations affected by anhydride and formaldehyde modification of wood. ACS Omega, 7 (46), 42199-42207. 10.1021/acsomega.2c04974.


V. Altgen, Michael; **Muhammad, Awais;** Altgen, Daniela; Klüppel, André; Koch, Gerald; Mäkelä, Mikko; Olbrich, Andrea; Rautkari, Lauri. 2023. Chemical imaging to reveal the resin distribution in impregnation-treated wood at different spatial scales. Materials & Design, 225, 111481. 10.1016/j.matdes.2022.111481.
Author’s Contributions

Publication I: Hyperspectral near-infrared image assessment of surface acetylated solid wood

MA conceptualised and designed the experiments with co-authors MAlt, MM, and LR. MA performed the experimental work, collected chemical imaging data, performed the formal analysis and visualisation, processed the imaging data with Matlab software, and wrote the original manuscript under the supervision of MAlt, MM, and LR. DA prepared the samples for confocal Raman imaging and contributed to the illustrations. MAlt and MM contributed to the chemical imaging and results analysis and critically reviewed the manuscript. LR supervised the work and reviewed the manuscript.

Publication II: Quantitative prediction of moisture content distribution in acetylated wood using near-infrared hyperspectral imaging

MA conceptualised and designed the experiments with MAlt, MM, and LR. MA performed the experimental work, chemical imaging, formal data analysis, and visualisation, processed the imaging data with Matlab software, and wrote the original manuscript under the supervision of MAlt, MM, TB, and LR. TB contributed to the reviewing and editing of the manuscript. MAlt and MM contributed to the chemical imaging, interpreted the results, and critically reviewed the manuscript. LR oversaw the work and reviewed the manuscript.

Publication III: Wood-water relations affected by anhydride and formaldehyde modification of wood

MA conceptualised and designed the primary part of the experiments with MAlt. MA performed the experimental work, formal data analysis, and visualisation, processed the imaging data with Matlab software, and wrote the original manuscript under the supervision of MAlt and TB. TB contributed to the confocal Raman imaging and reviewed the manuscript. VT contributed to the experimental work. MM supervised the work and reviewed and edited the manuscript. DA analysed the results and contributed to the visualisation. MN provided the resources for automated sorption balance measurements and reviewed the manuscript. LR supervised the work and reviewed the manuscript.

Publication IV: Distribution and curing reactions of melamine formaldehyde resin in cells of impregnation-modified wood

MA contributed to the formal image analysis and results interpretation and co-wrote the imaging section of the manuscript. MAlt was responsible for the conceptualisation, investigation, and design of the experiments and for writing the
original draft. DA contributed to experimental work, results analysis, and visualisations. AK contributed to the experimental work and results analysis. MM supervised the work and contributed to writing, reviewing, and editing the manuscript. LR supervised the work, provided resources, and reviewed and edited the manuscript.

**Publication V:** Chemical imaging to reveal the resin distribution in impregnation-treated wood at different spatial scales

MA performed the chemical imaging and formal analysis of the imaging data and wrote imaging section of the manuscript under the supervision of MAlt. MAlt conceptualised the design of the experiments, performed the analysis and visualisation of the results, and wrote the original manuscript. DA contributed to the experimental work, results analysis, and visualisation. AK contributed to the analysis of the results and co-wrote the manuscript. GK provided the resources for UV microspectrophotometry and co-wrote the manuscript. MM contributed to the chemical imaging and supervision and co-wrote the manuscript. AO performed the analysis of the results and wrote the manuscript. LR supervised the work and provided the resources.
1. Introduction

Wood and other lignocellulose materials encompass a hierarchical porous structure with numerous hollow cells and pronounced anisotropy (Chen and Hu, 2021). Like most bio-based materials, wood is hygroscopic, absorbing and desorbing water in direct contact with it or through the water vapour in the surrounding environment to equilibrate its moisture content (MC) (Thybring et al., 2022). The complex formation of the cellular matrix offers various pathways for the mass flow or diffusion of reagents at different spatial scales. These multiscale channels (or diffusion pathways) are generally utilised to chemically modify the wood to improve its inherent properties, such as dimensional stability (Kajita et al., 2004), durability (Klüppel, 2017), and resistance to decay (Biziks et al., 2021). The intricate wood cell wall structure generates an inhomogeneous distribution of chemical reagents (Zhang et al., 2022). One must determine the wood regions with insufficient chemical uptake to develop an efficient treatment method.

Standardised gravimetric methods are often used to estimate bulk chemical change, but these methods are insensitive to determining the localised distribution of chemicals within the wood. An alternative approach is to employ spectroscopic-based imaging methods to evaluate the physiochemical changes at an acquirable spatial resolution. A combination of imaging methods and sensing technologies can be utilised to determine the process-dependent distribution of chemical reagents from the scale of several millimetres (macro-scale) to the cellular-level distribution in microns. We used wood modification methods to create chemical gradients and evaluate the structural changes caused by the chemical treatment with near-infrared (NIR) hyperspectral imaging and confocal Raman imaging techniques. The acquired images are in a three-dimensional format called image hypercubes, containing two-dimensional spatially correlated pixels and corresponding spectral channels. Hyperspectral imaging data can be coupled with chemometric methods in a multivariate way to visualise selective chemical information concerning the concentration and presence of components at each pixel level (Amigo and Grassi, 2019). Besides sample-to-sample variations, hyperspectral imaging revealed the chemical changes within the sample, such as the chemical differences in the earlywood and latewood cells. Moreover, since chemical imaging methods are not limited to the smaller wood block, the quantitative prediction of components for the larger wood panels or wood blocks is also possible. With this, we offered to utilise chemical imaging
tools for wood and other bio-based materials where the location of chemical reagents in the structure is decisive.

1.1 Objective of the dissertation

This dissertation’s main aim was to analyse the suitability of NIR hyperspectral imaging and confocal Raman imaging to visualise the inhomogeneous distribution of chemical reagents within the wood at a macroscopic scale to the cellular scale of a few microns. Its primary focus was understanding the distribution of modification agents and water in the wood. We used chemometric methods of data decomposition, classification, and regression analysis on hyperspectral imaging data to monitor the inter- and intra-sample variations caused by the modification reagents at different spatial scales. Moreover, the effect of chemical modification on wood moisture uptake was also predicted. The results acquired by the chemometric analysis were validated with dynamic vapour sorption (DVS) and swelling measurements. With this aim, the dissertation addressed the following research questions: How are the modification agents distributed throughout the wood’s hierarchical structure at different spatial scales? Which chemical imaging methods are suitable to locate the modification reagents within the wood at different length scales? And how do chemometric methods based on chemical imaging data support the analysis to determine the chemical changes caused by the modification in wood?

The dissertation consists of five peer-reviewed articles. Publications I, II, and V investigate the process-dependent heterogeneity caused by the chemical wood modification using chemical imaging methods (NIR hyperspectral imaging and confocal Raman imaging). Publication II analyses the effect of chemical modification on the MC of the wood using NIR hyperspectral imaging. Publications III and IV evaluate the cell wall changes caused by chemical modification with confocal Raman imaging. Publication III further investigates the hydroxyl accessibility and consequent cell wall swelling with automated sorption balance and simultaneous 2D imaging. The NIR imaging approach was not restricted to determining the chemical variations in small wood blocks but applied on commercial-sized larger board sections in Publication V. The graphical illustration of the chemical imaging methods on wood at different spatial scales is shown in Figure 1.
Figure 1. The graphical illustration of the chemical imaging methods on wood at different spatial scales. Reproduced with permission from (Awais et al., 2022b). Copyright 2022 Springer Nature.
2. Background

This chapter includes the wood’s anatomical structure and composition, chemical modification, chemical imaging, and wood-water interaction. The wood’s complex structural formation causes the heterogeneous distribution of chemicals within the wood. Chemical imaging methods can detect these physicochemical changes at different spatial resolutions. The theoretical background of the wood structure is presented first, followed by the in-depth theory of chemical imaging techniques to understand the driving mechanisms for heterogeneity in wood. Then the background details of statistical and multivariate data analysis techniques are reviewed.

2.1 Wood morphology and its composition

Wood is complex, comprising plant tissues composed of various distinct types of cells. The formation of these cells provides several functional properties, such as water transportation, dissolved minerals supply, and mechanical support (Gibson, 2012; Pereira Oliveira Moreira et al., 2020; Ruelle, 2014). The wood cross-section can be visually distinguished into sapwood, heartwood, and pith. Sapwood is the physiological portion of the tree and transports water and nutrients into the tree. Heartwood is the central construction section of most wood stems and contains inactive or dead cells. The pith is at the tree’s centre and is formed in the first year of growth (Tokareva, 2011).

Wood can be categorised into softwood and hardwood (Parham and Gray, 1984). Hardwood and softwood differ in morphological structure. Softwood consists of long cell types called longitudinal tracheids (90-95% of the cells in softwood are tracheids), which are responsible for the translocation of water and mechanical support in trees. But hardwood cells contain more specialised vessel elements, joined end-to-end to form efficient water channels (Parham and Gray, 1984). Hardwood has many parenchyma cells and a narrow-lumined vessel tracheid. Tracheids are sometimes present but never abundant in hardwoods. Tracheids are considered to be a relatively early form of cell type that eventually evolved into both vessel members and fibers. Vessel members and fibers are present and oriented in axial direction in the hardwood. The parenchyma cells are axial oriented but seldom absent in hardwood. In certain species of softwood, axial parenchyma can be observed, but radial parenchyma cells are
always present and forms the rays, often in conjunction with radial tracheids (Fukuda and Ohashi-Ito, 2019). And they are one of the reasons for anisotropic behaviour with different directional properties in longitudinal, radial, and tangential directions (Hosford, 2013). The anisotropy in the structure continues in the growth rings as cell layers develop. In softwood growth rings, the wide-lumened thin cell wall earlywood tracheids transition gradually into the narrow-lumened thick cell wall latewood cells. However, the growth rings in hardwood are more apparent in ring-porous species than in diffuse-porous species. In diffuse-porous hardwoods, the vessel elements are smaller but homogeneously distributed throughout the growth rings. The pits conduct the water between the neighbouring cells. Softwood tracheids include bordered pits with a torus and a disc-like membrane in the adjoining lignified cell walls. Compared to other homogeneous materials, the complex morphology of wood shows that the structural elements are heterogeneously distributed hierarchically, with numerous hollow cells and a pronounced anisotropy (Richter, 2015; Rowell, 1984). Figure 2 shows the softwood’s anatomical structure.

![Figure 2. Model of softwood showing the wood’s anatomical structure at different spatial scales.](image)

### 2.2 Wood cell wall ultrastructure

The wood cell wall is a multi-component system consisting mainly of cellulose, hemicellulose, and lignin, and the structural complexity is reflected at the cellular scale (see Figure 3). The cross-section of longitudinal tracheid cells can be visualised with an optical and electron microscope. The cell wall is formed in a multilayer structure containing primary and secondary cell walls. The cell wall is connected with the middle lamella, which is not an integral part of the cell wall. The middle lamella and the primary cell wall are generally grouped as a single unit called the compound middle lamella. The primary cell is ultrathin, comprising a large quantity of lignin and a small amount of cellulose. The middle lamella layer contains lignin and acts as a bridge between the cells. Because of the similar structural units, it is sometimes challenging to distinguish
between the middle lamella and the primary cell wall (Hill, 2006; Zhong et al., 2019).

The secondary cell wall is subdivided into three layers: S1, S2, and S3. These layers include cellulose microfibrils at different orientation references from the axillary axis. In latewood and earlywood cell walls, the S2 layer occupies the most substantial volume and contributes extensively to the wood’s properties. The S2 layer is formed by several lamellae composed of a close network of microfibrils in a helical winding pattern. The microfibrils angle varies between 5-10° in latewood cells and 20-30° in earlywood. The S1 and S3 layers of microfibrils are oriented at angles of 50-70° and 50-90°, respectively. The space between the microfibrils is filled with lignin and hemicellulose. The region between the microfibrils is commonly identified as microvoids or micropores. In a fully swollen state, these micropores are open and provide accessibility to internal cell wall regions. During the drying process, water is removed from these spaces, and the micropores begin collapsing (Fengel and Wegener, 1983; Hill, 2006; Sjöström, 1993a).

The polymeric constituents of the cell wall are cellulose, hemicellulose, lignin, and a minor quantity of other components called extractives. The structure and components are briefly discussed here.

Cellulose content varies from 40 to 50% by volume in wood. It comprises linear polymer macromolecules formed by cellobiose units connected with glycosidic linkage resulting in cellulose polymer chains (degree of polymerization = 10^4). These polymer chains bundle together with the hydrogen bonds network and form microfibrils. The microfibrils contain crystalline and amorphous
components and act as a reinforcing unit for the cell wall in the wood structure. The presence of crystalline cellulose in the core of microfibrils makes them thermally stable and unreactive. The crystalline region is surrounded by amorphous cellulose content (Brigham, 2018; Hill, 2006).

