THESIS FOR THE DEGREE OF LICENTIATE OF ENGINEERING

Kraft pulping of birch

Insights into delignification and xylan removal

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CHALMERS UNIVERSITY OF TECHNOLOGY

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Cover:
Scheme illustrating the distribution of wood components within chips and the differences between bulk and pore liquor during kraft pulping.

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ABSTRACT

Kraft pulping is a well-established process in the production of many wood-based materials, holding a strategic position in the transition towards a bioeconomy. Nevertheless, further process development is needed to increase its resource-efficiency, assimilate new feedstocks and meet new specifications. Thus, the two studies presented in this thesis focused on investigating the mechanisms governing the removal of lignin and carbohydrates during kraft pulping of birch, aiming to identify the conditions leading to evenly delignified pulps.

The first study assessed the influence of mass transport on pulping by using impregnation liquors with different compositions and by comparing black liquor fractions collected from the bulk (surrounding the wood chips) and from the pore system of wood. The results showed that the transport of OH^- and SH^- during impregnation led to more extensive delignification throughout cooking. Moreover, when analyzing the black liquor fractions, higher concentrations and an initial accumulation of lignin and xylan were observed in the pore system. Also, the structure of the dissolved wood components in the pore and bulk fractions differed, highlighting the influence of mass transport resistance in the cell walls and pore system of wood.

The second study evaluated the local evolution of pulping within model wood chips under different cooking conditions. The extent of lignin and xylan removal strongly depended on the available hydroxide content within the chips. In addition, lignin was removed more uniformly from wood when using low cooking temperatures (145°C). Lastly, pulping was shown to progress faster in the longitudinal direction of the chips.

Keywords: kraft pulping, delignification, xylan, hardwood, black liquor, mass transport.

LIST OF PUBLICATIONS

This thesis is based on the following appended papers:

Paper I **Kraft cooking of birch wood chips: differences between the dissolved organic material in pore and bulk liquor**

Linus Kron, Carolina Marion de Godoy, Merima Hasani, Hans Theliander

Holzforschung 77: 598-609, 2023.

Paper II **Kraft pulping of model wood chips: local impact of process conditions on hardwood delignification and xylan retention**

Carolina Marion de Godoy, Merima Hasani, Hans Theliander

Manuscript, submitted.

CONTRIBUTION REPORT

Paper I Shared first-authorship together with Linus Kron. Both first authors were responsible for planning and conducting all experimental work, except performing the HSQC NMR measurements. Results were analyzed together with co-authors. Both first authors were responsible for drafting the manuscript, which was revised together with co-authors.

Paper II First author. Planned and conducted all experimental work, with the exception of HSQC NMR, size-exclusion chromatography of holocellulose samples and the preparation of sapwood sections for microscopy. Results were analyzed together with co-authors. Drafted the manuscript, which was revised together with co-authors.

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1 Introduction

The forest products sector has a privileged position when considering the transition towards a bio-based economy, as it can provide substitutes and/or alternatives to products and materials currently manufactured via carbon-intensive processes (UNECE/FAO, 2022). In this scenario, kraft pulping plays a major role. Pulp mills are already responsible for supplying a broad range of pulp grades, addressing the needs of many cellulose-based products, from paper and cardboard to textiles. In addition, the process can generate electricity and its by-products, such as lignin, can be used in different applications. Yet, to increase this potential and ensure the long-term competitiveness of pulp mills, it is necessary to address the main issues faced by the kraft process.

A well-known limitation of kraft pulping is its low resource-efficiency, particularly during the cooking step. Despite the continuous improvement of the process, the incidence of rejects and low cooking yields (about 45-55% in most modern mills) still pose challenges (Bajpai, 2015; Mboowa, 2024). This problem is also aggravated by the rising price of pulpwood (SKOGSSTYRELSEN, 2023). Moreover, current trends in forestry may demand changes or adjustments in the kraft cooking operation. For example, increasing the capacity for hardwood processing and the number of species considered for pulp production may be necessary (Hujala *et al.*, 2013; FOREST EUROPE, 2020).

Therefore, two present opportunities for development in the kraft process are: 1) to increase cooking yields and the uniformity of lignin content in the pulps produced, 2) to adjust the operation to support different raw materials. However, in order to meet these needs, it is imperative to have a thorough understanding of the phenomena influencing the progression of pulping – especially to answer the first demand, in which the local behavior inside the chips is particularly relevant. Yet, despite the extensive research regarding kraft pulping, the relative importance of different factors (e.g., reaction kinetics, topochemistry, mass transport) on the rates of delignification and carbohydrate losses remains unclear. Also, as most studies have focused on wood species traditionally used by pulp mills (e.g., Norway spruce), data focused on potential new pulpwoods is limited.

1.1 Aim

In this context, the studies presented in this thesis aimed to investigate the main phenomena taking place during kraft pulping of hardwood, focusing on

the progress of delignification and the accompanying xylan removal in the wood chips. Birch was the selected wood species, given its widespread distribution in Sweden (and in Europe in general) and given the fact that it is already used by some pulp mills in the production of paper pulp and dissolving pulp. As specific goals, the following research topics were explored:

1) To study how the advection of white liquor during impregnation affects the cooking step and assess the impact of performing impregnation using liquors with increased sodium content.
2) To evaluate the influence of mass transport during cooking by comparing the composition of black liquor fractions collected from the bulk and from the pore system of wood.
3) To study local changes in the composition of wood chips throughout kraft pulping using different conditions (namely, temperature and hydroxide concentration).
4) To assess the impact of wood structure on the evolution of pulping inside the wood chips.

2 Background

2.1 Wood

Wood is the lignocellulosic material that forms the xylem of trees from both gymnosperms (softwoods) and angiosperms (hardwoods). The xylem (and thus wood) can be divided into two parts: sapwood, the outer portion that is responsible for the transport of sap, and heartwood, the inner portion that is made exclusively by dead cells and works as a support tissue. In addition, both sapwood and heartwood are organized in concentric growth rings. Each ring contains earlywood, which is formed in the beginning of the growth season, and latewood, which is formed later and presents cells with considerably ticker walls (Sjöström, 1993a; Daniel, 2009). The detailed macrostructure of the xylem is depicted in Figure 2.1, together with the other components of the stem (outer bark, phloem/inner bark, cambium and pith).

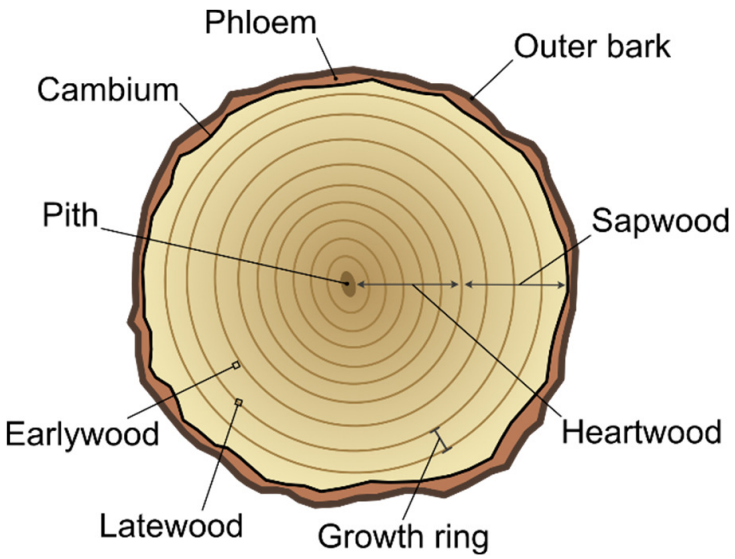

Figure 2.1. Scheme illustrating the different tissues present in the stem of a tree.

Wood can be further characterized according to its microstructure, ultrastructure and chemical composition, with substantial differences between softwoods and hardwoods, or even between different species within the same genre. Distinct characteristics can also be found when comparing sapwood and heartwood. Although, in this case, they are mainly caused by the deposition of extractives in the heartwood tissue, which may obstruct pits and vessels (refer to Section 2.1.1) and decrease permeability (Biermann, 1996a).

More details about wood morphology and composition are described in the following sections.

2.1.1 Microstructure

The microstructure of wood involves the different types of cells and anatomical features present in the tissue, as well as their spatial distribution. In general, wood cells can be divided into four main groups: a) parenchyma cells, whose main function is associated with storage; b) tracheids, which are responsible for support and conduction in softwoods and conduction in hardwoods; c) fibers, which provide support (only found in hardwoods); d) vessels, which are cells specialized in transport (only present in hardwoods). Other relevant anatomical features, especially in the context of wood permeability, include pits (recesses in the cell wall of adjacent cells, with specific shapes and distribution patterns) and the occurrence of tyloses (organic deposits that obstruct vessels in heartwood) (Koch, 2006; Daniel, 2009).

Softwood is formed by longitudinal and ray tracheids (accounting for up to 95% of the tissue) and parenchyma cells (mainly ray parenchyma). Another characteristic feature that is present in some softwood species are resin canals - intercellular spaces in which resin is stored (Sjöström, 1993a).

Hardwood, on the other hand, displays a much more complex microstructure. Besides possessing all four main types of wood cells, each of them may differ significantly in size and distribution from one hardwood species to another.

Usually, fibers and vessels account for most of the hardwood tissue, with rays being also significant in some species. The hardwood fibers are shorter than longitudinal tracheids of softwoods and are often smaller in diameter as well. The vessels are characterized by having thin walls and being quite wide when compared to fibers (Daniel, 2009). Moreover, the distribution and size of vessels along the growth ring (commonly referred to as porosity) is used to distinguish wood species, with three arrangements being possible: diffuse-porous, semi-ring porous and ring-porous. Diffuse-porous species have vessels with approximately the same diameter spread uniformly throughout the growth ring, whereas ring-porous species show larger vessels in earlywood than in latewood, with the change in the diameter of the vessels occurring abruptly. Semi-ring porous species can display two behaviors: the diameter of the vessels can decrease gradually between earlywood and latewood, or the vessels can remain similar in size, but being more closely packed in the earlywood than in the latewood (Ruffinatto and Crivellaro, 2019).

Table 2.1 summarizes some of the main anatomical characters of birch and other hardwoods of interest to the pulp mills.

Table 2.1. Wood morphology of selected hardwoods (Biermann, 1996b; Foelkel, 2007; Ruffinatto and Crivellaro, 2019).

Species	Porosity	Vessel diameter	Rays[1]	Notes
Alder *Alnus glutinosa*	Diffuse-porous	< 80 µm	5-12	-
White poplar *Populus alba*	Diffuse-porous / Semi-ring-porous	< 80 µm, 80-130 µm	> 12	-
Beech *Fagus sylvatica*	Diffuse-porous / Semi-ring-porous	< 80 µm	≤ 4	Rays can be very broad.
Birch *Betula pendula*	Diffuse-porous	< 80 µm length: 0.4-0.7 mm	5-12	-
Eucalyptus grandis	Diffuse-porous	130 - 250 µm length: 0.2-0.6 mm	-	Tyloses are common.

[1] Rays / mm, measured on the transverse surface (cross section).

2.1.2 Ultrastructure (cell wall)

The walls of wood cells are layered structures composed mainly of cellulose, hemicellulose and lignin (refer to Section 2.1.3 for more details regarding wood composition). Essentially, cellulose microfibrils assemble into larger macrofibrils, forming a matrix that is then surrounded by lignin and hemicelluloses. In turn, this fibrillar structure is arranged in distinct ways (layers), depending on orientation and composition (Koch, 2006).
One generally accepted cell wall model is illustrated in Figure 2.2. According to it, three distinct regions can be identified: middle lamella, primary wall and secondary wall (subdivided in S1, S2 and S3 layers).

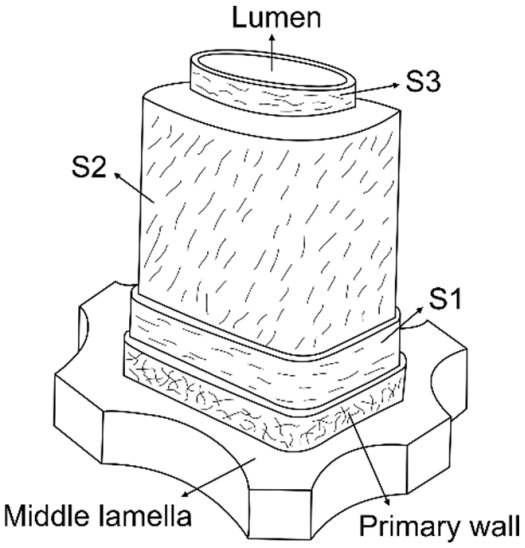

Figure 2.2. Scheme illustrating the cell wall. Adapted from Sjöström (1993a).

In this scheme, the middle lamella is located between adjacent cells, and it is heavily lignified (especially in the cell corner portion). The primary wall is a thin layer (usually around 0.1-0.2 µm) that is characterized by an irregular network of cellulose fibrils in its external portion. In turn, the secondary wall differs from the primary wall due to its fibrils being aligned in specific angles, moreover the secondary wall is thicker, being divided into different layers. Both the outer (S1 layer) and inner (S3 layer) portions are thin (about 0.1-0.3 µm), displaying fibrils aligned in relatively wide angles (50-90°). In contrast, the intermediary layer (S2) is thick – usually up to 5 µm – with fibrillar alignment in small angles (5-30°) (Sjöström, 1993a).
Yet, it is worth noting that the characteristics of the cell wall layers also vary according to the type of cell. For instance, the secondary wall in latewood cells is thicker than in earlywood, and the S2 layer in vessel elements is thinner than in fibers (Daniel, 2009). Nevertheless, in the context of pulping, the most relevant regions of the cell walls are the middle lamella and the S2 layer, as they account for most of the lignin in the cell.

2.1.3 Composition

Wood composition varies substantially among different species, but it can also differ from individual to individual, being affected by parameters such as the age of the tree and its place of origin (i.e., climate, soil conditions, etc.). Nonetheless, cellulose, lignin and hemicelluloses can be pointed out as the main components of both softwoods and hardwoods. Furthermore, the majority of trees exhibit organic extractives and minerals (ash), which, when combined, can represent up to 10% of wood (Pettersen, 1984).
The next sections describe the three main components (cellulose, hemicelluloses and lignin) in more detail.

2.1.3.1 Cellulose

Cellulose is a linear polymer formed by β-D-glucopyranose units connected by (1→4) glycosidic bonds, as shown in Figure 2.3. The cellulose chain is very long when compared to other polysaccharides: in wood, its native structure usually exhibits degree of polymerization above 10000, which can then be decreased to about 1000 after pulping and bleaching (Biermann, 1996a; Henriksson and Lennholm, 2009).

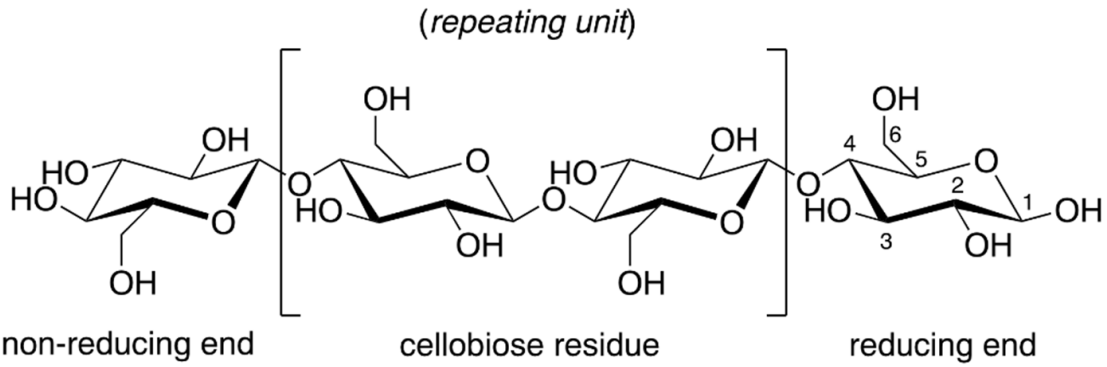

Figure 2.3. Molecular structure of cellulose, highlighting the (1→4) glycosidic bonds between β-D-glucopyranose and the repeating units of cellulose (cellobiose residues).

Despite its rather simple molecular structure, cellulose can display quite complex spatial arrangements. These supramolecular structures are a consequence of the different intra and intermolecular interactions of the cellulose chains, such as van der Waals bonds and hydrogen bonds. The hydrogen bonds are particularly relevant, as they stabilize the glycosidic linkages and provide stiffness, moreover, they can lead to the formation of cellulose crystallites (Koch, 2006; Henriksson and Lennholm, 2009). Ultimately, the cellulose bundles aggregate into microfibrils containing both highly crystalline regions and less ordered regions. The microfibrils are then further organized into macrofibrils (Sjöström, 1993b).
The role of cellulose in wood is mainly related to the structural and mechanical properties of the cell walls, with the highest concentrations of cellulose usually being found in the S2 and S3 layers and the lowest concentrations occurring in the middle lamella. Furthermore, cellulose accounts for most of the wood tissue: about 45-50% in softwoods and 40-50% in hardwoods (Biermann, 1996a). In birch, the cellulose content has been reported to be between 37 and 47%, depending on the species (Pettersen, 1984; Olm *et al.*, 2009; Santos *et al.*, 2011).

2.1.3.2 Hemicelluloses

Hemicelluloses are heteropolysaccharides with a relatively low degree of polymerization (about 50-200) and chains with different levels of branching. Overall, their structure is formed by hexoses (mainly glucose, mannose and galactose) and/or pentoses (such as xylose and arabinose). In addition, acetyl groups, rhamnose and acid groups (like 4-O-methyl-D-glucuronic acid) may also be present (Koch, 2006).

In the cell walls, hemicelluloses are usually found in the fibrillar structure, displaying different levels of association with the cellulose fibrils (Dammström *et al.*, 2009; Gomes *et al.*, 2020). They account for 20-35% of the wood tissue, contributing to the mechanical properties of the cell wall and potentially performing biochemical functions. It is possible that they may also play an important role in facilitating the interactions between cellulose and lignin (Teleman, 2009).
In softwoods, the most common hemicelluloses include: glucomannan (10-15%), arabinoglucuronoxylan (7-15%) and galactoglucomannan (5-8%). Hardwoods, on the other hand, contain mainly glucuronoxylan (15-35%) and small amounts of glucomannan (2-5%) (Teleman, 2009). Birch, in particular, usually presents 20-29% of glucuronoxylan and 1.5-4.0% of glucomannan (Pettersen, 1984; Olm *et al.*, 2009; Santos *et al.*, 2011).

Glucuronoxylan: the general structure of hardwood xylan is illustrated in Figure 2.4. Basically, it is formed by a linear backbone of β-D-xylopyranose units linked by (1→4) bonds. The xylose units are usually highly acetylated (about 4-7 *O*-acetyl groups / 10 xylose units), mainly in the C-2 and C-3 positions. In addition, the backbone carries 4-O-methyl-D-glucuronic acid groups, which are linked to the xylose units via (1→2) bonds (Sjöström, 1993b; Teleman, 2009).

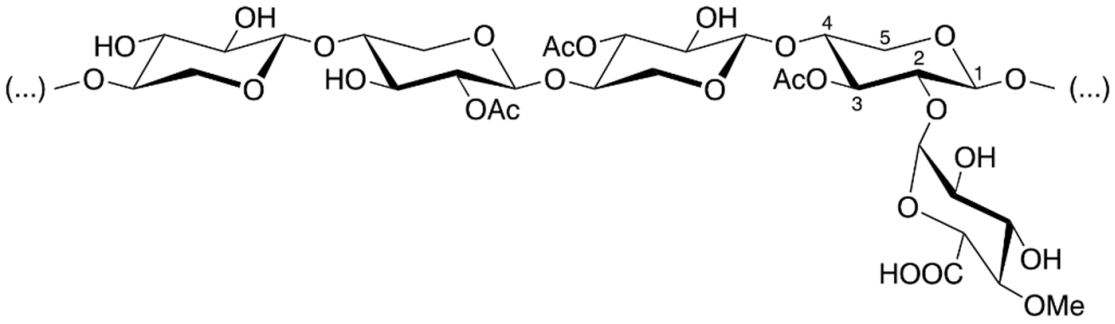

Figure 2.4. Schematic structure of glucuronoxylan.