Hemicelluloses are primarily polysaccharides that contain different sugar units. The structure of hemicelluloses is less organised than cellulose (Berglund et al., 2020). Hemicelluloses differ from cellulose in that they contain sugars other than glucose. And their structure is branched rather than linear and comprises some naturally occurring acetyl and carboxylate groups (Chen, 2014). Hemicelluloses contain amorphous structures with more accessible hydroxyl sites in the cell walls, which make them more moisture sensitive and thermally unstable than cellulose and lignin. The viscoelastic nature of hemicelluloses is an interfacial coupling agent between the highly polar microfibrils surfaces and the least polar lignin matrices. They form a hydrogen bond with the surface of the microfibrils and covalent bonds with the lignin. The degradation of hemicelluloses can cause brittleness in a wood structure (Hill, 2006).

Lignin is a complex and amorphous phenolic polymer. It has no definite structure because of its random nature of polymerization. But the frequency of each bond is well-defined. Lignin connects individual cells via the middle lamella and provides stiffness to cell walls. It acts as a rigid component at normal temperature but transitions at around 140 °C (glass transition temperature). Moisture also influences its properties by opening its structure and serving as a plasticizer for its polymeric network. It contains fewer hydroxyl sites than hemicelluloses. The earlywood regions contain more lignin than the latewood sections (Donaldson et al., 2017; Nimz, 1984).

Extractives can be low molecular mass and non-structural components in wood. Various extractive contents can be found in a tree (depending on the site, species, and age). These components can be removed from the wood using polar and non-polar solvents. The common extractive components are based on waxes, fats, terpenoids, and phenolics (Sjöström, 1993b; Verkasalo et al., 2022). Extractives provide tree protection against pathogen attacks. Some compounds in sapwood act as food reserves and resist water and decay resistance. The presence of extractives can hinder the chemical modification of wood or cause an inaccurate determination of weight percentage gain (WPG).

### 2.3 Wood-water interaction

Wood, like other natural materials, is hygroscopic, meaning that it absorbs and desorbs moisture from the surrounding environment until reaching an equilibrium. The interaction of wood with water can be in direct contact with liquid water or with water vapours in the surrounding atmosphere. The presence of water in wood compromises its mass, dimensions, density, mechanical strength, and thermal and biological properties (Azwa et al., 2013; Mvondo et al., 2017). Wood-water interaction is complex and can be subsequently mostly understood by knowing the basic mechanisms of moisture uptake at a molecular level.
All polymeric constituents of wood, such as hemicelluloses, lignin, and cellulose, contain hydroxyl groups and can interact with moisture. A substantial number of hydroxyl groups are present in a confined and compact formation of aggregated cellulose microfibrils. These hydroxyl groups are inaccessible under normal conditions. The hydroxyl groups at the surface of the microfibrils can interact with moisture and are considered accessible sorption sites. Hemicelluloses contain most of the accessible hydroxyl groups, then lignin and cellulose, with the least accessible OH groups. The accessible OH groups can be deuterated with D$_2$O and gravimetrically measured by the mass increase associated with the exchange of protium for the heavier deuterium in OH groups that form hydrogen bonds with D$_2$O (Altgen and Rautkari, 2021; Pönni et al., 2014; Uimonen et al., 2020). Moreover, the microfibrils push apart or contract while absorbing or desorbing moisture under specific relative humidity (RH) conditions (Zabler et al., 2010).

The sorption behaviour of the wood can be determined by the relationship between the equilibrium moisture content (EMC) and the change in RH, which is called sorption isotherms. Wood displays the sorption hysteresis phenomenon, meaning that the EMC differs in the absorption and desorption processes. The moisture is in the cell walls within the hygroscopic range, 0-97% RH. And it interacts with the OH groups of cell wall biopolymers by forming hydrogen bonds. The sorption of water swells the wood cell walls. The upward bend in the sorption isotherm at 60-70% RH corresponds to the softening of the amorphous polymers. The transition of amorphous polymers decreases the viscosity and rigidity of the polymer network and increases the cell wall space to accommodate the water molecules (Mauze and Stern, 1984; Vrentas and Vrentas, 1991). The softening of hemicelluloses takes place at around 75% RH at normal temperature. However, the temperature and MC severely affect the softening of the hemicelluloses (Engelund et al., 2013). The water in the cell lumen and microvoids can be related to capillary condensation (Thomson, 1871). The capillary condensation depends on the pore size and geometry. The presence of larger microvoids causes the capillary condensation process to occur at the over-hygroscopic range, above 99%. The moisture uptake in cell walls and the outside occurs simultaneously within this range, but the contribution of uptake outside the cell wall region is significantly higher (Fredriksson and Thybring, 2019). It has also been reported that the water molecules interacting with accessible hydroxyl groups are solely responsible for moisture uptake. However, a poor correlation has been found between the MC and hydroxyl accessibility, indicating that an additional factor of cell wall space substantially contributes to moisture uptake in the wood (Rautkari et al., 2013). Figure 4 shows the wood-water interaction and the mode of action against different cell wall modifications.
Figure 4. Wood-water interaction concerning volumetric changes and the mode of action with different cell wall alterations. The illustrations above show the wood ultrastructural changes caused by the cell wall bulking and cross-linking.

2.4 Wood modification

Wood can be modified by chemical, mechanical, and high-temperature heat treatment methods (Rowell et al., 2009). The chemical modification of wood reduces moisture uptake and improves its resistance to degradation by regulating MC. Generally, chemical modification occurs through two methods: active and passive. In an active modification, the wood cell walls’ polymers, such as hemicellulose, lignin, and cellulose, form a covalent bond with a chemical reagent. The added chemicals form a new chemical bond between the hydroxyl groups in wood or among other chemicals (Rowell and Dickerson, 2014). Some chemicals comprising more than one bonding position form cross-linking in the cell wall. The cross-linking formation depends on the applied reaction conditions or wood material. The passive modification involves no covalent bond formation with wood-polymeric constituents in the cell walls. However, some low molecular weight resin enters the wood, forming macromolecules via polycondensation, thus resulting in a fixation (Sandberg et al., 2021).

2.4.1 Acetylation

Acetylation involves the transportation of acetic anhydride through the tracheid, pits, and diffusion pathways deeper into the cell walls’ accessible hydroxyl groups in wood, resulting in hydrophobic acetyl groups forming ester bonds and acetic acid as by-products (Jones and Sandberg, 2020). The simplified reaction is shown in Figure 5. The added acetyl groups reduce the blocking effect toward the moisture uptake by occupying the available sorption site and causing cell wall bulking (Guo et al., 2022). Solid wood can be acetylated with various procedures depending on the wood species. At the commercial scale, acetylation is
performed by impregnating wood with acetic anhydride in a pressurized autoclave, which can enhance the flow of reagent through the porous wood material and into the cell walls. The presence of moisture in the structure can produce acetic acid. A small amount of acetic acid supports the acetylation by swelling the wood cell walls and providing room for the reagent to react with the reactive groups (Rowell and Dickerson, 2014). But acetylation with the gaseous method is undesirable and inefficient at a larger scale. It requires a higher induction time and has low permeability. The reaction rate can be improved by using more reactive reagents, such as acetyl chloride, as a catalyst (Koso et al., 2022).

![Figure 5. A schematic illustration of acetylation reaction with the wood.](image)

On a laboratory scale, smaller wood blocks are acetylated by first impregnating the acetic anhydride, and the reaction is induced by placing the wood in heated (ca. 120°C) acetic anhydride. At elevated temperatures, the reaction with accessible hydroxyl groups in cell walls biopolymer occurs and is replaced with acetyl groups, forming acetic acid as a by-product (Mantanis, 2017). The acetylation reaction can be controlled in a single step by monitoring reaction conditions. The targeted spatial locations in the cell wall can be modified, and the related MC is controlled (Digaitis et al., 2021).

### 2.4.2 Formalisation

The formalisation of wood with paraformaldehyde (pF) is performed in a vapour phase reaction in a closed vessel. Upon heating, formaldehyde molecules penetrate the cell walls and react with up to two hydroxyl groups to form a cross-linking between the reacted groups. The formalisation reaction is a two-stage reaction process: the formation of unstable hemiacetal with the OH group and a reaction with the second OH group to form a stable acetal group (Rowell, 1984). The gaseous formaldehyde molecules with lower molecular weight distribute uniformly into the wood cell wall. The reaction is triggered by pre-treatment with Lewis acid as a catalyst or by simultaneous vapour treatment with sulfur dioxide (Stevens and Parameswaran, 1981). The pre-treatment with an acidic catalyst, such as sulfur dioxide or hydrochloric acid, hydrolyzes the cell walls' biopolymers and causes the slight degradation of polysaccharides in the hemicellulose. Hemicelluloses contain the highest number of hydroxyl groups,
and the subsequent degradation can reduce moisture uptake. However, the primary cause of moisture reduction is attributed to cross-linking. Cross-links formed by the reaction with formaldehyde restrict the swelling of cell walls (Himmel and Mai, 2015). This protocol is not applied as a commercial modification method.

2.4.3 Resin impregnation

The resin impregnation process includes the penetration of resin (phenol, melamine, or urea-based, and others) into the wood under vacuum and/or pressure, then dried and cured. The process involves soaking the wood into an aqueous solution of low molecular weight resin and impregnation under pressure until the complete saturation of the wood with the impregnation solution. The impregnated wood is dried at a moderate temperature to remove water, followed by heating up to 60-150 °C to cure the resins to large macromolecules. The curing of resin can be performed in a controlled manner to avoid a heterogeneous distribution (Klüppel and Mai, 2013). Depending on the drying conditions applied, the resin can migrate toward the surface before curing. The phenol and melamine formaldehyde (MF) resins can polymerize with the cell walls' biopolymers (Sarika et al., 2020). Melamine resins can penetrate the cell walls and deposit into the cell walls without chemically reacting with the biopolymer matrix. The resin fills space in the cell walls otherwise occupied by water, keeping the wood swollen. Figure 6 demonstrates the cell wall changes due to acetylation, cross-linking (formalization), and resin impregnation in relation to water molecules.

![Figure 6. Wood ultrastructural changes caused by the acetylation, formalisation, and resin impregnation concerning water vapour sorption. Reproduced with permission from (Awais et al., 2022a). Copyright 2022, American Chemical Society.](image-url)
2.5 Chemical imaging

The hierarchical structure of wood comprises density variation in earlywood and latewood at its macrostructure down to the cell walls containing cellulose, lignin, and hemicelluloses at the micro level. The structural buildup of wood renders the heterogeneous distribution of chemical reagents and MC. Identifying and visualising inter- and intra-sample variations in chemical distribution requires modern chemical imaging tools to estimate the chemical changes at different spatial lengths. The standard gravimetric methods determine the bulk chemical changes in wood but have difficulty determining spatial differences. With the advancement in instrumentation, hyperspectral imaging — or multi-spectral imaging (MSI) — provides the opportunity to accurately estimate the chemical heterogeneity on the macroscopic level of wood, down to a few millimetres and across cells on a micron scale.

Hyperspectral instrumentation is continuously developing in sensing technology and includes a broader range of the electromagnetic spectrum (Aval et al., 2019). The basic configuration is a mixture of conventional imaging techniques and single-point spectroscopy in different modes of action. Several spectroscopic methods, such as ultraviolet-visible, NIR, Mid-infrared, Raman, X-ray, and confocal laser fluorescence microscopy, are available to link with imaging. But the formulation depends on the instrument limitations and end-user application (Amigo and Grassi, 2019). This dissertation includes well-developed chemical imaging methods based on NIR hyperspectral and confocal Raman imaging.

2.5.1 Near-Infrared hyperspectral imaging

NIR imaging is evolving from an analytical imaging technique to a more economically accessible daily usage quality assessment tool. The robustness, higher computational capabilities, and data processing make it an efficient method for solving real-world problems. The analytical NIR region includes a wavelength range from approximately 700 to 2500 nm. This region contains absorbance bands related to the stretching and deformation of chemical bonds in wood, including O-H, C-H, and N-H bands, as well as the fundamental vibrations of the skeletal structure of the cellulose, hemicellulose, and lignin molecules. Moreover, NIR imaging requires sample surface preparation and is a rapid, reproducible, and non-destructive technique. The absorption bands related to the overtones and combinations of chemical bonds (such as O-H, C-H, and N-H bands) are often broad and overlapping. The overlapping and broadening of absorption bands are due to various factors such as the complexity of the chemical structure of the material being analysed and the influence of other chemical components in the sample (Workman and Weyer, 2008). The fundamental principle behind the NIR imaging is the interaction of light with the target’s molecules. The interaction can be related to chemical information (lignin, cellulose, and hemicelluloses in wood) or the physical properties (surface roughness, cracks) of the target. The photon from a light source contains specific energy and trajectory affected by the interference with the target, resulting in lower energy and
trajectory change depending on the chemical properties of the material. The photon captured by the detector contains molecular fingerprints in spectral form. The trajectory of the photon after hitting the target can be absorbed completely, reflected, or transmitted. The reflection of light is the most common way to measure the difference in energies and trajectories of photons. The schematic illustration in Figure 7 shows the light interaction with the target in different modes.

![Figure 7. Light interaction with target and possible trajectories.](image)

The classification of a NIR hyperspectral camera depends on the image acquisition modes and its mechanical procedure. The prevalent acquisition modes of scanning are global imaging (area or plan scanning), point or line scanning, spatio-spectral scanning, and more. The short wave near-infrared (SWIR) hyperspectral camera presented in this dissertation was manufactured by Specim, Spectral Imaging Ltd. The operational mechanism for collecting hyperspectral data was line scanning mode, which records the target’s complete spectral and spatial information line by line (also called push broom technology). The main components of a line-scanning hyperspectral camera are an imaging spectrograph, a grayscale camera, and an objective. All these components must be optimised by the camera wavelength range. The typical recording of a black-and-white image requires only the objective and the camera units. The objective projects the target to the camera sensor, which records the image in black and white. In hyperspectral image recording, the imaging spectrograph is added, which contains an input slit accommodating optics, a dispersive unit, and a focusing lens. The objective forms an image to input a slit that limits the incoming information. The input slit requires accurate spectra measurement, which depends on the input split size. The narrower the input slit, the more precise the data achieved. The following component is collimating optics that direct the light signals from the slit to the dispersive unit. The dispersive unit spreads the incoming light into the spectra. The spectra are focused on the grayscale camera with a focusing lens. The grayscale camera records the intensities of the dispersed light and acquired spectral image data. This process is repeated by
moving the stage until the whole target is scanned line by line. The hyperspectral image acquisition is illustrated in Figure 8.

**Figure 8.** The operational mechanism for collecting hyperspectral data.

**Calibration**

Like other spectroscopic techniques, NIR hyperspectral imaging is calibrated to measure reliable spectral images. The spatial information must be well-correlated with the ground coordinates (x and y dimensions). Several parameters, such as light source illumination, the sensing devices layout, distance from the target, and more, must be optimised to acquire unbiased spectral images. Thereby, calibrating the camera is essential and must be considered before and during the collection of spectral images (Mäkelä et al., 2020). The spatial calibration is performed to determine the spatial resolution and the range (field of view). But many methods can spatially calibrate the device depending on the acquisition modes. In a push-broom system, spatial calibration can be performed by adjusting the focal distance and the speed of the movable stage and capturing the image at specific wavelengths using a customized printed checkerboard with known spatial information. The reflectance calibration can be performed by correcting the dark 0% and 100% reflectance values for the detector. The dark response can be collected by recording an image of a non-reflective opaque lens cover. The high reflectance values are recorded using high-reflectance standards or white ceramic spectralon targets. These two recorded images are used to determine the relative reflectance of the sample from the raw spectral image. The relative reflectance \( I \) is calculated by Eq. 1:

\[
I = \frac{I_o - D}{W - D} \quad (1)
\]

\( D \) and \( W \) are the values of dark and white reflectance images, respectively, and \( I_o \) is the measured reflectance value. The reflectance can be converted into absorbance \( A \) by Eq. 2:

\[
A = -\log_{10} \left( \frac{I_o - D}{W - D} \right) \quad (2)
\]
2.5.2 Confocal Raman imaging

Raman spectroscopy requires a monochromatic (single frequency) light source and the Raman effect or Raman scattering (Salzer and Siesler, 2014). The light scatters during the interaction with atoms or molecules of substrate is shown in Figure 7. Most of the photons scatter elastically and contain the same energies and wavelength as the incident photon, referred to as Rayleigh Scattering. But a few photons scatter, with energies typically lower than the frequency of incident photons. This interaction is inelastic, meaning photons carry information about the molecular vibrations in the substrate. The Raman effect is caused by the absorption and the subsequent emission of the photons, which corresponds to the excitation from the intermediate energy state to the virtual energy state. This exchange of energies is interpreted in terms of the molecules’ vibrational and rotational energy levels. The Raman scattering comprises two components: Stokes and the anti-stokes. The energy absorbed by the molecules results in lower energy photons and generates a stoke line on the red side of the incident spectrum. However, when the molecules lose energy, the incident photons are shifted to the blue side of the spectrum, resulting in anti-stokes (Figure 9). The polarizability is determined by the distortion of the molecules in a material. And the Raman shift occurs due to a change in the polarizability concerning vibrational and rotational energies (Zoubir, 2012).

Confocal Raman spectroscopy attained a remarkable development through the advancement in laser technology as an excitation source, coupled with a high-output monochromator and a highly sensitive photo-detector, i.e., a charge-coupled device camera (CCD). The Raman microprobe is a conventional Raman spectrometer and a confocal microscope. The confocal microscope functions to project the exciting light on the sample precisely to a size of 1-2 µm and gathers the scattered light, transmitting it to the slit of the spectrometer. The modern confocal Raman is equipped with single or multiple lasers, a high-quality

![Figure 9. Energy diagrams of Raman and Rayleigh scattering. The horizontal lines correspond to the energy levels (S₀, S₁, S₂, and S₃) associated with the different vibrational modes of the molecules. The dashed horizontal lines indicate virtual energy state.](image-url)
objective lens with a longer working distance, and a specialised motorized spectrometer lens that automatically adjusts with different configurations, the highest quality diffraction gratings, and thermoelectrically cooled CCD detector. Combining these devices enables the collection of the Raman spectra of one-dimensional profiles, two-dimensional images, or three-dimensional render volume. In Raman imaging, the spectral data is gathered in a hypercube by two imaging methods: point-by-point and line-focus imaging. In the point-by-point imaging approach, the laser source focuses on a spot, and the motorized stage moves and sequentially records the defined area of interest. The spatial resolution can be controlled by combining the laser spot size and the spacing between the acquisition points.

Raman Spectra include various features relating to the different aspects of the material, such as the relative number of components in the material, layer thickness, crystallinity, tension or compression, and temperature (Renishaw, 2021). These features are interpreted from the changes in the spectra, such as height (relative intensities), width, and the position of Raman bands. The variation in spectral fingerprints at a specific point of a map reveals the changes in the uniformity of the material. The Raman spectrum of crystalline material is relatively simple to interpret because it contains identical atoms in the same configuration, and a dominant Raman band can be observed (Guieu and Lagugné-Labarthet, 2012). However, natural composites, such as wood, comprise complex and less symmetrical arrangements of molecules. The vibrational frequencies depend on the atomic masses and the strength of their bonds. The Raman shifts are low for heavy atoms, and weak bonds, such as the C=C bond, have a low vibrational frequency. The vibration from the C=C double bond has a higher frequency than the C-C single bond (Renishaw, 2021).

2.5.3 Chemometric and multivariate data analysis

The acquired data from NIR hyperspectral imaging and confocal Raman imaging is visualised as an image hypercube containing two-dimensional spatial pixels of the measured surface. And the third dimension includes chemical information in spectral channels. The hypercube data structure is a multi-component system. The corresponding spectrum to each pixel usually contains mixed information of more than one polymeric component of wood such as lignin or cellulose. Irrespective of the helpful chemical information, the measured data has some artefacts, such as noise signals, spectral interference, and redundant dead or saturated pixels. The unwanted information must be removed from the image hypercube. There are various algorithms available to process the data in a way that integrates with multivariate data analysis methods.

The primary reason for implementing hyperspectral imaging in plant sciences, recycling, waste management, the textile industry, food technology, mining and oil, pharmaceuticals, and others is its augmentation with chemometrics and multivariate data analysis methods. Chemometrics is fundamentally a data mining technique comprising mathematical, statistical, and data analysis methods to employ formal logic or the optimised design of experiments (DoE) to extract maximum relevant chemical information by analysing chemical data in a
multivariate way (Héberger, 2008). In hyperspectral imaging, chemometric methods are used to determine the concentration of specific compounds, quantitative analysis, or the identification or presence of chemical constituents in a qualitative manner. The chemometric approach varies depending on the nature of the acquired data, DoE, analysis type, and the end target. There are no fixed procedures to employ on any dataset. The path followed in this dissertation to determine the heterogeneous distribution of chemical analytes in wood is shown in Figure 10. The main building blocks to develop the chemometric models were image processing (segmentation and calibration, preprocessing), principal component analysis (PCA), partial least squares regression (PLSR), and classification.

Figure 10. A comprehensive flow chart of the chemometric methods and data processing techniques applied to wood samples in this dissertation.
2.5.4 Chemical imaging in wood

Chemical heterogeneity is an established challenge in wood, including wood modified with chemical reagents or impregnation treatments. Wood comprises a complex hierarchical buildup that creates process-dependent chemical heterogeneity in distributing chemical reagents at different spatial dimensions (Klüppel and Mai, 2013; Smith and Cockcroft, 1961). Chemical imaging techniques, such as NIR hyperspectral imaging and confocal Raman imaging, enabled us to analyse the chemical properties of wood at different spatial resolutions. On a macroscopic scale, wood is a compact solid containing features at all length scales: cell walls composed of microfibrils comprising the molecular architecture of celluloses, lignin, and hemicelluloses (Toumpanaki et al., 2021). The chemical makeup is a governing factor in causing the heterogeneity of chemicals in the wood. By combining imaging techniques, we can explore the chemical changes and the structural properties concerning the heterogeneity in wood at various spatial dimensions. Other analytical (microscopic) imaging techniques show anatomical and morphological forms of wood, while spectroscopic techniques measure the chemical and structure. Hyperspectral imaging combines anatomical and chemical features. Scanning electron microscopy (SEM), ultraviolet (UV), and fluorescence microscopy can be used to visualise morphological features at high resolution but are insensitive to chemical variation (Abe et al., 1991; Daniel, 2016; Zimmermann and Sell, 2003). The chemical composition of wood can be measured with Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) (Frihart and Beecher, 2005; Salzer and Siesler, 2014). FT-IR spectroscopy detects the chemical bond in wood and identifies the chemical changes that occur in the wood due to the treatment (Salzer and Siesler, 2014). FTIR imaging analyses wood’s spatially resolved chemical composition (Salzer et al., 2000). Here, we selected two spectroscopic-based imaging methods to visualise and quantify the chemical changes detected as spectral signals with corresponding spatial dimensions.

NIR imaging is used to identify and quantify the chemical composition of wood at a macroscopic scale. Wood is an organic chemical complex containing three major polymers, celluloses, lignin, and hemicelluloses, which serve as the main skeletal units in the wood matrix. NIR spectroscopy has been used widely in wood sciences (dos Santos et al., 2021; Kelley et al., 2004; López et al., 2017; Poke and Raymond, 2006; Tsuchikawa and Kobori, 2015), but imaging has rarely been performed. Interpreting NIR spectra requires the fundamental knowledge of underlying chemistry and functional groups in wood that absorbs distinct wavelengths (Schwanninger et al., 2011a). The assignment of NIR bands is typically based on the analysis of spectroscopic signals, which arise from a combination of fundamental vibrations and overtones of those vibrations. The spectral signals of pure components contain symmetrical and distinct bands. But the isolation and purification of wood components are difficult and lead to chemical alteration compared to the native wood. However, several NIR spectroscopic methods have been developed to characterize the cellulose and lignin contents in solid wood (Ai et al., 2022; Elg-Christofferson et al., 1999; Hodge and Woodbridge, 2004; Schwanninger et al., 2011a; Schwanninger and
Hinterstoisser, 2002). NIR imaging data can readily be coupled with chemometrics and multivariate data analysis techniques to extract the desired chemical information (Çelen et al., 2008; dos Santos et al., 2021; Haddadi et al., 2015; Inagaki et al., 2015; Schwanninger et al., 2011b; Stefansson et al., 2021).

Confocal Raman imaging is an effective technique to characterize the spatially resolved heterogeneity in the structural organisation and composition of wood cell walls at a microscopic scale (Agarwal, 2006; Andrew and Hancewicz, 1998; Hu, 2008). The chemical composition is of interest. However, it also provides the structural features and composition of cell wall layers and the orientation of the microfibrils (Cao et al., 2006; Jarvis and McCann, 2000). Confocal Raman imaging maps the spatial distribution of lignin and cellulose-rich regions (Agarwal, 2006). Besides the distribution of other cell wall biopolymers, the molecular structural formation of these components can also be investigated (Agarwal, 2019). The excitation of lignin's aromatic and conjugated structure produces the Raman effect, which enables the prediction of the lignin structure and identifies its traces in the wall matrix (Czaja et al., 2006; Halttunen et al., 2001; Nuopponen et al., 2004; Saariaho et al., 2005, 2003). Cell wall imaging showed the presence of higher lignin content in the middle lamella and the cell wall corners compared to the secondary cell walls (Xu et al., 2006). It was also observed that a small amount of lignin localised in the outer and inner regions of the secondary cell walls (Agarwal, 2006). Cellulose contribution in the cell walls is detected by the band integration around 380 and 2898 cm\(^{-1}\), which shows the high localisation of cellulose content in the secondary cell walls. Confocal Raman imaging also detects the cell wall changes caused by the modification treatment with chemical reagent and impregnation treatment (Digaitis et al., 2021; Dong et al., 2020; Mäkelä et al., 2021; Saletnik et al., 2021; Thybring et al., 2020).