In birch, about 7 methyl glucuronic acid residues occur every 100 xylose units and most of the substituted xylose units also carry an acetyl group in C3 (Pinto *et al.*, 2005a; Teleman, 2009).

2.1.3.3 Lignin

Lignin is a complex biopolymer whose main function in the wood tissue is associated with preserving the wood structure. Besides embedding the cellulose fibrils and hemicelluloses, lignin serves as a glue between different

cells. In addition, by surrounding the polysaccharides, lignin creates a hydrophobic barrier that protects the fibers from water, enzymes and microbial attack (Henriksson, 2009). Yet, the extent of the interactions between lignin and carbohydrates is still not fully understood, with the possible existence of covalent linkages between native lignin and polysaccharides (LCCs) being the topic of extensive research, e.g., Lawoko *et al*. (2005), You *et al*. (2015), Giummarella and Lawoko (2016), and Zhao *et al*. (2020).

Regarding its structure, lignin can be seen as a network formed essentially by monolignols linked via ether bonds (mostly β-O-4, 4-O-5 and 1-O-4) and carbon-carbon bonds (mainly 5-5, β-5, β-β and β-1). Figure 2.5 shows the most common monolignols found in lignin: *p*-coumaryl alcohol (H), coniferyl alcohol (G) and sinapyl alcohol (S).

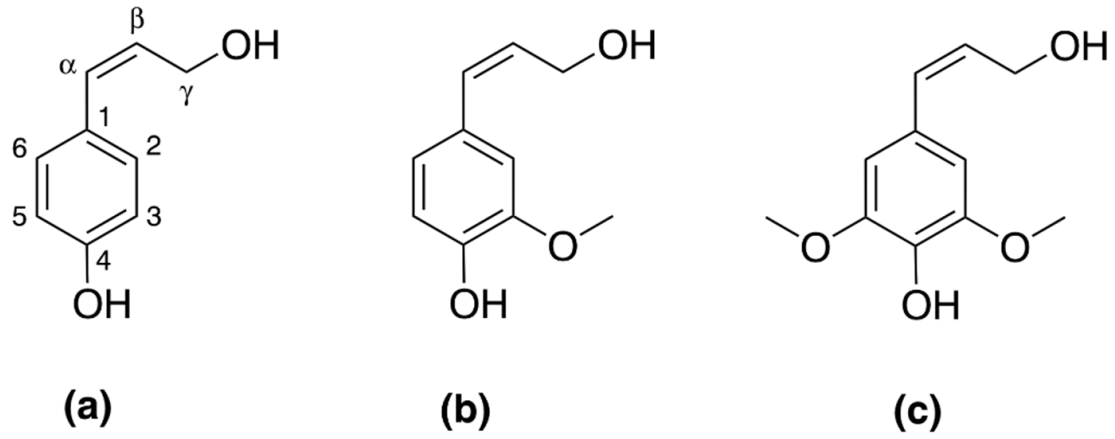

Figure 2.5. Main monolignols in lignin: a) *p*-coumaryl alcohol, b) coniferyl alcohol and c) sinapyl alcohol.

However, the abundance of these monolignols depends on the kind of plant. In grasses, lignin displays significant amounts of all three monolignols, whereas the lignin of softwoods is formed almost entirely by coniferyl alcohol (guaiacyl lignin). In hardwoods, both coniferyl alcohol and sinapyl alcohol are found in large quantities, with the S/G ratio varying between different species and different wood cells. Usually, the lignin found in parenchyma cells and in the secondary wall of fibers is rich in S units (syringyl lignin), but the S/G ratio decreases in the middle lamella. In contrast, the lignin in vessels is mostly guaiacyl lignin. Still, the average S/G ratio in hardwood lignin has been reported to be between 1:1 and 3:1 for most species (Sjöström, 1993c; Santos *et al*., 2012; Yamashita *et al*., 2016).

2.2 Kraft pulping

Kraft pulping is the prevalent method in the pulp and paper industry: globally, it accounts for the production of about 98% of all chemical wood pulp and 82% of wood pulp in general – recovered fibers not included (FAO, 2023). Some of the main reasons for the success of kraft pulping include its ability to generate pulps with high strength, well-established strategies for the recovery of energy and chemicals during the process, and its flexibility regarding raw materials (Mboowa, 2024).

In the process, defibration is achieved by removing lignin from the lignocellulosic matrix, which is accomplished through the use of an aqueous solution containing hydroxide and hydrogen sulfide ions (white liquor) at elevated temperatures (> 140°C). However, between the wood logs and the final product, several steps take place, such as chipping, screening, cooking, washing, bleaching, among others. For the purpose of this thesis, two operations will be in focus: impregnation and cooking.

Impregnation is the step that precedes the kraft cooking operation. Its main goal is to ensure that the initial distribution of cooking chemicals is even between and within the wood chips before the cooking temperature is reached, which is essential to minimize the occurrence of underdelignified material (shives) and/or overcooked fibers. In pulp mills, impregnation takes place after the wood chips were subjected to steaming (i.e., after they have been pre-heated, and the air has been removed from their pore system). During the process, the chips are treated with white liquor (usually supplemented with black liquor) at 100-110°C, which promotes the penetration of liquor within the pore system of the wood and the diffusion of chemicals from the bulk liquor towards the cell walls. In addition, a significant fraction of the alkali is already consumed in this step (mostly to neutralize acidic groups) (Brännvall, 2009; Foelkel, 2009).

Cooking is the step in which delignification takes place. It can be carried out using a wide range of temperatures (historically, 170-160°C were applied for softwoods and 150-160°C for hardwoods), nonetheless, modern pulp mills tend to utilize mild conditions (145-155°C). The mode of operation can be either batch or continuous. Also, it is in this stage that most of the alkali is consumed: 25-30% are used in lignin reactions, whereas the rest is mainly spent in reactions involving carbohydrates (neutralization, peeling, etc.) (Sjöström, 1993d; Brännvall, 2009; Bajpai, 2015).

The next sections discuss some of the most important phenomena taking place during impregnation and cooking, as well as a brief description of the most common approaches used to model these operations.

2.2.1 Reactions

2.2.1.1 Delignification

During kraft pulping, lignin units can undergo different reactions. Nevertheless, the degradation/dissolution of lignin fragments occurs mainly through the cleavage of ether linkages (most common bonds between the monolignols). In phenolic units, which are more reactive than nonphenolic ones, the process begins with the ionization of the phenolic groups. Next, a group in the alpha position is eliminated, forming quinone methide (intermediate). This step is a result of the alkaline cleavage of α-O-4 ether bonds, or elimination of other substituents (e.g., hydroxyl group). Then, the quinone methide can be subjected to different reaction pathways. The preferred one is the nucleophilic attack of hydrogen sulfide, which cleaves β-O-4 ether bonds and liberates phenolic groups, effectively fragmenting the lignin structure. This pathway highlights the importance of HS^- in the kraft process: in the absence of hydrogen sulfide (soda pulping), the main reaction path would involve the elimination of terminal hydroxymethyl groups, forming an alkali-resistant structure. Condensation reactions and electron-transfer reactions are also viable pathways after the formation of quinone methide, but only occur to lesser extents (Gierer, 1980; Potthast, 2006).
In nonphenolic units, the cleavage of β-O-4 and α-O-4 ether bonds is relatively slow. It encompasses the formation of intramolecular epoxides followed by nucleophilic attack by OH^- or HS^-. In addition, to avoid the formation of alkali-resistant bonds during the nucleophilic attack, nonphenolic units require higher alkalinity and temperature than phenolic ones (Potthast, 2006).

2.2.1.2 Reactions with carbohydrates

Carbohydrates, especially hemicelluloses, are quite sensitive to the alkaline conditions utilized in kraft pulping, so much so that the loss of carbohydrates is one of the main factors contributing to the low pulp yields in the kraft process. Peeling and alkaline hydrolysis of glycosidic bonds are the main causes of said losses, alongside reactions affecting side groups in the polysaccharide chains, such as deacetylation (particularly in glucuronoxylans and galactoglucomannans). Other relevant reactions taking place include the formation of hexenuronic acid and stopping reactions (that interrupt peeling) (Sjöström, 1993d; Potthast, 2006). Dissolution and re-adsorption of hemicelluloses (mainly xylan) are also significant, with the former occurring early in the process (still at low temperatures) and the latter being more prevalent at the end, when the alkali content is lower and the fibers are more exposed (Danielsson and Lindström, 2005; Pinto *et al.*, 2005a).

Peeling affects the reducing ends of polysaccharide chains. The terminal monosaccharide unit is cleaved off and rearranged into an isosaccharinic acid, while a new reducing end is formed in the carbohydrate chain. Given its relatively low activation energy (about 103 kJ/mol), significant peeling can occur even during the heating-up period (temperature > 80°C), being interrupted when metasaccharinic acid end groups (or other stable structures) are formed in the polysaccharide chain (stopping reaction). Alkaline hydrolysis, on the other hand, demands harsher conditions than peeling (at least 140-150°C) and contributes to decrease the viscosity of the pulp. Moreover, it creates new reducing ends in the polysaccharide chains – which initiates a new cycle of peeling (secondary peeling) (Gierer, 1980; Sjöström, 1993d; Potthast, 2006).

Yet, it is worth highlighting that each polysaccharide behaves differently during pulping. For instance, cellulose is degraded to a lesser extent than hemicelluloses, given its high degree of polymerization and crystallinity. Furthermore, glucomannans are heavily degraded by peeling, whereas in xylan the reaction is retarded by the presence of substituent groups (Sjöström, 1993d).