The combined chemical imaging techniques provide a comprehensive analysis of wood by revealing chemical heterogeneity, structural properties, and the chemical makeup of wood at different spatial scales. However, there are some limitations in both imaging techniques in characterizing the wood. NIR Hyper-spectral Imaging has a limited depth of penetration. The penetration depth of radiation is close to 0.3 mm (or a few millimetres) into the wood, and it absorbs 70 to 90% of the incident infrared radiation (Conners and Banerjee, 2019). The NIR measurements are highly dependent on the preparation of the sample, including the surface smoothness, uniformity, and orientation of the wood (dos Santos et al., 2021). These factors can affect the NIR spectra and introduce variability in the predicted results. The presence of moisture, resins, and extractives in the wood will impact the spectra, and will interfere with any signal from the wood itself (Tsuchikawa, 2007). The NIR spectra are complex and contain overlapping signals from different compounds, making it challenging to identify specific compounds in the wood (Tsuchikawa, 2007). Wood contains compounds that cause fluorescence under laser excitation, which can interfere with Raman signals and reduce the accuracy of the analysis (Wei et al., 2015). Despite the limitations of chemical imaging techniques, these tools provide a reliable estimation of chemical composition, structural features, and heterogeneity in
chemical distribution at different spatial dimensions. Overall, we expect to utilise similar chemical imaging methods with certain upscaling that we used here in the field of wood sciences.
3. Materials and methods

This thesis’s core was chemical imaging coupling with chemometrics to reveal the wood modification and its sorption behaviour at different spatial scales. These techniques were performed on native and modified wood with acetic anhydride, pF, melamine-formaldehyde, and phenol-formaldehyde. Chemical modifications were utilised to create heterogeneity in MC and its detection through chemical imaging methods. This chapter briefly describes the chemical changes, moisture uptake in wood, chemical imaging, and multivariate data analysis techniques.

3.1 Preparation of the samples

The wood species used in Publications I to IV was Scots pine (*Pinus sylvestris* L.), and European beech (*Fagus sylvatica* L.) was used in Publication V. The samples with different dimensions were cut from commercially kiln-dried boards, and they did not contain heartwood, knots, or visible defects. The sample dimensions used in Publications I to V are shown in Table 1 and Figure 11.

The number of samples and the treatment levels were selected based on the DoE levels reported in wood modification literature, (see relevant publications for details). In Publication I, samples with dimensions of $12 \times 12 \times 70$ mm³ (radial, R × tangential, T × longitudinal, L) were cut, and special care was taken for the homogeneous distribution of earlywood and latewood. Publication II included samples with dimensions of $15 \times 15 \times 15$ mm³ (R × T × L). In Publication III, two sample sets were prepared, with dimensions of $20 \times 20 \times 10$ mm³ (R × T × L) and $30 \times 30 \times 5$ mm³ (R × T × L).

Publication IV included samples with dry dimensions of $25 \times 25 \times 10$ mm³ (R × T × L). In Publication V, two sets of samples were made of small cubes with an edge length of 15 mm and board sections with dimensions of $75 \times 15 \times 15$ mm³ (R × T × L).
Materials and methods

3.1.1 Surface acetylation

Surface acetylation was performed in Publication I by drying the samples at 103 °C for 24 hours. First, the samples were extracted with fresh acetone to remove the extractives from the wood blocks using vacuum impregnation at 0.04 MPa and room temperature for two hours. Then they were soaked in fresh acetone for 72 hours under standard room conditions in a closed container, followed by air-drying the samples in fume hood for 24 hours. The samples were then oven-dried at 103 °C for 24 hours, and the initial dry mass was recorded.

Wood blocks were sealed with aluminium tape (except for the tangential surface), and the mass of sealed samples was measured. The unsealed, tangential surface was exposed to neat acetic anhydride for different time intervals of 0.5, 1, 2, 3, 6, 12, 24, 48, 72, 144, 216, and 288 hours. The exposed samples were wrapped in aluminium foil and subjected to a hot press with a pressing plate temperature of 120 °C for three hours. The samples were again soaked in fresh acetone for 24 hours and air-dried under the fume hood. This step was performed to remove unreacted acetic anhydride and acetic acid. Oven drying at 103 °C for 24 hours was repeated, and the final dry mass was measured. The degree of acetylation was measured by gravimetric WPG calculated by Eq. 3:

$$WPG = \frac{m_m - m_i}{m_o} \times 100$$

where $m_m$ and $m_i$ are the final and initial dry mass (in g) of wood blocks with aluminium tape. The initial dry mass (in g) without aluminium tape is $m_o$. 

Figure 11. Wood species and the sample dimensions (mm) used in each publication.
3.1.2 Bulk acetylation

The bulk acetylation of wood blocks was performed in Publications II and III. The samples were first Soxhlet extracted with acetone for six hours, followed by oven drying at 103 °C for 24 hours. The initial dry mass of the samples was recorded. Wood blocks were impregnated with neat acetic anhydride under a vacuum pressure of 0.04 MPa at room temperature for two hours. Fresh acetic anhydride was heated to 120 °C in a reaction flask under the flux. Wood blocks were added into the reaction flask after 0, 20, 30, 60, and 360 min (Publication II) and 0, 10, 20, 50, 100, and 360 min (Publication III). The differences in sample size required variation in time steps for the same WPG. The reaction was terminated by quenching the flask in an ice bath. The samples were rinsed with acetone and soaked in fresh acetone overnight. Soxhlet extraction was again performed, and samples were dried in the oven. The final dry mass (in g) was measured, and the WPG was calculated as Eq. 4.

\[
WPG = \frac{m_m - m_o}{m_o} \times 100
\]

where \( m_m \) and \( m_o \) represented the final and initial dry mass (in g) of the wood blocks.

3.1.3 Formalisation

Formalisation was conducted by first Soxhlet extracting the samples with acetone (Publication III), then drying them at 103 °C for 24 hours. Afterwards, they were impregnated with an aqueous solution of Lewis acid (1.5% ZnCl2) at 0.04 MPa for two hours. Then the samples were dried in the oven at 103 °C, and the initial dry mass was measured. The reaction was initiated by heating the 1000 cm\(^3\) desiccator at 100 °C, and 20 g of paraformaldehyde (CH\(_2\)O)\(_n\) was placed in an aluminium pan. The wooden samples were positioned to ensure no direct contact between the pF and the wood blocks. Five similar experimental setups were designed to evaluate the time variations of 6, 12, 24, 30, and 48 hours. After the allocated reaction time, the desiccators were removed from the oven and kept at room condition for two hours. A reference set of ten replicates was also prepared. The replicates were treated only with Lewis acid. The samples were again Soxhlet extracted and oven-dried, followed by measuring the final dry mass. The WPG was calculated by Eq. 3.

3.1.4 Impregnation with melamine-formaldehyde resin

Wood samples were impregnated with an aqueous solution of lower molecular weight MF resin (Madurit MW 840; INEOS Melamines GmbH, Germany) at 0.01 MPa for one hour. Methylol groups in MF resins were partly methylated and provided as an aqueous stock solution (pH 10.1). The stock solution was dried separately at 103 °C for 24 hours to determine the non-volatile matter concerning percentage residue. And this was used to prepare the solutions with a solid content of 10% and 25% by dilution with deionized water, and pure deionized water was considered as a reference 0%. After the impregnation, the
samples were kept in the solution at normal pressure for one hour. The samples were removed from the solution and heated at temperature sequences of 20, 40, 60, 80, 40, and 103 °C for 24 hours. In wet curing, the samples were wrapped in aluminium foil during the first four steps of 20, 40, 60, and 80 °C. The dry-cured samples were exposed to direct heat without covering them in aluminium foil for all temperature sequences. Five replicates per sample group of solid content were prepared, and all samples were vacuum impregnated with deionized water and leached for approximately one week after the modification. During leaching, the water was replaced regularly.

3.1.5 Phenol formaldehyde

The samples with an edge length of 15 mm were oven-dried at 103°C and kept in a desiccator at room temperature for two hours. First, the samples were impregnated with an aqueous solution of low molecular weight PF resin (P554; Surfactor Germany GmbH) with a solid content distribution of 10, 15, 20, 25, and 30%. Sodium hydroxide (NaOH) was included as 3% of the solid resin content to keep the resin soluble and facilitate curing. The reference samples were treated only with deionized water with no added sodium hydroxide. The samples were impregnated with the solution under a vacuum at 0.01 MPa for one hour and then 1.2 MPa for two hours. Then the solution was removed, and the surface of the sample was cleaned with a dry cloth. All samples were exposed to the temperature sequence of 20, 40, 60, 80, 40, and 103 °C, with each temperature step kept constant for 24 hours. For wet curing, controlled drying was performed by wrapping the samples in aluminium foil for the first four temperature sequences. The samples were exposed to the temperature without wrapping them in aluminium foil for dry curing. Five replicates for each solid content percentage were prepared according to the DoE.

Larger board sections were treated using the aforementioned process but only with the aqueous PF resin solution with 15% of solid content. During the heat-curing process, the cross-sectional surfaces of half of the board sections were sealed with aluminium foil. This action was performed to control the water evaporation from the cross-sectional surfaces during the heat curing stage to replicate the commercial-size boards. The samples were cut in half before the NIR measurements to avoid measuring the excess resin at the outer surfaces. The schematic illustration of wood with different chemical modifications is shown in Figure 12.
3.2 Hyperspectral imaging and chemometric

The process-dependent distribution of chemical agents in wood was determined within the scale of several millimetres (macroscopic) down to the micron scale at a cellular level. NIR hyperspectral imaging (Publications I, II, and V) and confocal Raman imaging (Publications I, II, and IV) were implemented to identify and visualise the chemical changes at different spatial scales. The hyperspectral imaging data was coupled with chemometric methods to decompose high-dimensional data into useful chemical information in a multivariate fashion. Chemometric-based models were developed to reliably quantify the concentration of wood polymers, chemical agents in wood, and MC.

3.2.1 Near-Infrared Hyperspectral Imaging

Reflectance images of the wood blocks were recorded with high-speed short-wavelength infrared (SWIR) hyperspectral camera (Specim, Spectral Imaging Ltd) equipped with OLES macro-objective lens (Publication I, II, and V).
The field of view (FOV) was set to approximately 10 mm. The operational speed of the camera was adjusted to record square pixels with dimensions of 26 × 26 µm². Imaging was performed in line scanning mode and measured 384 spatial pixels on 288 spectral channels within the wavelength range of 950 to 2550 nm. Two halogen lamps with polychromatic light sources were positioned in a line to illuminate the surface of the sample. A HgCdTe detector aligned with a grading prism monochromator collected the reflected wavelengths from the wooden samples. The integration time was set to acquire the maximum signal (approximately 90%) strength from the highest reflectance target. Sample images were collected along with a reflectance target placed on the stage, which moved past the spectral camera. The raw images were median filtered and corrected with external reflectance targets (75, 99%) (Mäkelä et al., 2020). Potential dead and extreme pixels were eliminated from the images, and a region of interest (ROI) was selected depending on the sample dimensions. The samples were scanned from the middle with 10 mm (FOV), and the region outside was eliminated to reduce the edge effect. The detailed operational setup of the NIR hyperspectral is shown in Figure 13.

![Figure 13. NIR hyperspectral imaging setup.](image)

### 3.2.2 Confocal Raman Imaging

Confocal Raman spectra and images (Publications I and IV) were acquired with a WItec alpha 300 RA Raman microscopy (WItech, Ulm, Germany) equipped with a 532 nm frequency-doubled Nd: YAG laser and a DU970-BV EMCCD camera behind a 600 lines/mm grating. The samples for the scan were prepared from the cross-section with a thickness of 25–35 µm (Publication I) and 20 µm (Publication IV). The sections were prepared with a rotary microtome and carefully placed on an objective slide with a drop of deionized water covered with a glass coverslip (with a thickness of 0.17 mm). A small weight was applied from the top to straighten up the section, and the edges of the coverslip were sealed with nail polish. The Raman images were acquired in Publication I with a 20× objective (numerical aperture (NA) = 0.4). Each image was captured with a dimension of 70 × 70 µm², with 185 lines per image and 185 points
per line. An integration time of 0.3 seconds was applied, and the area selected for imaging was at the annual ring border with equal proportions of earlywood and latewood. In *Publication IV*, a 100× immersion oil objective (NA = 1.25, coverslip correction = 0.17 mm) was used to scan images with dimensions of 40 × 40 µm². Each image was recorded with 175 lines and 175 points per line from the latewood cells with an integration time of 0.3 s. Besides imaging, single spectra of pure MF resin were acquired using 20x air objective (NA=0.4) and an integration time of 0.5 seconds and ten accumulations.

### 3.2.3 Image calibration and transformation

NIR images were collected in reflectance mode consisting of the background, exposed sample surface, and the reflectance target. The raw images were processed with the PCA (Wold et al., 1987), and the background was removed using the pixel scores threshold. The image data was filtered with a median filter (Tan and Jiang, 2013) comprising a moving window of 3 × 3 pixels in the horizontal and vertical directions. The median filter removed the effect of saturated and dead pixels from the images. Each pixel was then corrected with the recorded spectral reflectance target and the dark current intensities (Mäkelä et al., 2020). The ROI was selected by choosing the centre of the sample and the equivalent number of pixels in a rectangular coordinate manner. The ROI size was based on the sample dimensions, and the edges of the sample were excluded from the extracted image. The images were converted into absorbance using $A = \log_{10}(1/r)$, where $A$ is the estimated absorbance and $r$ is the unitless reflectance value. Five replicates samples were prepared based on the DoE in *Publications I, II, and V*. A separate test image set was prepared from the larger board section in *Publication V*. A sample from each design location was selected based on the WPG value and the standard deviation. The selected samples were then fused into a test image mosaic. Chemometric methods, such as PCA, PCA-based clustering, and PLSR, were applied to the final test image mosaics. Step-by-step image transformation details are shown in Figure 14.

Confocal Raman images were extracted using WiTech Suite 5.2 software (*Publications I and IV*) and Renishaw InVia confocal Raman microscope supporting software WiRE 5.3 (*Publication III*). Cosmic rays were removed using a despike median filter. Raman images were also fused into a test image mosaic. Finally, the cell lumen fillings were removed based on the threshold score values calculated with PCA.
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3.2.4 Principal component analysis

Before quantitative analysis, the differences in NIR test images from the wood blocks were visualised using PCA. The wavelengths within the range of 1000-2500 (Publication I), 1100-2400 (Publication II), and 1000-2400 nm (Publication V) were considered. The unfolded data hypercube from the image mosaic was first preprocessed with a standard normal variate (SNV) (Barnes et al., 1989), followed by the mean centring. SNV transforms the variables in rows to mean zero and unit standard deviation. Confocal Raman images were unfolded into a two-dimensional array with pixels in row objects and respective wavenumbers in columns (Publication I and IV). The wavenumbers within the 300-3600 cm\(^{-1}\) range were considered for analysis. The data was preprocessed by removing cosmic rays, applying polynomial baseline correction with degree n, followed by normalisation by unit vector and mean centering. Normalisation with unit vector involves to first calculate length of each vector using summed squared value of each component of spectrum, then divide each component of the vector by its length. The preprocessed data were then analysed using PCA (Bro and Smilde, 2014). The general PCA model is defined in Eq. 5:

\[
X = t_1 p_1^T + \cdots + t_n p_n^T + E_n,
\]

where \(X\) is the unfolded preprocessed data matrix with spatial pixel spectra, \(t\) represents orthogonal score, \(p\) contains orthonormal loading vectors, and \(E_n\) is a residual matrix. The score values were refolded to image dimensions and interpreted with corresponding loadings vectors. Data analysis was performed by combining in-house MATLAB R2022b (MathWorks, Inc.) scripts and
commercial functions from the PLS Toolbox 8.7 (Eigenvector Research, Inc.). The graphical representation of PCA is shown in Figure 15.

![Image of PCA](https://via.placeholder.com/150)

**Figure 15.** Images data decomposition with PCA.

### 3.2.5 K-means clustering

The PCA scores were segregated into a predefined number of clusters (Publication IV), as shown in Figure 16. The k-means clustering method was employed to identify a defined number of distinct samples (pixels) within an image, and their corresponding responses were utilised as the target spectra for the resulting clusters (Hartigan and Wong, 1979). The algorithm separates the pixels into a number of predefined classes based on their correlation with the mean of each cluster. The Euclidean distance was used to determine the first centroids furthest away from the mean of the score space (Sparks, 1973). The average correlation of the clusters was calculated to define an optimal number of clusters. The corresponding mean-centred average class spectra were calculated, and the class vector was folded back into image dimensions.
Partial least squares regression

The hyperspectral images were acquired based on the DoE. Five replicates were prepared at each design location, from which three images were assigned to the calibration set and two to the test set. A separate test image mosaic was prepared to apply the developed model and predict the concentration of chemical analytes at the pixel level. The image hypercubes were first unfolded into two-dimensional arrays. The dataset included a considerably larger number of objects than the wavelength variables, and from each image (Publications II and V), three mean spectra were obtained. In Publication I, the heterogeneity was intentionally created by restricting the flow of acetic anhydride into the wood surfaces. A single average spectrum was calculated from each image hypercube. In Publication II, the samples were recorded in EMC, and the moisture variation was not an issue. Similarly, bulk sample treatment with phenol-formaldehyde (PF) was used in Publication V. The average objects were split into calibration and test sets. The calibration set was preprocessed with SNV and mean centring. The WPG values were allocated to the response target matrix (y).

Partial least squares models were developed based on the calibration objects and the response variables using the SIMPLS algorithm (Andersson, 2009; de Jong, 1993). SIMPLS calculates the PLS factors based on the maximised covariance criteria while obeying certain orthogonality and normalisation constraints. The general equation for regression is shown in Eq. 6:

\[ y = Xb + e \]  

(6)
where \( y \) is the \( n \times 1 \) matrix containing the average concentration of analytes, \( X \) is the \( n \times m \) matrix that includes preprocessed average objects, \( b \) is regression model coefficients with \( m \times 1 \) dimensions, and \( e \) represents model residuals. The model performance parameters were determined by the root mean squared error of calibration (RMSEC) and the prediction (RMSEP) based on the test set. Moreover, the maximum model complexity was evaluated by calculating the root mean squared error prediction of the pixel population (RMSEP_{img}) from test images (Publications I and II) and the root mean squared error of cross-validation (RMSECV) (Publication V). A prediction map was generated on test images by applying the regression vector with different LV, and the residual prediction images were calculated (Gowen et al., 2014). The RMSEP_{img} was determined with Eq. 7:

\[
RMSEP_{img} = \sqrt{\frac{\sum_{i=1}^{k} \sum_{p=1}^{n_p} (y_i - \tilde{y}_i)^2}{kn_p}}
\]

where \( y_i \) is the WPG measured after the treatment or moisture uptake, \( \tilde{y}_i \) represents predicted WPG values of each pixel, \( n_p \) is the total number of pixels per image, and \( k \) is the total number of test images. Moreover, the optimal number of LV was further evaluated with RMSECV. Cross-validation performance was determined by splitting the training objects into five groups and using only one group per iteration as a validation set. This procedure was repeated until all had been excluded once. PLS regression was applied with an in-house Matlab script and by using commercially available PLS toolbox functions.

### 3.3 Wood-water vapour sorption

Wood-water interaction was further evaluated with gravimetric methods, such as automated sorption balance and simultaneous mass and dimensional changes. The mechanisms of hydroxyl accessibility were determined by deuterium exchange in an automatic sorption balance. The dimensional changes and MC were recorded in two-dimensional images, and the swelling was estimated.

#### 3.3.1 Sorption isotherms

Sorption isotherms were measured on samples isolated from the earlywood and the latewood section of wood blocks in Publication II. These sections were carefully sectioned with a razor blade under a light microscope. In Publication III, the samples with the highest WPG were milled into a fine powder using a laboratory mill. The sorption isotherms were measured with an automated sorption apparatus (DVS intrinsic, Surface Measurement Systems, UK). The automated sorption balance measured the sample mass under nitrogen gas flow mixed with water vapour. The initial sample mass was approximately within the range of 15-18 mg. The samples were placed on the sample pan at 25°C under the 200 sccm flow of nitrogen (grade 5.0; \( \leq 3 \) ppm \( \text{H}_2\text{O} \)). In Publication II, only absorption isotherms were recorded, whereas both absorption and
desorption isotherms were measured in Publication III. The RH was raised in
ten discrete steps (0, 5, 15, 25, 35, 45, 55, 65, 75, 85, and 95%), and each step
was maintained until the change in mass per minute (dm/dt) was less than
0.0005% min⁻¹, determined by linear regression in a ten-minute window. The
desorption isotherms were measured with the same steps in reverse order. MC
was calculated using Eq. 8 and later corrected by a factor of WPG as Eq. 9:
\[
MC(\%) = \frac{m_{RH} - m_d}{m_d} \times 100
\]  
\[
MC_R = MC \times \left(1 + \frac{\text{WPG}}{100}\right)
\]

Samples mass (g) after conditioning at specific RH is \(m_{RH}\); the initial dry mass
(g) is represented as \(m_d\). The correction factor in MC correction relates to the
water mass to the dry wood mass without added chemical reagents.

3.3.2 Deuterium exchange

In Publication III, hydroxyl accessibility was measured with automated
sorption balance (DVS ET, Surface Measurement Systems, UK). The measure-
ments were performed on laboratory-milled powder samples. The sample
was placed on the sample pan with a mass of approximately 15-18 mg,
and the nitrogen flow (grade 5.0 ≤ 0.5 ppm H₂O) was kept at 200 sccm. The
samples were followed through a set of sequences with A) sample drying by
pre-heating at 60 °C for six hours and cooling to 25 °C for two hours, B)
exposure of deuterium oxide (D₂O) vapour for 12 hours to reach the target
RH of approximately 95% and C) measuring dry mass by again heating at
60 °C for six hours and cooling to 25 °C for two hours. Two replicates of each
sample were prepared, and the concentration of accessible hydroxyl groups
was calculated using Eq. 10:
\[
\text{OH}_{\text{acc}}(\text{mmol g}^{-1}) = \frac{m_f - m_d}{m_d \times \Delta M} \times 100
\]  

where \(m_i\) and \(m_f\) are the initial and final dry mass (mg) of the samples. \(\Delta M\) is
calculated as the difference in the atomic mass of deuterium and protium, which
is 1.006 g mol⁻¹. The hydroxyl accessibility was corrected by the factor of WPG,
as shown in Eq. 11. The concentration of absorbed D₂O molecules was estimated
in Eq. 11:
\[
D_2O_{\text{absorbed}} = \frac{m_{D_2O} - m_d}{m_i \times M_{D_2O}} \times 100
\]

The mass (mg) change caused by the condition with D₂O vapors is \(m_{D_2O}\). The
molar mass of D₂O is represented by \(M_{D_2O}\), which is 20.028 g mol⁻¹. The concen-
tration of absorbed D₂O was also corrected with the correction factor of WPG,
as presented in Eq. 9.
3.3.3 Simultaneous mass and dimensional changes during water vapor sorption

In Publication III, the simultaneous mass, dimensional changes, and water vapour sorption were determined with a gravimetric sorption system equipped with a high-resolution camera. A separate set of samples with 20×20×5 mm³ dimensions was prepared, and absorption behaviour within the range of 0-85% RH was measured. Before the sorption analysis, the samples were soaked in de-ionized water for 24 hours, stacked between two metallic meshes to prevent curling, and dried at 20 °C and 65% RH. After drying, the edges were smoothed with a wood shaper, and a 2 mm hole was drilled at the centre of each sample. The samples were held flat and fixated on the custom-designed sample holders (Nopens et al., 2019). The Lewis acid, acetylation, and formalisation treated samples were placed on the large round panel of the automated sorption balance (SPSx-μ-High-Load, ProUmid, Germany). The samples were exposed to the RH sequence of 0, 5, 15, 25, 35, 45, 55, 65, 75, and 85% at 20 °C, which was generated by mixing water vapours with dry air. The mass of the samples was measured every 15 mins using a balance with an accuracy of 1 µg. Each RH step was kept constant until the mass change of each sample was less than 0.01% h⁻¹. Images of each sample were captured simultaneously with mass measurement from the top view of the cross-sectional dimension with a CCD camera (BASLER acA2040-25gc). A floating average in a one-hour window was used as a reference mass to estimate the slope. After meeting the equilibrium criterion, the RH steps at 0 and 85% were used for five more days.

3.3.4 Dimensional changes – Image analysis

A customized batch macro was created using Fiji, an ImageJ distribution, to transform RBG images into single-colour images. The cross-sectional image area was calculated based on the intensity difference threshold with a pixel resolution of 0.016 mm/pixel. The sample outlines were generated and exported as a raster graphic. These images were imported into Matlab, and the location indices of each pixel were identified. A linear fit was generated based on 1000 pixels on each edge of the samples, and the distance between two parallel lines was calculated in both radial and tangential dimensions. Eq. 12 calculated the dimensional changes (S_A) of each sample:

\[
S_A = \frac{A_t - A_0}{A_0} \times 100
\]

where \(A_t\) and \(A_0\) are the cross-sectional area (mm²) at the given time, and the area at the end of the initial drying step at 0% RH.
4. Results and Discussion

This chapter describes the main results and their discussion, which reflects the main objectives concerning the applied methodology. Various wood modification methods were used to evaluate the capability of chemical imaging methods to detect the chemical reagent whether reacted with the wood biopolymeric components or effected the overall properties of wood. The treatment levels caused by the modification agents were first determined with gravimetric methods by measuring the WPG. Following this, the distribution of chemical agents within the hierarchical structure of wood was visualised by the hyperspectral imaging techniques from the spatial scale of several millimetres down to the micron level. The treatment levels influenced the moisture properties of wood that were measured by humidity-dependent mass changes. Finally, the MC, as a function of treatment levels, was quantified by NIR hyperspectral imaging and chemometric methods.

4.1 Treatment levels caused by the modification

The amount of modification reagent absorbed by the wood was determined by measuring the mass change with gravimetric techniques. The added chemical reagents either react with cell wall polymers to create bonds or impregnate the wood and polymerize within the cell wall space. The residual reagents are leached out, and the wood is dried and weighed. The change in mass is used to calculate the concentration of added chemicals in wood to identify the treatment levels.