2.2.2 Mass transport and wood chemistry

2.2.2.1 Transport of ions and dissolved wood components

Mass transport is a key factor during kraft pulping. The transfer of OH^- and HS^- from the bulk cooking liquor towards the interior of the wood chips (i.e., through the pore system and cell walls) affects the availability of cooking chemicals in different regions of the chip. Meanwhile, the overall rate of lignin and carbohydrates removal is influenced by the rate of transport of the dissolved material (e.g., lignin fragments, degradation products, etc.) in the opposite direction (from cell wall towards bulk).

In the transport of cooking chemicals, two mechanisms are relevant: penetration of liquor (mainly during the impregnation step) and diffusion of ions (Sjöström, 1993d; Brännvall, 2017). The former is mainly affected by pressure gradients while the latter is governed by differences in concentration, being highly impacted by the consumption of alkali throughout the process. Furthermore, the mass transfer in the cell wall can be influenced by the local ionic strength within the pore system of wood, as described by the Donnan equilibrium (Bygrave and Englezos, 2000; Bogren, 2008; Nieminen *et al*., 2014).

In the transport of dissolved wood components, diffusion is the main mechanism (at least during impregnation and cooking), with substantial differences being reported between the concentration of dissolved material

within the pore system of wood and in the bulk liquor (Simão *et al.*, 2011; Pakkanen and Alén, 2012; Brännvall and Rönnols, 2021). Again, the local chemical environment inside the chip (e.g., ionic strength and pH) can affect the transport rates, especially in the cell walls. It can alter solubility and promote higher interactions between the dissolved fragments and/or between fragments and the lignocellulosic matrix – leading to changes in diffusivity or even promoting the aggregation of fragments or their deposition over the fibers (Dang, 2017; Kishani *et al.*, 2020; Roujin, 2022). In addition, the molecular weight of the dissolved material has also been described as a relevant factor. For example, during kraft cooking of Scots pine woodmeal, Dang (2017) verified that small lignin fragments were released to the bulk liquor earlier than large ones.

The transport of both ions and wood components is also impacted by the characteristics of the wood chip, such as the dimensions and wood morphology. For instance, properties like permeability vary not only between species, but also between different directions, i.e., longitudinal, radial and tangential (Siau, 1984). Not surprisingly, appropriate thickness has been pointed out as a determining factor to ensure uniform pulping of wood chips (Brännvall, 2017; Tripathi *et al.*, 2018). In general, to reduce the occurrence of shives and overcooked material, it is recommended to avoid over-thick and fine chips: preferably the thickness should be between 2 mm and 5 mm (Foelkel, 2009).

The elucidation of liquor penetration as a function of wood structure has also been the subject of a variety of studies. Wardrop and Davies (1961), for instance, found that pulping liquors entered softwood samples through the lumen of tracheids or through resin canals, whereas in hardwood the penetration started mainly through the lumen of the vessels. Adjacent cells were then reached via pits, and further lateral penetration proceeded through rays, with transport occurring faster in cells with thin walls and numerous pits. Similar results were achieved later by Tondi *et al.* (2013) and Wagih *et al.* (2022).

In addition, Wardrop and Davies (1961) evaluated the transport of pulping media in the cell walls, concluding that the reagents diffused from the lumen across the wall, that is, from the S_3 layer to the middle lamella. However, this pathway seems to be dependent on the type of liquor, more specifically its pH. When comparing alkaline and acidic pulping liquors, Koch *et al.* (2003) identified delignification patterns that suggested significant penetration through pit chambers in samples subjected to acidic medium (likely due to less swelling), which probably exposed the middle lamella to the active ions earlier than in alkaline conditions. Moreover, the mass transport pathways and the overall mass transfer resistance may also change substantially

throughout cooking, as cracks and the separation of cell walls become prevalent (Wagih *et al*., 2022).

2.2.2.2 Wood composition and topochemistry

The evolution of pulping within wood can also be affected by its chemical composition and how the different components are distributed in the microstructure.

When studying the topochemistry of black spruce, Whiting and Goring (1981; 1982) observed that isolated secondary layer tissue was delignified faster than isolated middle lamella, which the authors argued could be related to the differences in lignin structure in the two tissues (e.g., degree of crosslinking and amount of phenolic hydroxyl groups) and differences in hemicellulose concentration. It was suggested that the removal of hemicelluloses in the early stages of kraft cooking could increase the porosity of the secondary wall (as it has higher hemicellulose content than the middle lamella) facilitating delignification.

Similar results were reported by Takada *et al*. (2021) when evaluating delignification during supercritical methanol treatment of Japanese beech. The authors found that lignin removal occurred earlier in the secondary wall of fibers than in the secondary wall of vessels and in the middle lamella, which they justified by the existence of lignin with different characteristics in each region (condensed-type structures prevail in the middle lamella, whereas ether-type linkages dominate in the secondary wall). In addition, their results may hint at the effect of differences in the physical organization of the secondary wall in vessels and fibers or the effect of the syringyl/guaiacyl (S/G) ratio over delignification, although further investigation is necessary.

The degree of condensation and the S/G ratio were also highlighted by Pinto *et al*. (2005b) to explain the differences in delignification behavior between different hardwood species. The authors concluded that the original concentrations of lignin and carbohydrates in the wood, despite being significantly different among the species, could not explain the differences in pulping. Instead, the performances seemed to be affected by structural features of wood and its biopolymers. Xylan retention, for instance, was connected to the molecular weight and to the abundance and type of uronic acid moieties (i.e., terminal or substituted moieties). In the case of delignification, the S/G ratio and the percentage of condensed lignin had a larger impact over the process than the overall percentage of lignin in the wood, the lignin average molecular weight in the wood/pulps and the content of β-O-4 structures. Still, it is worth mentioning that their experiments were conducted utilizing different alkali loads between the species, which complicates the analysis of the results.

Finally, external factors, such as growth circumstances, may alter the characteristics of wood and, thus, the pulping performance. The content of calcium, for example, was shown to be affected by the growth site and to impact negatively the kraft process: Vegunta *et al*. (2022) studied batch kraft pulping of *Eucalyptus dunnii* and concluded that, depending on the origin of the tree, the wood samples could present high amounts of calcium crystals in the lumen of fibers, resulting in decreased delignification, increased percentage of rejects and enhanced degradation of carbohydrates.

2.2.3 Modeling

As mentioned in the Introduction (Chapter 1), there is no consensus regarding the limiting mechanisms governing kraft pulping. This is reflected in the many attempts to describe the operation, with the resulting models varying in complexity, accuracy and predictive power. The models can consider different aspects of the system. Usually, they include the mass balances for relevant species (e.g., lignin, OH^-, SH^-, etc.), which requires equations for the reaction rates of alkali consumption, delignification and, depending on the approach, equations for the degradation/dissolution of carbohydrates. Expressions for mass transfer may also be considered (e.g., to describe the impregnation of chips and the diffusion during cooking). Furthermore, if the goal is describing a large system (e.g., industrial reactors), the models can also incorporate equations for the flow of liquor and the heat exchange.

When focusing on the impregnation of chips, two main approaches can be identified. The first one can be exemplified by the work of Kazi *et al*. (1997). In this case, the model assumes that no relevant reactions occur, given the low impregnation temperature (≤ 100°C). Thus, impregnation is described exclusively by mass transport equations. The remaining assumptions in this kind of model concern how to account for the initial advection of liquor, how to describe the anisotropic nature of wood and how to attain the transport coefficients. Kazi *et al*. (1997), for instance, did not account for capillary flow (as the water in their raw material was above the fiber saturation point), describing the mass transport as purely diffusion (according to Fick's second law) in two directions (axial and radial/tangential). Diffusivities were independent of position, being a function of the wood species, temperature and pressure. This is a fairly simple approach, which provides a good description for the concentration of inactive ions.

The second approach to model impregnation is more suitable to describe the hydroxide distribution. In this case, due to the alkali uptake during impregnation, the hydroxide concentration is assumed to follow a shrinking core model (Zanuttini *et al*., 2000; Brännvall and Reimann, 2018). One example of a model considering the impact of the reactions during

impregnation was developed by Costanza *et al*. (2001). To describe the distribution of OH^-, the authors incorporated both diffusion (one-dimensional) and consumption by deacetylation (non-linear reaction rate). In addition, the diffusivity was assumed to be a function of temperature and alkali concentration.

When modeling the cooking step, even more options are found in literature. The influence of mass transport can be described as a simple correction factor in the reaction rates, as proposed by Dang and Nguyen (2008) when trying to account for the impact of chip thickness on delignification. Alternatively, the models can consider different phases (e.g., solid, entrapped liquor and bulk liquor) in which the chemical species can diffuse from or to, as suggested by Wisnewski *et al*. (1997) and Gilbert *et al*. (2021). Furthermore, Bijork *et al*. (2022) proposed a model with an even higher level of complexity by considering three-dimensional mass transfer between each phase, in an attempt to include the differences in mass transport resistance in the longitudinal, radial and tangential directions of wood chips.

To describe the rates of reaction, most models adopt a version of either the 3-stage model (Gustafson *et al*., 1983) or the parallel reaction model (Smith, 1974). In the 3-stage model, the cooking time is divided into three intervals (initial, bulk and residual), each one of them with their own equations for lignin and carbohydrates removal. On the other hand, in the parallel reaction approach (also known as the Purdue model) the equations describing reaction rates are the same throughout the whole cooking time, but the wood components are often divided into fractions with different reactivities (Nieminen and Sixta, 2012).

The delignification rate is commonly expressed as a linear function of lignin concentration, with an Arrhenius like dependency on temperature. In addition, the equations are often a function of the concentrations of hydroxide and hydrogen sulfide ions (Nieminen and Sixta, 2012). Moreover, the concentration of inactive ions (e.g., sodium) and correction factors based on wood properties (e.g., S/G ratio) can also be featured in the reaction rates (Bogren, 2008; Correa *et al*., 2023). The equations describing reaction rates involving carbohydrates vary significantly among the models available in literature. They have been formulated as simple functions of lignin concentration (Gustafson *et al*., 1983), with expressions analogous to the one utilized for delignification, or with distinct equations based on temperature, the concentrations of the own polysaccharides and hydroxide content (Andersson *et al*., 2003).

Thus, given the large variety of models available for pulping, it is important to understand the main mechanisms influencing the system under study in order to choose the most appropriate approach to describe it.

3 Material and Methods

This chapter covers the methodology utilized to develop the experiments performed in Papers I and II. It presents the main analytical procedures that were used and the reasoning behind the pulping experiments (e.g., selection of cooking conditions).

3.1 Wood samples

All wood used in this work was kindly provided by Södra Skogsägarna.

3.1.1 Industrially cut wood chips - Paper I

In the first paper, a mix of industrially cut birch chips (*B. pendula* and *B. pubescens*) from southern Sweden was used as raw material. They were screened to be free of bark and knots, and chips with thickness lower than 2 mm or higher than 6 mm were discarded. Before being subjected to any experiments, the chips were air dried (average dry content: 93.2±0.5%, w/w) and kept at room temperature.

3.1.2 Wood log - Paper II

The experiments in the second paper were conducted with hand-cut sapwood chips. The starting point to prepare these chips was a log from a 27-year-old birch tree (*Betula pubescens*) grown in southern Sweden. The log was 1 m high and had a diameter of approximately 16.5 cm. The hand-cut chips (refer to the procedure in Section 3.3) were air dried and stored at room temperature. The average dry content of the final material was 94.6±0.2% (w/w).

3.2 Chemical Reagents

All chemicals utilized in this work were of analytical grade. The Pullulan standards (PL2090-0100, 0.180 to 708 kDa) used in the analysis of molecular weight distribution of lignin and extracted xylan were purchased from Varian Inc.

3.3 Hand-cut sapwood chips - Paper II

Model chips were prepared by following the procedure shown in Figure 3.1. Basically, the process involved five steps:

i. Cutting the log into disks (height: 30±1 mm) with a vertical bandsaw (SM/420, Mössner Rekord).
ii. Separating the inner heartwood-rich part of each disk (region with approximately 64 cm^2) from the sapwood-rich fractions.
iii. Slicing the sapwood-rich pieces into slabs (8±1 mm thick).
iv. Removing bark and cutting the slabs into smaller pieces (45±1 mm long) using a hand saw (Sandvik 268, BAHCO).
v. Fixing rough edges and coarse surfaces with a hand plane (220 Block Plane, Stanley Hand Tools), and discarding chips containing knots.

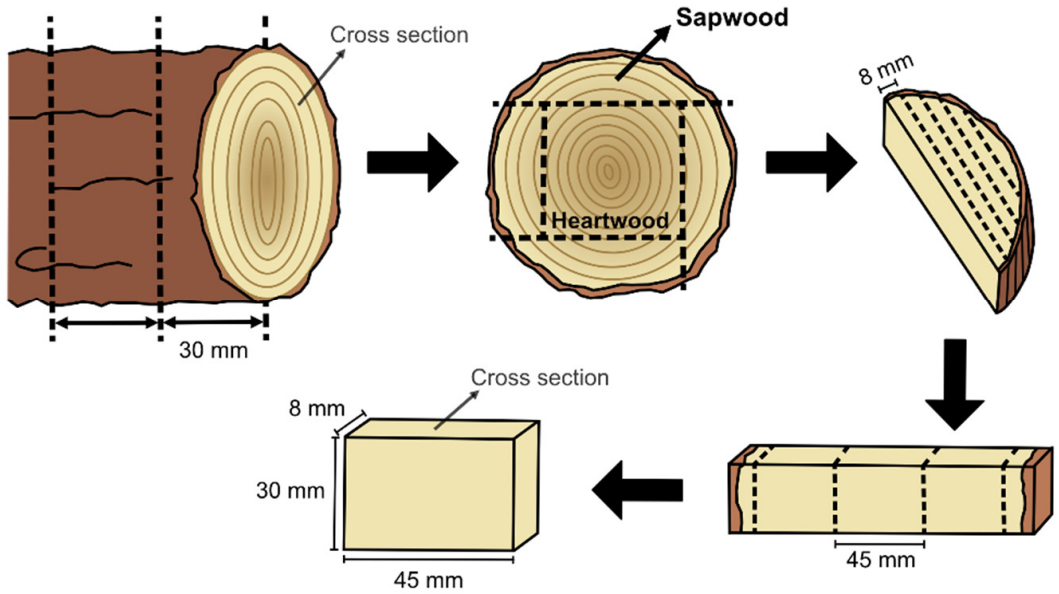

Figure 3.1. Scheme describing the preparation of sapwood model chips. Final dimensions: 30±1 mm (length) x 45±1 mm (width) x 8±1 mm (thickness).

3.4 Batch kraft cooking

All kraft cooking experiments were carried out in autoclave vessels (1.5 L). Heating took place in a PEG-bath, in which the autoclaves took approximately 25 minutes to reach the target cooking temperature, as measured by Bogren *et al.* (2007). After reaching the desired cooking time, the vessels were placed in a water bath for about 10 minutes, in order to terminate the process. The autoclaves were then opened, and the black liquor was separated from the treated chips by vacuum filtration using a polypropylene mesh.

The details regarding the impregnation and cooking conditions used in each paper are described in Sections 3.4.1 and 3.4.2. Nevertheless, a few remarks can be made about both sets of experiments:

a) The impregnation step was conducted at room temperature to minimize the loss of carbohydrates before achieving a suitable impregnation of the wood chips.
b) The cooking temperatures were selected to cover a range that is relevant for hardwood processing.
c) High liquor:wood ratios were applied during the experiments in an attempt to maintain the concentration of cooking chemicals relatively constant throughout pulping.
d) High effective alkali (hydroxide content) was used during cooking in order to ensure an excess of cooking chemicals and, in the case of Paper II, to avoid excessively low diffusion rates into the model chips.

3.4.1 Process conditions - Paper I

In Paper I, impregnation liquors of different compositions were tested. During impregnation, the autoclave vessels were loaded with 25 g of industrially cut chips and 250 g of one of the four liquors presented in Table 3.1. These liquors were selected so that both the impact of the advective/diffusive transport of cooking chemicals (i.e., OH^- and HS^-) and the impact of additional sodium ions (increased ionic strength) could be studied.

Table 3.1. Composition of the impregnation liquors.

Liquor[1]	OH^- & HS^- (mol/kg liquor)	NaCl (mol/kg liquor)	Total Na^+ (mol/kg liquor)
WL	0.60 & 0.15	-	0.75
WLNa	0.60 & 0.15	1.25	2.00
W	-	-	0
WNa	-	2.00	2.00

[1]WL = white liquor, WLNa = white liquor with addition of NaCl, W = water, WNa = water with addition of NaCl.

The loaded vessels were first deaerated (vacuum for 5 min), and then pressurized with nitrogen (5 bar / 15 min). Once the impregnation was completed, the spent impregnation liquors were removed via filtration, and 500 g of fresh cooking liquor was added.
The cooking conditions are summarized in Table 3.2.

Table 3.2. Cooking conditions utilized in Paper I.

Parameter	Condition
Temperature (°C)	160
OH^- in cooking liquor (mol / kg liquor)	0.60 (EA = 48%)
SH^- in cooking liquor (mol / kg liquor)	0.15 (S = 40%)
Wood:liquor (w/w)[1]	1:20
Cooking time (min)	10[2], 20[2], 30, 60, 90 and 120

[1]Value is based on the original mass of wood (the mass of impregnation liquor still present in the chips was ignored). [2]Process terminated before reaching the cooking temperature. EA = effective alkali (expressed as NaOH). S = sulfidity.

At the end of pulping, two fractions of black liquor were collected and stored in a freezer. Vacuum filtration provided the bulk fraction (bulk liquor, BL), whereas centrifugation of the moist pulps (3500 rpm / 10 min) delivered the fraction still trapped in the pore system (centrifuged liquor, CL) – as described by Brännvall and Rönnols (2021). The solid residue attained after centrifugation was washed and leached with distilled water (until neutral pH). Then it was dried overnight (105°C) and stored at room temperature.

3.4.2 Process conditions - Paper II

In Paper II, four model chips (approximately 24 g of wood) were used in each cooking experiment. Table 3.3 presents the process conditions considered.

Table 3.3. Cooking conditions utilized in Paper II.

Parameter[1]	Level (-1)	Level (0)[2]	Level (+1)
Temperature (°C)	145	155	165
$[OH]^-$ (mol / kg liquor) (EA, %)	0.25 (22)	0.40 (35)	0.55 (48)
Liquor:wood (w:w)[f]	22:1	22:1	22:1
$[SH]^-$ (mol / kg liquor)[f] (S, %)	0.25 (100)	0.25 (77)	0.25 (63)
Na_2CO_3 (mol / kg liquor)[f]	0.10	0.10	0.10
Cooking time (min)	15[3], 30, 45, 60, 90 and 120		

[1]Parameters marked with "f" were kept fixed. [2]Center point conditions applied in one experiment conducted with triplicates to provide the repeatability of the test. [3]Process terminated still in the heating-up period. EA = effective alkali (as NaOH). S = sulfidity.

It is worth mentioning that the levels of sulfidity shown in Table 3.3 are considered high. This choice was made to allow the same content of bisulfide ions in all experiments and still maintain the hydroxide content (studied parameter) as the limiting cooking chemical (if lower sulfidity levels were employed it would be difficult to isolate the effects of OH^- and SH^-). In addition, sodium carbonate was added at low concentrations in order to minimize the effect of metal ions naturally present in the wood (mainly calcium), as they have been shown to decrease the delignification rate in hardwoods (Saltberg *et al*. 2009, Brelid *et al*. 2011, Vegunta *et al*. 2022).
Regarding the impregnation procedure, the model chips and the cooking liquor were loaded in the autoclave vessel. The system was sealed and deaerated (vacuum for 10 min), followed by pressurization with nitrogen (5 bar / 20 min). The longer impregnation time (when compared to the one applied in Paper I) was utilized in an attempt to ensure a suitable impregnation of the model chips.
Afterwards, the pressure was released, and the vessels were placed in the heating bath. At the end of the process, the black liquor was separated, and the treated chips were washed and leached in distilled water (11-14 days – until neutral pH and colorless leaching water were observed).