4.1.1 Gravimetric estimation and reaction kinetics

The reaction kinetics was followed by measuring the WPG of individual samples. The surface acetylation permits the single-sided penetration of acetic anhydride at different time steps. A temperature of 120 °C was applied to the exposed surface to initiate the reaction between anhydride molecules and the cell wall hydroxyl groups. Wood blocks gained 4% of WPG after soaking in acetic anhydride for less than three hours but gradually levelled off and attained a higher WPG of 12% after soaking the blocks for 288 hours. But bulk acetylation was conducted by first impregnating the wood blocks in neat acetic anhydride for 24 hours and then soaking them in fresh acetic anhydride in a reaction flask at 120° C. Acetic anhydride penetrated the wood, reached the cell walls through
wood macropores and the diffusion pathways, and formed ester bonds with accessible hydroxyl groups at elevated temperature. The reaction in a flask under the flux was rapid, increasing by 8% WPG within the first hour. It slowed with time, and 16% WPG was achieved after six hours (Figure 17a). Both acetylation processes were performed differently. Surface acetylation was performed by soaking the woodblocks in neat acetic anhydride and heating in hot press at 120°C. For bulk acetylation, the woodblocks were boiled in acetic anhydride at 120°C under a flux. Although the woodblocks underwent different treatment process, the underlying chemical reaction that resulted in varying levels of WPG was the same. It must be noted in Figure 17a that the time for surface acetylation refers to the soaking time, while time for bulk acetylation refers to the reaction time at 120°C. The mechanism between the WPG changes of the two acetylation techniques are different.

The formalisation reaction was performed in the vapour phase in the presence of an acidic catalyst (1.5% aqueous solution of ZnCl₂). Upon heating to 100°C, the formaldehyde molecules entered the cell walls and formed covalent bonds with the hydroxyl groups. Formaldehyde molecules can react with up to two hydroxyl groups and make a cross-link between them. The formaldehyde reaction caused an increase of 4.5% WPG in the first 12 hours. The reaction rate then slowed and increased by 6% WPG after 48 hours (Figure 17b). Acetylation and formalisation differ in reaction kinetics, and at a given WPG, more formaldehyde molecules reacted with OH groups than the anhydride molecules due to the larger molecular weight of anhydride groups. Each formaldehyde molecule added less mass than the acetyl group in wood.

Figure 17. The WPG of treated wood (a) WPG measured from surface and bulk acetylation as a function of time. Noted that the time for surface acetylation refers to the soaking time, while time for bulk acetylation refers to the reaction time at 120°C. (b) WPG for formalised wood measured against time. Please note that different periods were used for the treatments. The green axis shows the time in minutes for bulk acetylation, and the purple axis is the time course of surface acetylation in an hour. Similarly, the black axis shows the time (in hours) for the formalisation process. The dotted lines show an exponential fit. The vertical lines show the error bars, which were calculated based on the standard deviation between
The MF impregnation treatment was quantified by measuring the change in sample mass as WPG against the increase in solid resin content of the impregnation solution. The continuous growth in the WPG, with the added solid content of MF, indicated the fixation of resin in the wood blocks, irrespective of the curing conditions (Figure 18). The influence of curing conditions was also compared, which did not significantly affect the WPG of the resins (Figure 18a, b). After leaching the MF-impregnated samples for two weeks, a slight loss in WPG was observed, possibly due to the loss of unreacted MF resin. Moreover, the leaching of untreated wood also exhibits a slight decrease in WPG, which is associated with the removal of soluble extractives from native wood. Nevertheless, the WPG of the highest solids content treatment corresponds to 25-27% after the leaching cycles.

![Figure 18. WPG obtained by the MF impregnation treatment of wood. (a) WPG as a function of the solid resin content of MF upon dry curing and a two-week leaching period. (b) WPG as a function of the solid resin content of MF upon wet curing and a two-week leaching period. The dotted lines show the linear fit. The vertical lines show the error bars, which were calculated based on the standard deviation between the replicates. Reproduced with permission from (Altgen et al., 2020). Copyright 2020, Springer Nature.](image)

The impregnation treatment with PF was also measured with the WPG and the increase in the concentration of PF resin load (Figure 19). An average WPG per sample group was measured, which correlated positively with the added solid content of PF resin in impregnated solution, independent of the curing conditions (Figure 19a, b). It has been reported that the WPG increases slowly at the higher concentration of PF resin in solution, which relates to the reduced permeability of PF solution at higher viscosity in wood (Wang et al., 2019). However, our results show a linear correlation between the WPG and the highest solid resin content in the solution. The samples were then water-leached for two weeks, after which a slight decrease in WPG was observed. The continuous leaching with the water removed the unreacted PF resin and the water-soluble
extractives. At the highest solids content (ca. 30% WPG), the large concentration of PF resins remained within the treated wood blocks after leaching cycles.

Figure 19. WPG obtained by the phenol-formaldehyde impregnation treatment of wood. (a) WPG against solid resin content of PF resin upon dry curing and a two-week leaching period. (b) WPG as a function of solid resin content of PF upon wet curing and a two-week leaching period. The dotted lines show the linear fit. The vertical lines show the error bars, which were calculated based on the standard deviation between the replicates. Reproduced with permission from (Altgen et al., 2023). Copyright 2022, Elsevier.

4.1.2 Chemical imaging at different spatial scales

The gravimetric methods are an excellent indicator to determine the concentration of chemical reagents in wood. However, these methods are insensitive to the location of chemicals within the hierarchical structure of wood. For this, imaging-based spectroscopic methods must visualise and quantify the spatial distribution of chemicals and their interaction with wood at multiple scales. NIR hyperspectral imaging was used to quantify the chemical changes caused by the treatment at a scale of a few millimetres or larger, and confocal Raman imaging was performed to study the cell wall alteration by the modification agents at the micron level.

Visualising the acetylation of wood over time

The surface acetylation of wood over time was determined at a macroscopic level using NIR hyperspectral imaging. The acetylation degree was controlled by exposing the single-sided surface of wood blocks to acetic anhydride at different times. The cross-sectional surfaces were scanned with a NIR camera and analysed with PCA (Figure 20). PCA identifies the degree of acetylation and the wood biopolymers within the images mosaic. The first two principal components (PCs) explained ca. 80% of the variation in image data (Figure 20a). The score images were interpreted with their respective loading vectors (Figure 20b, c). PC1 loading vector contained wavelength band regions of 1212-1225 nm and 1477-1484 nm (Figure 20b), which were assigned to cellulose and the absorbed water molecules (Ali et al., 2001; Schwanninger et al., 2011a). The bands that
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appeared at 2255-2260 nm correspond to the acetyl group, resulting from the acetylation of wood. The bands within the 2490-2493 nm region emerged by the C-H and C-C stretching of the cellulose (Schwanninger et al., 2011a). PC1 explained the highest variation in data (ca. 69%) and revealed chemical information related to the wood cell wall polymeric constituents (Figure 20d). Additionally, it also showed the differences between the earlywood and latewood cells and the surface roughness.

![Figure 20](image)

*Figure 20. PCA to indicate changes caused by the single-sided flow of acetic anhydride. (a) The number of PCs with corresponding variation in data by each component (b) PC1 loading vector mostly showed the bands assigned to wood polymeric components (c) PC2 loading vector indicated the bands primarily assigned to acetylation and wood polymeric components (d) Score image mosaic of PC1 arranged at distinct time steps (hours). (e) Score image mosaic of PC2 arranged at distinct time steps (hours). The colour scale denotes pixel score values. Reproduced with permission from (Awais et al., 2020). Copyright 2020, American Chemical Society.*

The bands observed in the PC2 loading vector mostly correspond to acetyl groups (Figure 20c). Hemicelluloses contain acetyl ester groups, and the bands that appeared in region 1160-1164 nm indicated the presence of acetyl groups. The intensity of these bands increases with the increase in acetylation degree (Alves et al., 2012). These bands are assigned to C-H stretching vibrations from methyl groups. The band region ca. 1370-1370 nm also belongs to CH₃ acetyl ester groups in the hemicellulose. And it is tentatively assigned to the first overtone vibration of combined C-H stretching and C-H deformation of methyl groups (Schwanninger et al., 2011b). In addition, the amplitude of the 1720 nm band increased with the increase in methyl groups caused by the increase in
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Acetylation degree. The bands in this region are assigned to the first overtone vibration of C-H stretching vibration from CH3 groups present in hemicelluloses in wood (Schwanninger et al., 2011a). Bands at 2250-2255 nm correspond to the acetyl groups present in all components of acetylated wood (Schwanninger et al., 2011a). PC2 explained 11% of the variation, and the score images exhibit acetylation-related information within the wood components (Figure 20e).

PCA results showed gradual chemical changes with time, which are attributed to the acetylation degree within the wood blocks. The calibration model was created based on the average spectra of the wood blocks and the corresponding measured WPG. The model accuracy was evaluated by the RMSEC, RMSEP, and RMSE_img. Two LV were selected to avoid model overfitting, which provided a satisfactory performance in predicting the test set and test image set (Figure 21a, b). The regression vector contains spectral bands with chemical meaning and separate positive bands related to acetylation at ca. 1165, 1351, 1721, and 2253 nm from the wood biocomponents bands at 1452 (lignin) and 2057 nm (cellulose) (Figure 21c).

The calibration model with two LV was used to predict the WPG caused by the time-dependent flow of acetic anhydride (Figure 21d). The test image set comprises sample images in an image mosaic placed in the order of different treatment times. The prediction image shows sample-to-sample variation in WPG complementing the gravimetrically measured WPG. Besides sample-to-sample variations, the predicted image also indicated the intra-sample variations caused by the one-sided exposure to anhydride between the earlywood and latewood regions. However, the average predicted WPG was close to the average measured WPG except for samples with shorter exposure time. And this could be related to the non-linear distribution of WPGs, shown in Figure 17. Average spectra per sample image were used to develop the calibration model and added prediction error that could cause the overestimation in average predicted WPG. The predicted WPG per pixel is shown by histograms in Figures 21d and e. Moreover, the predicted WPG was higher in the earlywood regions, demonstrating that the thin-walled early wood cells acetylate faster than the thick-walled latewood cells. Earlywood tracheids presumably provide shorter diffusion pathways to acetic anhydride, resulting in earlywood cells acetylated first. And this was validated with confocal Raman imaging at a cellular level in the later section.
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Figure 21. Results obtained from the PLSR model and predicted WPG based on surface acetylated wood blocks. (a) RMSEC, RMSEP, and RMSEP\_img set as a function of LV, (b) Measured and predicted values of WPG after implying calibration model with two LV. The dotted line is a reference line with slope 1 and passes through the origin, (c) the Regression vector based on the calibration model with two LV, (d) The predicted image mosaic indicating sample-to-sample variations at different time steps (hours), (e) the corresponding histograms of pixel extracted from predicted image samples mosaic. Reproduced with permission from (Awais et al., 2020). Copyright 2020, American Chemical Society.

Phenol formaldehyde resin distribution in impregnation-treated wood

NIR hyperspectral imaging provides a reliable estimation of acetic anhydride distribution and identifies the related chemical changes at a surface level within wood blocks. Therefore, its application was further expanded in Publication V to determine the inhomogeneous distribution of phenol-formaldehyde resin in impregnation-treated wood blocks and the larger board sections. The differences in curing conditions and the treatment levels were first determined by the PCA. Publication V demonstrates the detailed results after applying PCA on an image mosaic. The images were positioned in a mosaic by the differences in solid PF resin content (0-30%) in impregnated solution. The first three PCs were selected, which explained 78% of the variations within the data. The score images were folded back into image dimensions and interpreted with corresponding loadings vectors. The first PC primarily explained the physical aspects caused by the sample preparation, such as surface roughness. The second PC identifies the sample-to-sample chemical variations caused by the PF resin treatment. The third PC distinguishes the differences in the chemical structures
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of the resin under the different curing conditions in samples containing ≥ 20 % resin load.

PCA isolates useful spectral information related to resin content from unwanted chemical information in a qualitative manner. Thereby, the distribution of the PF resin was quantified using the PLSR model (Figure 22). A calibration model was developed based on the average NIR image spectra (three average spectra per sample) and the corresponding gravimetrically measured WPG. The calibration and validation accuracy were determined at different numbers of LV with RMSEC, cross-validation (RMSECV), and RMSEP (Figure 22a). Four LV provided a satisfactory prediction of the calibration (0.93%) and test set (0.92%) errors (Figure 22b). The test set error was smaller than the calibration set because a few outliers were removed from the test set. A slight underestimation in predicted WPG was noticed in samples above 30% WPG due to including a broad range of WPG and the more substantial deviation within the sample group with higher solid content.

The model regression vector with four LV shows the positive bands at ca. 1113, 1659, 2141, and 2281 nm correspond to the NIR bands acquired from the cured PF resin in stock solution (Figure 22c). The negative band at ca. 1385 nm is attributed to hindered OH groups (Ali et al., 2001), whereas the bands at 2035 and 2236 nm remained unidentified. The negative bands were associated with the MC, which indicates the reduction in MC caused by the treatment in the image context. The calibration model was used to predict the test image set (Figure 22d). The results show the variation in WPG between the samples, which were in line with the gravimetrically measured WPG. The predicted image revealed no distinctions between samples cured with different conditions. The average predicted pixel count (predicted WPG) per sample corresponded to the measured WPG and showed a maximum difference of 1.4% (Figure 22e), except for the dry-cured sample with 30% solid resin content (overestimated 2.4% points). Moreover, no notable gradient from the centre toward the edges of the samples was observed in smaller wood blocks.
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Figure 22. Results of PLSR model and predicted PF resin distribution based on WPG. (a) Root mean squares error of calibration, cross-validation, and the test set prediction as a function of LV, (b) Measured and predicted values of WPG after applying the calibration model with four LV. The dotted line is a reference line with slope 1 and passes through the origin, (c) Regression vector based on the calibration model with four LV, (d) Predicted image mosaic indicating sample-to-sample variations in WPG and curing conditions at different solid resin content in impregnated solution, (e) The corresponding histograms of pixel count, extracted from predicted image samples mosaic. Reproduced with permission from (Altgen et al., 2023). Copyright 2022, Elsevier.