3.5 Lignin precipitation - Paper I

In Paper I, in order to study the characteristics of the lignin dissolved during kraft cooking, lignin samples were isolated from black liquor. To achieve this, a modified version of the procedure described by Dang *et al*. (2016) was used. Sulfuric acid was added to samples of bulk (BL) and centrifuged (CL) black liquor, until reaching pH 3.0. The samples were then frozen overnight, thawed, and the precipitated material was collected via filtration on glass fiber filters. The solid residue was dried in an oven at 40°C for three days.

3.6 Sectioning of cooked chips - Paper II

In Paper II, after leaching the cooked model chips, they were divided into sections, according to Figure 3.2. The sectioning strategy was inspired by the work of Wojtasz-Mucha *et al*. (2017). First, while the chips were still wet, a hand saw (Sandvik 268, BAHCO) was used to divide each one of them into nine pieces (classified as corners, sides and center) of about 1 cm x 1.5 cm x 0.8 cm. Then, with the aid of a microtome (Jung AG, Heidelberg), each piece was divided into layers (outer, intermediate and inner).

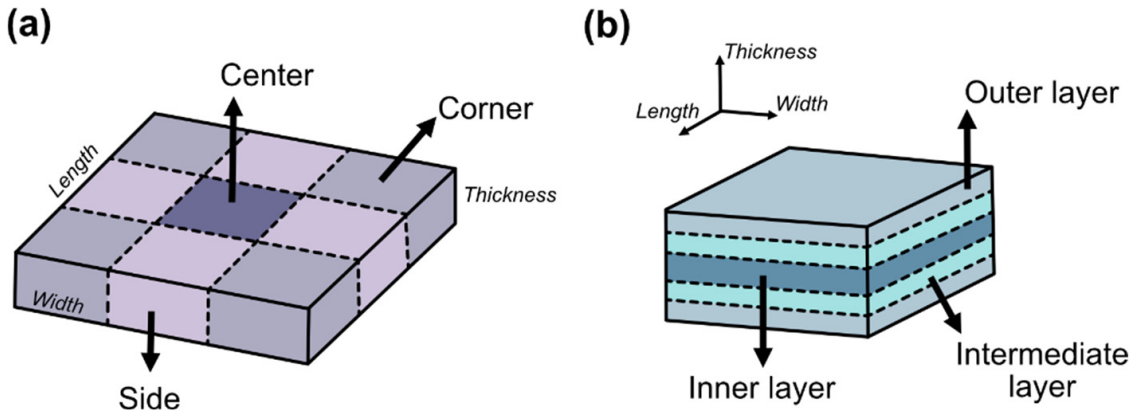

Figure 3.2. Sectioning procedure: (a) division into pieces, (b) sectioning of each piece into three different layers (outer and intermediate layers with about 3 mm and inner layer with around 2 mm).

The nine samples (sections) were then dried at 105°C overnight and stored at room temperature.
In the center-point experiment (refer to Table 3.3), four different regions were considered during sectioning: center, corner, side I and side II, as presented in Figure 3.3.

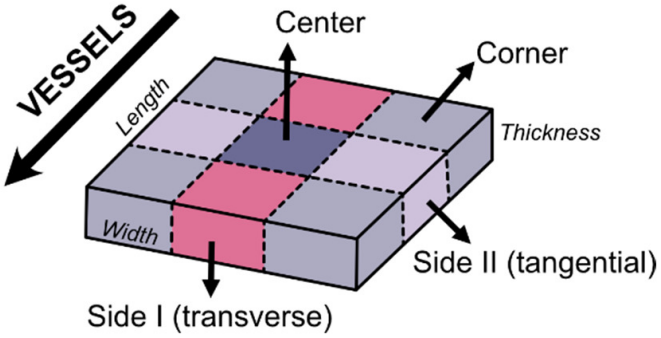

Figure 3.3. Regions in the sectioning strategy utilized in model chips cooked under Level (0) conditions.

This sectioning strategy was adopted to allow the comparison between the lateral pieces with different exposed surfaces: transverse (side I) vs tangential (side II). Thus, in this case, twelve sections were generated.

3.7 Compositional analyses

The following sections present the methods utilized to determine the content of wood components (Klason lignin, acid-soluble lignin and anhydro sugars). The estimated repeatability of the experiments performed in Paper II (based

on replicates pulped applying the center-point conditions mentioned in Table 3.3) is reported in Appendix – A1.

3.7.1 Klason lignin

In both papers, Klason lignin was quantified using adapted versions of the methodology reported by Sluiter *et al*. (2012). Notably, the extractives were not removed prior to the Klason procedure, which, consequently, resulted in slightly overestimated lignin values in wood and pulps. However, as shown in Appendix – A2, this issue does not seem to compromise the comparison between different samples.

In short, a certain amount of sample was weighted (in duplicates) and sulfuric acid (72%, w) was added. In the case of solid samples (like wood or pulp), the material was first milled in a Wiley mill (particle size < 1 mm) and dried at 105°C overnight. After adding acid, the duplicates were stirred with glass rods and, in the case of solids, they were subjected to 15 min of vacuum. Then, the samples were placed in a water bath (Isotemp, Fisher Scientific) at 30°C for 1 h, and the mixture was stirred every 20 min. Next, the system was diluted with distilled water, covered with aluminium foil, and placed in an autoclave (CV-EL 125/140°C, CertoClav) at 125°C for 1 h.

After hydrolysis, the duplicates were vacuum filtered in glass microfibre filters (Whatman GF/A, diameter: 24 mm). The filtrate was collected, diluted with distilled water, and stored for the analyses of acid-soluble lignin (ASL) and carbohydrates content. The solid residue was dried overnight (105°C) and weighted.

3.7.2 Acid-soluble lignin (ASL)

The measurement of the ASL content was based on the absorbance of the filtrate solutions obtained after the Klason procedure. The solutions were placed in 1 cm quartz cuvettes and analyzed at 205 nm in an UV spectrophotometer (Specord 205, Analytik Jena). The absorptivity constant was 110 $dm^3g^{-1}cm^{-1}$, as described by Dence (1992). If the absorbance values were outside the 0.2-0.7 range, the measurements were repeated with different dilutions.

3.7.3 Carbohydrates (anhydro sugars)

The filtrate solutions attained during the sample preparation for Klason lignin analysis were also utilized to quantify the carbohydrates content. The solutions were analyzed via anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD Dionex ICS-5000, Thermo Fisher Scientific). A gold reference electrode and Dionex CarboPac PA1 columns

(2 x 50 mm guard column and 2 x 250 mm analytical column) were used. Elution conditions: 0.26 mL/min (lower pump) and 0.13 mL/min (upper pump) at 30°C for 25 minutes (+ 13 min. of column washing). Mobile phase: H_2O/200 mM NaOH (for elution) and 200 mM NaOH/200 mM NaOH + 170 mM sodium acetate (for washing). Injection volume: 10 µL.
All samples were filtered with 0.2 µm PTFE filters. The concentrations of monosaccharides were corrected for the acid hydrolysis yield based on previously measured values (Wojtasz-Mucha *et al*. 2017) and the final results were expressed as anhydro sugars (Janson, 1974).

3.7.4 Yield and local composition - Paper II

In order to compare the compositional changes in the different parts of the cooked model chips, it was imperative to account for the yield of pulping in each section. Equation 3.1 was used to estimate these yields and Equation 3.2 provided the concentrations of the wood components (i.e., Klason lignin, ASL and anhydro sugars) in grams per gram of dry wood (g/g odw).

$$Yield_i = \frac{G_{wood}}{G_i} \tag{3.1}$$

$$X_i = x_i(Yield_i) = x_i\left(\frac{G_{wood}}{G_i}\right) \tag{3.2}$$

With $Yield_i$ = yield of pulping in a given section *i*, G_{wood} = content of glucan in the untreated wood (g of glucan/g of wood) and *Gi* = content of glucan measured in the section (g of glucan/g of cooked material in section *i*). *Xi* stands for the content (g/g odw) of *X* (*X* = Klason lignin, ASL or anhydro sugars) in section *i* and *xi* is the content of *X* in section *i* given in g/g of cooked material.
The assumption behind Eqs.3.1-3.2 is that the degradation of glucan is virtually negligible, which is justified by the short pulping times and/or mild pulping conditions utilized in Paper II. Furthermore, the assumption is corroborated by molecular weight measurements in holocellulose samples prepared from the cooked model chips (which showed only small shifts in the molecular weight distribution of cellulose between different samples), and by glucose dissolution results reported by Pinto *et al*. (2005a) when kraft cooking birch.

3.8 Preparation of holocellulose - Paper II

In Paper II, holocellulose samples were prepared to investigate and compare the extent of carbohydrates degradation in different layers of kraft cooked

chips. The procedure utilized to prepare these samples was based on peracetic acid delignification experiments carried out by Chang and Holtzapple (2000) and Kumar *et al*. (2013). This methodology was selected as the delignification was shown to be selective, and, moreover, resulted in high recovery of carbohydrates (Kumar *et al*., 2013).
The delignification was performed in beakers with 5% (w/w) of dry solids (sapwood meal or milled kraft cooked sections). The acid to solids ratio was 5.5 g of peracetic acid / g of solids. The beakers were covered with parafilm and left under agitation (300 rpm) at 25°C for 48 h. Then, the slurry was vacuum filtered in a glass microfiber filter (Whatman GF/A 55 mm, Cytiva) and washed with distilled water. The residue was washed with an ethanol/acetone mixture (50:50, v:v) and dried in an oven (40°C) for four days. Measured yields: 74%-95%.
To avoid working with low amounts of material, different pieces belonging to the same layer (i.e., center, corners and sides) were combined to prepare the holocellulose fractions of model chips. Hence, each cooked sample resulted in three different holocellulose fractions.

3.9 Extraction and isolation of xylan - Paper II

O In order to further characterize the residual xylan in pulped model chips, in Paper II xylan was extracted from the prepared holocellulose samples using the procedure described by Evtuguin *et al*. (2003). The time of extraction, however, was increased to 20 h, as mentioned by Pinto *et al*. (2005a). In the process, holocellulose was extracted at 50°C utilizing dimethyl sulphoxide (solid to DMSO ratio: 1:60, w) under nitrogen atmosphere and constant agitation (300 rpm). The extract was separated from the solid residue by vacuum filtration in a glass microfiber filter (Whatman GF/A 55 mm, Cytiva) and an extra volume of 25 mL of fresh DMSO was added to wash the solid residue.
To precipitate xylan, ethanol was added to the filtrate (2:1, v) together with enough acetic acid to form a suspension. Then, the system was kept at 4°C overnight, followed by centrifugation at 4500 rpm / 30 min / 4°C (Heraeus Megafuge 40R, Thermo Scientific), as described by Corradini *et al*. (2018). At last, the isolated xylan was freeze-dried (FreeZone Triad benchtop freeze dryer, Labconco) and stored in a desiccator. Xylan recovery (based on samples from holocellulose of untreated sapwood): 33%.
Besides the DMSO extraction, xylan was also isolated via alkaline extraction. The details regarding this procedure and its limitations are described in Appendix – A3.

3.10 Molecular weight distribution (MWD)

3.10.1 MWD of holocellulose - Paper II

The determination of MWD of holocellulose samples was executed by Södra Skogsägarna, Södra Innovation in Väröbacka. Air dried holocellulose samples (26 mg) were suspended in 300 mL of Milli-Q water. Next, the material was vacuum filtered and washed with ethanol. Then, solvent exchange to N,N-dimethylacetamide (DMAc) took place overnight, with the excess of DMAc being removed later via centrifugation (3700 rpm/ 2 min). Each sample was dissolved with 2 mL of fresh DMAc/LiCl 9% (v/w), followed by 24 h of agitation. Afterwards, the samples were diluted (0.9 mL sample / 2.7 mL dry DMAc) and filtered with 0.45 µm PTFE filters.
The MWD was measured using size exclusion chromatography (Agilent 1260 Infinity II HPLC system), with four Agilent PLgel Mixed-A columns (7.5 x 300 mm, 20 µm). Elution conditions: 1 mL/min at room temperature for 60 minutes. Eluent: DMAc/LiCl (0.9% v/w). Injection volume: 80 µL. Detection was accomplished using a multi-angle light scattering detector (Wyatt, Dawn 8, laser λ = 785 nm) and a refractive index detector (Wyatt, Optilab). Data processing was performed with Astra 8.2, considering dn/dc = 0.136 mL/g.

3.10.2 MWD of isolated xylan and precipitated lignin

Duplicates of xylan and lignin samples were dissolved in DMSO + LiBr (10 mM) overnight (10 mg/mL). Then, each sample was further diluted (final concentration: 2 mg/mL) and filtered with 0.2 µm PTFE filters. The analysis was conducted in a PL-GPC 50 Plus Integrated Gel Permeation Chromatography system (Polymer Laboratories, Varian Inc.). Two PolarGel-M columns (300 x 7.5 mm) and one PolarGel-M guard column (50 x 7.5 mm) were used. Elution conditions: 0.5 mL/min at 50°C for 45 minutes. Eluent: DMSO + LiBr (10 mM). Injection volume: 100 µL. Detection involved a UV detector (280 nm) and a refractive index detector. Data processing utilized a calibration curve based on Pullulan standards (0.180 to 708 kDa) and was carried out with the Cirrus GPC Software 3.2.

3.11 2D ^{1}H-^{13}C HSQC NMR

Xylan (Paper II) and lignin (Paper I) samples were dissolved in DMSO-d_6 (140 mg/mL), if any undissolved material was present, it was separated by centrifugation (12500 rpm for 5 min). The solutions were transferred to 3 mm tubes and analyzed via 2D NMR spectroscopy (heteronuclear single quantum coherence - HSQC) using a Bruker Avance III HD (Rheinstetten, Germany).

The test was performed at 25°C using a 5 mm TXO cold probe (800 MHz for ^{1}H and 200 MHz for ^{13}C). Pulse program: hsqcedetgpsisp2.3. Acquisition time: 96 ms for ^{1}H and 6 ms for ^{13}C. FID size of 3072 and 512 points (for ^{1}H and ^{13}C, respectively) and 24 scans with 1 s relaxation time. The software for data analysis was TopSpin 4.2.0 (Bruker).

3.12 Mäule reaction and microscopy - Paper II

In order to assess the anatomical features of *Betula pubescens*, 20 µm transverse and radial sections of sapwood were prepared. Sectioning was conducted by the Centre for Cellular Imaging (CCI) at the University of Gothenburg with the aid of a vibratome.

The sections were stained as described by Yamashita *et al*. (2016) to differentiate tissues with high (purple-red) and low (brown) content of syringyl lignin. In short, the sections were treated for 5 minutes with $KMnO_4$ (1%, w/v). Next, they were washed with distilled water and treated with HCl (1 M) for 30 minutes. Then, after a final washing step with destilled water, the sections were mounted in glass slides and color was generated by adding a few drops of 1 M Tris-HCl buffer (pH 8). Finally, the samples were observed under white light (light microscope Axio Imager Z2m, Zeiss).

4 Results and Discussion

In this chapter, the main findings unveiled in the study are presented and discussed. The first sections (4.1-4.2) focus on the results reported in Paper I, while the last ones (4.3-4.5) cover the results from Paper II.

4.1 Impregnation liquors and the impact of their composition on pulping

In Paper I, the impact of using active ions (hydroxide and bisulfide) in the impregnation liquor was investigated, as well as the influence of advection and diffusion during pulping. In addition, the effect of increasing sodium concentration in the impregnation liquor was examined. Four different impregnation liquors were tested (white liquor – WL, water – W, white liquor supplemented with NaCl – WLNa, and aqueous solution of NaCl – WNa), as described in Section 3.4.1.
The contents of Klason lignin and xylan determined in the impregnated wood chips throughout kraft cooking are shown in Figure 4.1.

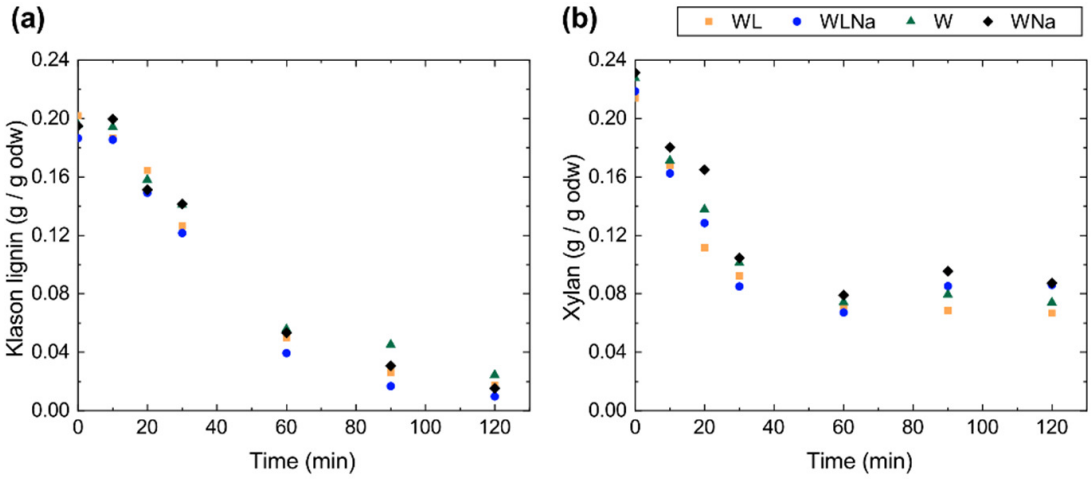

Figure 4.1. Composition of the pulp samples collected throughout cooking in g/g of dry wood: (a) Klason lignin, (b) xylan. Values at 0 min represent samples analyzed after impregnation. WL = samples impregnated with white liquor, WLNa = samples impregnated using white liquor supplemented with NaCl, W = samples impregnated with water, WNa = samples impregnated with NaCl solution.

Based on these delignification profiles, it was only possible to identify a minor influence of the different impregnation liquors over lignin removal (at the investigated conditions). Still, the extent of delignification in samples

impregnated without using active ions (samples treated with water and NaCl solution) was in general lower than in samples impregnated with white liquor (with or without addition of NaCl). Hence, the initial content of hydroxide and bisulfide transported via the advection of liquor during impregnation had a significant, albeit small, effect on the progress of the cooking process. The samples that relied exclusively on the transport of active ions via diffusion lacked the "memory effect" caused by the initial advection, i.e., their delignification profiles were delayed in comparison to the samples impregnated with white liquor, as there were no cooking chemicals readily available in the center of the chips in the beginning of pulping.

The experiments also revealed that, within the evaluated range, increasing the content of sodium utilized in the impregnation liquors had no significant impact over the delignification of samples impregnated without hydroxide and bisulfide ions. Nevertheless, when the active ions were present, the addition of extra salt resulted in slightly increased removal of lignin, indicating a possible shift in Donnan equilibrium. According to the Donnan equilibrium theory, during pulping the ionization of acidic groups and phenols creates a negative charge in the cell walls, which in turn decreases the local concentration of hydroxide and bisulfide close to the fibers. The addition of extra charged species in the liquor can therefore change the balance between charges and minimize this effect (Nieminen *et al*., 2014), resulting in the increase of delignification.

Still regarding the addition of extra sodium to the system, it is worth mentioning that the opposite behavior was reported when utilizing higher concentrations of salt than the ones applied in this study (Dang *et al*., 2013; Brännvall and Rönnols, 2021). In these cases, the decrease in delignification has been hypothesized to be associated with a decrease in lignin solubility caused by the excess of sodium, overshadowing the effect of the extra charges on the Donnan equilibrium.