The calibration model developed based on the cross-sectional surfaces of small wood blocks was utilised to determine the PF resin distribution in larger impregnated board sections (Figure 23). The board sections were impregnated with a 15% solid-content PF resin solution. The drying and curing conditions were changed for each sample group to target a gradient in PF resin distribution within the board sections that can later be identified by the PLS-regression model. The results showed that the predicted WPG in the larger board sections was dependent on the curing conditions (Figure 23a, b). The controlled heat-
curing step, where the cross-section of samples was sealed, caused an increase in predicted WPG from the core of the sample towards the surface region of the large sections. A gradual increase in predicted WPG was found in wet-cured board sections. In contrast, a sharp change in predicted WPG was observed from the centre to the edges of the board section under dry curing conditions (Figure 23a). The sealing of cross-sections caused the evaporation of water towards the radial and tangential surfaces, which act as a driving force for the capillary flow of water through the porous wood structure. And this transported the low-molecular-weight PF resin toward the surface where the resins are heat cured and act as immobile macromolecules. Thereby, the NIR prediction results revealed the strong migration of PF resins toward the radial and tangential surfaces after dry-curing than wet-curing. In unsealed samples, no gradient was observed in the predicted WPG between the center and the cross-sectional edges (Figure 23b). The water evaporated through the cross-sectional surface and the capillary flow of the solution was in the longitudinal and lateral directions. The transport of water in the longitudinal direction was faster than in the lateral direction, which resulted in the most PF resin migration toward the longitudinal direction (Bastani et al., 2015; Sedighi-Gilani et al., 2014). This was highlighted by the predicted NIR images of dry-cured, unsealed samples with lower WPG from the centre of the board section toward the cross-section. On contrary, higher predicted WPG was observed in wet-cured, unsealed samples. This was due to the prevention of translocation of PF resin toward the longitudinal direction.

Figure 23. Predicted WPG of images from treated larger board sections. (a) Board sections with sealed cross-sectional surfaces during heat-curing, (b) and board sections with unsealed cross-sectional surfaces during heat-curing. The arrows highlight the anatomical directions of the wood (R radial, T tangential). The placement of the images on the board sections guides the eye. Reproduced with permission from (Altgen et al., 2023).Copyright 2022, Elsevier.

Cell wall changes caused by the flow of acetic anhydride
The heterogeneous flow of acetic anhydride within the hierarchical structure of wood was examined at a spatial resolution in the micron or submicron range using confocal Raman spectroscopic imaging. NIR hyperspectral imaging indicated the process-dependent flow of acetic anhydride and the variations in the acetylation degree within the earlywood and latewood cells. Thereby, two sections near the annual ring border that contain an equal amount of earlywood and latewood cells were carefully selected. One section was picked near the exposed surface to acetic anhydride and the other from the bottom of the sample, shown in Figure 24a. Raman imaging collected a more substantial set of spectral data with a high spatial resolution of the selected region, which can be treated as a hyperspectral data cube and analysed in a multivariate fashion. An image mosaic was created and analysed with PCA. Before the analysis, the data were preprocessed with a median filter to remove the cosmic rays, polynomial-based baseline correction (n=3), vector normalisation, and mean centring. Moreover, only the wavenumbers range 300-3600 rel. cm\(^{-1}\) was included in the analysis, and the pixels belonging to lumen filling were removed using PCA to better visualise the cell wall-related changes.

The first four PCs explained 57% of the variations in data (Figure 24a). The score images were folded into image dimensions and interpreted by their corresponding loading vectors (Figure 24b). PC1 loading vector contains a negative band at 1597 rel. cm\(^{-1}\) due to the stretching of the aromatic ring of lignin in wood (Agarwal and Ralph, 1997). The positive band region 3200-3600 rel. cm\(^{-1}\) is assigned to OH stretching of water molecules (Blackwell et al., 1970; Larkin, 2018). There was a sharp shift in band region 2880-2940 rel. cm\(^{-1}\) observed, which relates to the disappearance of the positive band at 2887 rel. cm\(^{-1}\), resulting in an increase in band 2933 rel. cm\(^{-1}\). The 2933 rel. cm\(^{-1}\) band was due to the acetylation and assigned to the C-H stretching in acetoxy groups in acetylated lignin (Agarwal et al., 2011). The PC1 shows the higher acetylation degree in the region close to the exposed surface, the lignin-rich regions in the cell corners and middle lamella, and the residual water pixels. PC2 contains negative bands at 1592 and 3322 rel. cm\(^{-1}\), which were assigned to the aromatic ring stretching of lignin and the OH stretching in the water (Agarwal and Ralph, 1997; Larkin, 2018). The positive band at region 2839 rel. cm\(^{-1}\) is assigned to the carbohydrates associated with cellulose (Agarwal et al., 2011). PC2 separates the cellulose from the lignin-rich region and the leftover water-related pixels. PC3 loading vector comprises two positive bands at 1599 and 2889 rel. cm\(^{-1}\), allocated to lignin’s C-H stretching (Agarwal and Ralph, 1997). In addition, it contains noise signals and baseline shifts in the negative bands. PC4 considers being the significantly important principal component. It complements the differences in acetylation degree in earlywood and latewood regions predicted by NIR hyperspectral imaging, shown in Figure 21d. The shift in bands 2887 and 2933 rel. cm\(^{-1}\) indicated the acetylation reaction in wood (Agarwal et al., 2011). These bands had been previously used for the quantitative observation of acetylation reaction with lignin and cellulose. Moreover, the band at 1601 rel. cm\(^{-1}\) is assigned to the aromatic stretching of lignin and the band at 1144 rel. cm\(^{-1}\) associated with cellulose (Agarwal and Ralph, 1997). The section near the exposed
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Surface showed complete acetylation of the earlywood and latewood cells since it is difficult to know if the maximum acetylation degree has been reached. This is presumably because the acetic anhydride had sufficient time to diffuse into the thick-walled latewood tracheids. In contrast, the section taken near the bottom surface shows significant differences in the earlywood and the latewood cells. This shows the thin-walled earlywood cells acetylated faster, and the thick-walled latewood cells required a larger diffusion time to fully acetylate. Thereby, the diffusion time to reach the earlywood and latewood cells is an important parameter.

Figure 24. PCA on confocal Raman mapped images of the section close to the exposed surface to acetic anhydride and the section near the bottom edge. (a) Indicates first four PCs scores of image mosaic (b) Corresponding loadings vector of PCs showing the dominant bands in terms of wavenumbers. Reproduced with permission from (Awais et al., 2020). Copyright 2020, American Chemical Society.

Cell wall changes caused by MF resins impregnation at different curing conditions

Confocal Raman imaging was performed to analyse the cell wall changes caused by the impregnation of MF resin under different curing conditions. The imaging was recorded from the samples (25% solid resin content) that were cured under dry and wet conditions, and the images were fused in an image mosaic. PCA was applied to the image mosaic to evaluate the spectral changes due to the curing conditions. The data was preprocessed with image despiking using median filter to remove cosmic rays, baseline correction with polynomial fitting (n=3), normalisation with unit vector, and mean-centring. The wavenumbers between 300-3600 rel. cm\(^{-1}\) were included in the analysis, and the pixels belonging to lumen fillings were removed using PCA scores. The PCA was recalculated on the remaining pixels related to the wood cell walls. The first four PCs were
considered, which explains the 70% variation in data (Figure 25a). The score images were interpreted with their corresponding loading vectors.

PC1 loading vector contains two dominant bands: an intense negative band at 1592 rel. cm\(^{-1}\) and positive band at 2890 rel. cm\(^{-1}\). The band at ca. 1592 rel. cm\(^{-1}\) is assigned to the aromatics and associated with lignin in wood (Agarwal and Ralph, 1997). The band at 2890 rel. cm\(^{-1}\) corresponds to the CH/CH\(_2\) stretching vibrations and can be tentatively assigned to wood carbohydrates (Agarwal et al., 2011; Blackwell et al., 1970; Edwards et al., 1994). PC1 score image shows the negative score values to lignin-rich regions (in cell corners and middle lamella) and positive score values to carbohydrate-rich secondary cell walls. PC2 loading vector includes a strong contribution of bands at 379, 1096, and 1377 rel. cm\(^{-1}\), which relates to cellulose (Agarwal and Ralph, 1997; Gierlinger and Schwanninger, 2006). The PC2 score image indicated the crystalline region of the cellulose in the cell wall carbohydrates. The negative score values indicate the lignin in wood. The first two PCs also differentiate the cell wall differences caused by the dry and wet curing conditions. The secondary cell walls in the wet-cured wood have lower PC1 score values and higher PC2 score values. This presumably relates to removing some quantities of amorphous carbohydrates, such as hemicelluloses, from the secondary cell walls of wet-cured wood (Al-Dajani and Tschiner, 2008), increasing the portion of crystalline cellulose. PC3 and PC4 explained lower variations in the data, 6.1% and 1.6%, and primarily included the orientation sensitivity of cellulose and detecting residual lumen deposits of MF resins. The PC3 shows band ca. 1091 rel. cm\(^{-1}\), which is associated with the orientation sensitivity (S\(_1\) and S\(_3\) layers) of the cellulose (Gierlinger and Schwanninger, 2006). In the PC4 loading vector, the band at ca. 974 rel. cm\(^{-1}\) is assigned to the triazine-ring vibration of cured MF resin (Larkin et al., 1999).
Figure 25. Results from the PCA and PCA-based clustering of wood sections treated with MF resin (25% solid resin content) under different curing conditions. (a) The first four PCs with score images and respective loading vectors. The image on the left, bordered with red, was cured in dry conditions, and the image section on the right, with a blue border, was cured in wet conditions. (b) PCA-based cluster image indicating class pixels and the respective class average mean-centred spectrum. Reproduced with permission from (Altgen et al., 2020). Copyright 2020, Springer Nature.

The spectral changes detected by the PCA were classified into sub-classes using the K-means clustering approach (Figure 25b). The average-mean centred spectrum of each class was calculated, and the cluster image was interpreted, respectively. Class 1 shows the positive band at 1596 rel. cm\(^{-1}\), which relates to the lignin in wood (Agarwal and Ralph, 1997). Class 2 includes the dominant band at 1091 rel. cm\(^{-1}\), which was previously associated with orientation sensitivity due to the cellulose microfibrils angle in S1 and S3 layers (Gierlinger and Schwanninger, 2006). Class 3 and 4 separate the differences in secondary cell wall carbohydrates due to the different curing conditions. Class 3 highlights the spectral changes related to wet curing conditioning, potentially caused by removing numerous hemicelluloses. The dry-cured wood cell wall carbohydrates were grouped in class 4 which included a dominant positive band at 2890 rel. cm\(^{-1}\).

4.2 Wood moisture content

Wood, like several lignocellulosic materials, is hygroscopic. A large amount of hydroxyl groups is present within the aggregated cellulose microfibrils, which are limited to access under normal conditions. The MC of wood is controlled by altering the cell wall structure with the substitution of hydroxyl groups with the chemical reagents and the available cell wall space (Emmerich et al., 2020). There is a complex relationship between the absorption and desorption of water
molecules, the available sorption sites, and the cell wall space. Gravimetric methods based on the mass changes are used to determine the water vapour sorption in wood. These methods only indicated the bulk changes caused by the moisture adsorption/desorption and failed to determine the localised distribution of MC in wood. Therefore, imaging-based spectroscopic methods can be applied to determine the spatially resolved distribution of MC within the wood structure. In this section, we presented gravimetric and imaging spectroscopic-based methods to estimate the bulk and spatially resolved MC in the presence of modification reagents.

4.2.1 Gravimetric estimation of water vapour sorption

The water vapour sorption behaviour of wood was investigated with the gravimetrically determined sorption isotherms using automated sorption balance (Dynamic vapour sorption, DVS). The sorption isotherm measures the amount of MC compared to the given RH at a defined temperature and the environment. The equilibrium MC of wood is influenced by the accessibility of hydroxyl groups and the cell wall space within the hygroscopic range of 0-95% RH. We demonstrated here the variation in equilibrium MC caused by the acetylation and formalisation of wood. Additionally, it was noticed in Publication I and II that the wood shows differences in acetylation degree between the earlywood and the latewood regions. The water vapour sorption behaviour of the earlywood and latewood regions was also demonstrated in this section.

Sorption isotherms of unmodified and modified wood

Samples with the highest WPGs after acetylation and formalisation were selected. Sorption isotherms were collected using two different sorption balances (Figure 26). The direct comparison in MC is not meaningful because different samples' WPGs and dimensions were used in both measurements. The acquired MC data was corrected by their respective WPG values. A similar trend in absorption curves was observed, which were recorded with different sorption balances within the RH range of 0-85% (Figure 26a, b). In untreated wood, an upward bend in the absorption curve was noticed at 65% RH, which is associated with the softening of amorphous hemicelluloses in the cell walls (Himmel and Mai, 2015). And this reduces the overall viscosity and rigidity of the wood polymeric matrix and frees more space for the water molecules to enter the cell walls (Engelund et al., 2013). The wood sample treated with Lewis acid as a catalyst shows almost identical isotherm to the untreated wood. A slight difference in absorption at higher RH above 80% was noticed, which could be caused by the acid-hydrolysis of the cell walls biopolymers (Stevens and Parameswaran, 1981). Both acetylation and formalisation significantly reduced the MC over the entire hygroscopic range (0-95%).

The MC\textsubscript{R} ratio was calculated at each RH step to determine the effectiveness of the reduction in MC (Figure 26c, d). In acetylated wood, the MC\textsubscript{R} ratio increased until 50% RH and remained constant afterwards, until 95% or 85% RH, depending on the sorption balance used for the measurement. The MC ratio of
formalised wood increased between the RH range of 10-25% and then decreased with the increase in RH till the end. The magnitude of the MC\textsubscript{R} ratio differs in both measurements. And this might be caused by the difference in sample dimensions, mass, and treatment levels. Acetylation reduces the MC by occupying the accessible OH groups and the available cell wall spaces but does not restrict the swelling of the wood cell walls (Hill et al., 2010). Thereby, the cell wall swelling enabled moisture absorption at higher RH. In contrast, formalisation presumably caused the cross-linking among the cell wall polymers and restricted the swelling, resulting in increased stiffness of the cell wall matrix and reduced water uptake continuously with an increase in RH (Himmel and Mai, 2015).

Figure 26. Sorption isotherms and MC ratios of the treated and untreated wood were measured with two different sorption balances. (a) MC\textsubscript{R} (%) in absorption and desorption from 0 to 95% RH measured on wood powder. (b) MC\textsubscript{R} (%) in absorption from 0 to 85% RH measured on larger wood blocks. (c) MC ratio of modified wood samples in relation to untreated and Lewis acid-treated wood measured on wood powder. (d) MC ratio of modified wood samples relative to untreated and Lewis acid-treated wood measured on wood blocks. Reproduced with permission from (Awais et al., 2022a). Copyright 2022, American Chemical Society.

Sorption isotherms of the earlywood and the latewood regions
Chemical imaging revealed the difference in acetylation degree in the earlywood and the latewood regions in **Publication I and II**. The thin-walled earlywood regions acetylated faster than the thick-walled latewood cells. The acetyl groups occupy the accessible OH groups and reduce the cell wall space. The moisture uptake is expected to reduce in the earlywood regions. This was validated by measuring the sorption isotherms of carefully isolated earlywood and latewood sections with automated sorption balance (Figure 27). The sorption isotherms highlighted the reduction in MC with the increase in acetylation WPG and differentiated the MC variation in the earlywood and latewood regions within the hygroscopic range of 0-95% RH. The untreated reference samples show insignificant differences in the EW and LW regions. And the isotherms recorded were almost identical. However, the difference increased with the increase in acetylation WPG. The EW regions modified with 8.5% and 17% WPG showed lower MC\(R\) than the LW sections due to the stronger acetylation of the EW cells. Moreover, three replicates of each untreated, 8.5%, and 17% WPG-treated EW and the LW were measured, and the standard error over each RH step was calculated. The pooled standard error was 0.03%. The paired t-test was applied to the measured MC values of the EW and the LW regions and found statistically significant \((p < 0.01)\) differences within the acetylated samples with 8.5 and 17% WPG. However, the differences in the EW and LW regions of the untreated wood were statistically insignificant.

The MC measured with the automated sorption balance was corrected with the WPG of the wood block since the WPG of the earlywood and the latewood region was unknown. This might have increased the differences in the MC\(R\). The observed MC\(R\) of the EW regions could be slightly higher than the sample average, and the LW regions could be lower than the sample average. Overall, the differences were statistically significant and complemented with imaging-based spectroscopy in the later section.
Figure 27. Sorption isotherms of earlywood and the latewood regions of untreated reference, 8.5 and 17% WPG acetylated samples measured with automated sorption balance and wood block on the right highlighting the earlywood and the latewood regions for better understanding. Reproduced with permission from (Awais et al., 2022b). Copyright 2022, Springer Nature.

4.2.2 NIR hyperspectral imaging reveals the wood moisture content

NIR hyperspectral imaging was used to quantify the MC distribution within the wood blocks at a spatial resolution of a few millimetres. Wood blocks were acetylated to create a broad range of MC variations. The treatment levels (0-17% WPG) and the RH (0-95%) steps were set up with a DoE approach. The cross-sectional and radial surfaces were scanned with NIR hyperspectral camera at a conditioned state. An image mosaic was generated, and the samples were arranged based on DoE. The image mosaic was first analysed with the PCA (Figure 28). The first three PCs explain 95% of the variation in data in cross-sectional surface mosaic and 98% in radial surface mosaic. Only PC1 was included here, which shows the MC-related information in relation to the acetylation degree. PC2 and PC3 separately explain the difference in acetylation and MC in the wood blocks, presented in Publication II.

Both radial and cross-sectional surfaces indicated similar results, shown in Figures 28a and b. PC1 loading vector contains two positive band regions at 1390-1410 and 1905-1928 nm. The band at 1390-1410 nm is assigned to the first overtone of absorption of OH groups associated with all wood biopolymers. The intensity of this band decreases with the increase in acetylation degree in wood (Ali et al., 2001; Schwanninger et al., 2011a). The bands at 1923, 1969, and 2033 nm are related to water molecules that can be present in either free water state, one OH group is occupied in hydrogen bonding or two OH groups occupied in hydrogen bonding (Inagaki et al., 2008; Tsuchikawa and Tsutsumi, 1998). Several bands’ shifts were observed in the negative band region of 2268-2279 nm. The peak at 2267 and 2269 nm were assigned to aromatics lignin in wood, and
2272nm belonged to celluloses and hemicelluloses (Schwanninger et al., 2011a). PC1 also showed the difference in MC of earlywood and latewood regions.

Figure 28. PC1 score images and the corresponding loading vectors of the cross-sectional and radial scanned surfaces mosaics, (a) Cross-sectional surface (b) Radial surface of wood blocks. Reproduced with permission from (Awais et al., 2022b). Copyright 2022, Springer Nature.

PCA detected the spectral changes associated with the MC as a function of acetylation degree in wood. These spectral changes were quantified based on the gravimetrically measured MC using the PLSR model (Figure 29). The calibration model was built based on the 111 screened objects with corresponding MC (%) values in the response variable. The test set comprised 75 objects. The calibration model was validated with RMSEC, RMSEP, and the test image set (RMSE<sub>img</sub>) (Figure 29a). The model diagnostic suggested four LV with an RMSEC of 0.66% and RMSEP of 0.62% (Figure 29b). The regression vector with four LV highlights the dominant bands contributing to the prediction of MC and acetylation (Figure 29c).
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Figure 29. Results from the PLSR model to determine the MC distribution in relation to the acetylation, (a) Root mean squares error of calibration, test set, and the test image set prediction, (b) Measured and predicted MC values by applying the model with four LV and the dotted line is a reference line with slope 1 and passes through the origin, (c) Regression vector based on four LV, (d) Predicted image mosaic using PLS regression shows MC variation in relation to acetylation degree, (e) Histograms indicating predicted images pixels distribution (solid black line) and the average gravimetrically measured MC values (pink dotted line). Reproduced with permission from (Awais et al., 2022b). Copyright 2022, Springer Nature.

The regression vector contains a band region at 1181-1198 nm assigned to the second overtone stretching bond vibration and associated with CH$_3$ groups in the hemicellulose acetyl esters (Alves et al., 2012; Schwanninger et al., 2011a). The band at 1410 nm is assigned to the phenolic hydroxyl groups, and the crystalline regions of the cellulose were detected at band region 1564 – 1586 nm (Yonenobu and Tsuchikawa, 2003). A spectral shift from a positive band at 1860 nm to a negative band at 1910 nm was observed that segregates acetylation bands from water. The bands at 1923, 1969, and 2033 nm are critical and associated with the water (free water, one OH group is occupied in hydrogen bonding, or two OH groups are occupied in hydrogen bonding) (Fornés and Chaus sidon, 1978; Inagaki et al., 2008). The band region at 2267-2279 nm belongs to the CH$_3$ groups, cellulose, and hemicelluloses.

The regression vector with four LV was used to predict the MC distribution in the final image mosaic (Figure 29d). The image mosaic was preprocessed with the SNV transformation and mean-centring. The corresponding image pixel histograms were plotted, and individual samples’ pooled average was compared
with the gravimetrically determined MC, which indicated the performance of the developed model (Figure 29e). The predicted image showed that as RH increases from 0-95%, the MC also increases. However, the water uptake is lower in wood blocks with higher acetylation WPG. Moreover, the predicted images also demonstrated the difference in the MC distribution between the earlywood and the latewood regions, which is in line that the thin-walled earlywood section acetylates faster. Thereby, the latewood regions show higher MC than the earlywood regions. A similar phenomenon was observed in sorption isotherms of EW and LW sections measured with automated sorption balance (Figure 27).
5. Conclusion and future perspectives

This dissertation investigated the two chemical imaging methods to identify the process-dependent heterogeneity of chemical reagents within the different hierarchical structures of the wood at macroscopic and microscopic scales. The chemical imaging methods selected for the study were NIR hyperspectral and confocal Raman imaging. NIR and Raman spectroscopy have been used widely in wood sciences, but imaging has rarely been performed. The objective of the work was to analyse the suitability of these imaging techniques in understanding the distribution of modification agents throughout the wood’s hierarchical structure at different length scales. The standard gravimetric methods can be used to determine the amount of modification reagents added to the wood, but they are insufficient to identify the heterogeneity of these reagents. The wood regions with inadequate chemical uptake must be located to develop an efficient modification process.

The chemical modifications selected for the study were acetylation, formalisation, and impregnation modification with thermosetting resins (PF and MF). The added chemical reagents either reacted with cell wall polymers to create covalent bonds or polymerized to macromolecules within the cell wall space. These chemical changes were inhomogeneous throughout the wood structure. The combined chemical imaging techniques with different lateral resolutions identified the process-dependent heterogeneity in modified wood. Chemometric methods were necessary to process hyperspectral data because these datasets typically contain a substantial number of variables (e.g., spectral data from different wavelengths) for each spatial location in the image. Univariate data analysis techniques, which consider only one variable at a time, cannot analyse such complex datasets. The multivariate data analysis techniques applied to hyperspectral data in this dissertation include PCA, PLSR, and cluster analysis. PCA can be used to reduce the dimensionality of a dataset by identifying the most critical patterns or variations in the data. This can be useful for identifying regions of the wood that have undergone significant chemical changes due to the modification. PLS regression models the relationship between the chemical imaging data and variables, such as gravimetrically determined WPG of acetylation, PF resin load, and the MC. And this can be useful for quantifying chemical changes caused by the modification. Cluster analysis grouped regions of the wood that have undergone similar levels of chemical modification or for
comparing the chemical properties of different samples. Chemometric methods are essential to extract meaningful information from the chemical imaging data and to determine the variations within modified wood.

The NIR hyperspectral imaging studies revealed the difference in chemical uptakes between the samples and the corresponding MC. The predicted images showed the local variations within the wood blocks, most notably the differences in chemical uptake and MC in the EW and LW regions. We found in acetylated samples that the thin-walled EW regions acetylated faster than the thick-walled LW regions, resulting in a gradient in moisture uptake and reduced MC during conditioning. NIR imaging also revealed the distribution of PF resin in impregnation-treated wood under different heat-curing conditions. The results indicated the effect of drying conditions on the migration of the resin in larger board sections.

Confocal Raman imaging was used to visualise the local variations in the cell walls and the cellular level distributions of modification agents. In the case of heterogeneity in acetylated wood, Raman images showed a distinction in the acetylation degree between the EW and LW regions. In short diffusion time, the thickness differences in the cell walls of EW and LW had a strong effect on the acetylation degree. The cell wall changes caused by the acetylation and formalization showed that treatments influenced the cell wall and corner regions, and no noticeable variations in treatment intensity were observed. Following this, the affect of chemical modification on MC was measured by the dynamic measurement of mass change and the consequent swelling. The results indicated that the chemical modifications (acetylation and formalization) reduced the MC of untreated wood. The differences in MC between the EW and LW regions were also measured with dynamic vapour sorption, and we found statistically significant differences in the measured MC in EW and LW over the different RH levels. This was in-line with the results predicted by NIR hyperspectral imaging.

This thesis provided information on combining chemical imaging techniques in determining the process-dependent heterogeneity and the location of chemical reagents within the hierarchical structure of the wood at different spatial dimensions. Our approach addresses the major challenge of reliable estimation of chemical reagents in bio-based materials. The results presented the high significance that these chemical imaging techniques coupled with chemometric methods can be potentially applied to other bio-based materials, where the location of the chemical agents in the structure is decisive. The designed methodology is not limited to the small scale but has the relevance to apply to large board sections. In the future, the methods may be used to characterise other treatments and processes that affect the wood composition.


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How are the modification agents distributed throughout the wood’s hierarchical structure at different spatial scales? Which chemical imaging methods are suitable to locate the modification reagents within the wood at different length scales? And how do chemometric methods based on chemical imaging data support the analysis to determine the chemical changes caused by the modification in wood?