The trends concerning xylan removal (Figure 4.1b) were similar to the ones observed for lignin. The use of impregnation liquors containing hydroxide and bisulfide intensified xylan removal during cooking, whereas the increase in sodium content had no clear effect. Nevertheless, the profiles of delignification and xylan removal had a few differences. The maximum rate of delignification occurred around 30-60 minutes and the content of lignin continued to decline even after 120 minutes of pulping. Xylan, on the other hand, showed high rates of removal since the start of pulping, but after 30 minutes a pronounced decrease in xylan extraction was observed, with minor changes in concentration being detected in pulps collected after 60 minutes. Thus, a large portion of xylan was removed before reaching the final cooking temperature, which agrees with the fact that a substantial part of the xylan

chains is removed by dissolution. The rates of carbohydrate reactions (such as peeling) are also significantly higher than delignification at temperatures below 140°C and, thus, could have contributed to xylan removal (albeit, to a lesser extent). Moreover, the marginal changes in xylan content taking place after 60 minutes of pulping suggested that the fraction of xylan that remains in the pulp is less susceptible to the cooking conditions. Such behavior has been ascribed to the association between xylan and cellulose (Dammström *et al.*, 2009; Gomes *et al.*, 2020), which may be a result of the natural location of xylan in the lignocellulosic network, or it can be a consequence of changes in accessibility due to modifications in the native structure of xylan.

4.2 Differences in the dissolved material present in the bulk and pore fractions of black liquor

The same experiments utilized to evaluate the impact of the composition of impregnation liquors over pulping were also used to collect black liquor samples for subsequent characterization. Different fractions of black liquor were considered: bulk liquor (collected via filtration) and centrifuged liquor, which was assumed to represent the black liquor contained within the pore system of wood. The composition of the two fractions was analyzed throughout pulping to check if the use of different liquors during impregnation led to any significant differences between the samples. The concentrations of lignin and xylan in these black liquor samples are presented in Figure 4.2.

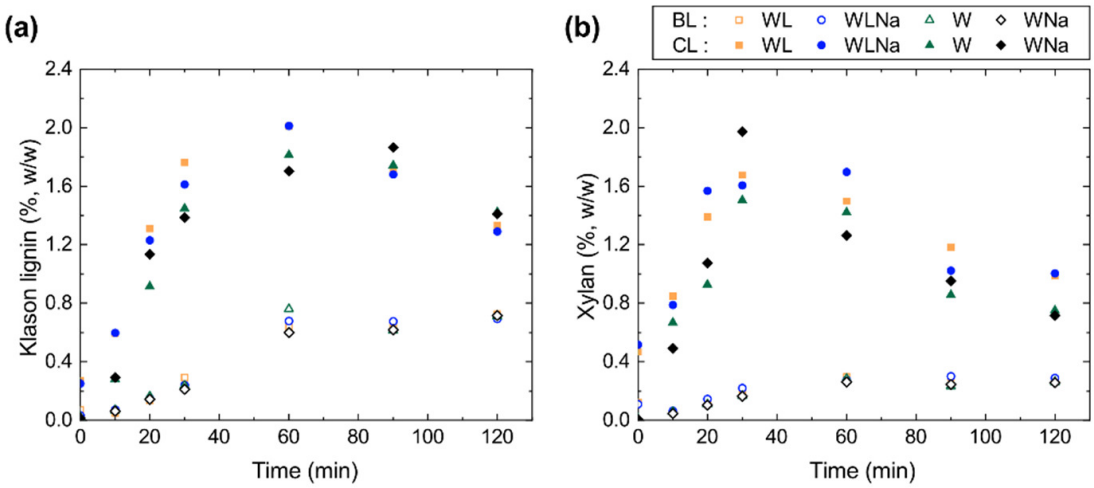

Figure 4.2. Concentration of Klason lignin (a) and xylan (b) in bulk (BL) and centrifuged (CL) black liquor samples collected during cooking. Values at 0 min represent the spent impregnation liquor. WL = impregnation with white liquor, WLNa = impregnation with white liquor + NaCl, W = impregnation with water, WNa = impregnation with NaCl solution.

While there was only a minor influence of the liquors utilized during impregnation over the composition of the black liquor fractions, both the lignin and the xylan profiles in Figure 4.2 showed striking differences between the bulk and the centrifuged liquors. The concentration of dissolved material in the bulk liquor increased continuously during the process, but it remained substantially lower than the one measured in the centrifuged liquor. In contrast, the composition of the centrifuged liquor was deeply affected by the balance between the rates of removal of wood components from the cell wall and the rates of transport of the dissolved material from the pore system to the bulk liquor.

Such behavior has also been reported in literature (Egas *et al.*, 2002; Simão *et al.*, 2011; Brännvall and Rönnols, 2021). At the beginning of pulping, the high rates of xylan removal and the increasing rates of delignification were faster than the rate of diffusion of the dissolved components towards the bulk liquor, causing the build-up of concentration seen in the centrifuged liquor. However, as pulping progressed, the rates of material being released from the cell walls decreased and, at the same time, microstructural changes in the wood may have contributed to increase the rates of transport inside the chip. Eventually, the rate of material leaving the pore system surpassed the rate of wood components being removed from the cell walls and, thus, the concentration of lignin and xylan in the centrifuged liquor decreased.

Besides the distinct concentration profiles, the bulk and centrifuged liquors also differed with regard to the structure of the dissolved lignin. To illustrate these differences, Figure 4.3 presents the molecular weight distribution (MWD) of precipitate material from the two liquor fractions after 30 and 90 minutes of pulping.

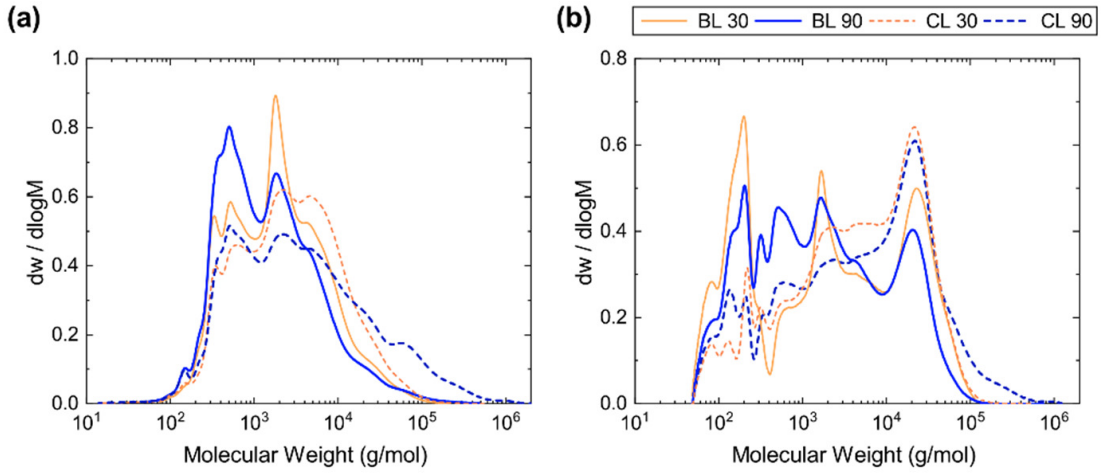

Figure 4.3. Molecular weight distribution of the material precipitated from bulk (BL) and centrifuged (CL) liquors, sampled after 30 and 90 min of pulping. Results based on (a) UV detection and (b) RI detection.

The data acquired through UV detection (Figure 4.3a) indicated the MWD of lignin in the different samples. By comparing the results of the material precipitated from the centrifuged liquors (dashed data) with the results achieved using samples precipitated from the bulk liquor (solid lines), it was evident that the former had higher average molecular weights (Mw). Moreover, while the portion of fragments with high molecular weight (> 10 kDa) increased with time in the centrifuged liquor, the MWD shifted towards low molecular weights for the samples isolated from the bulk liquor.

These results suggested that different phenomena played significant roles in each fraction of the liquor. In the bulk, the high percentage of low molecular weight lignin hinted at small lignin fragments being transported out of the pore system of the chips faster than large fragments. In addition, the decrease in Mw of lignin with time indicated that further degradation of the dissolved fragments was significant in the bulk liquor. These continuous reactions between dissolved lignin and cooking chemicals were supported by HSQC NMR results, as some of the chemical shifts associated with lignin (C_α in β-O-4 structures in particular) vanished in the spectra of the bulk sample after 90 minutes of pulping.

In the pore system, further degradation of the dissolved lignin was not as striking, which is reasonable given that lower concentrations of hydroxide and bisulfide ions are expected inside the chips (when compared to the bulk). Also, the fragments of lignin in the pore system were more recently released from the cell walls than the ones in the bulk (and, consequently, less degraded). The increase in the percentage of high molecular weight lignin with time can be explained by the accumulation of large fragments (since they require more time to be transported out) and by a likely increase in the molecular weight of the lignin being released from the cell walls as pulping advances.

The same trends were observed when assessing the data measured with the RI detector (Figure 4.3b), with additional information about carbohydrates. The data at low molecular weight (around 200 Da) indicated the presence of degradation products – probably precipitated together with lignin or formed during the precipitation procedure. The high molecular weight fraction (around 20 kDa) was related to a portion of the dissolved xylan that ended up precipitating (as corroborated by the chemical shifts detected via HSQC NMR). Notably, the peak associated to xylan shifted to lower molecular weights in the bulk sample after 90 minutes of cook, while no significant changes were seen in the other samples, reinforcing the notion that the material in the bulk was subjected to further degradation as time went by.

4.3 Effect of kraft cooking conditions: xylan retention in model chips

After investigating the differences between bulk and pore system in Paper I and recognizing the significant interplay between the rates of reaction and transport of ions and dissolved wood components during cooking, Paper II was focused on studying the local compositional changes in wood chips during kraft pulping.

In order to investigate how xylan removal progresses within wood chips, and how the final retention of xylan is affected by process parameters (namely temperature and hydroxide content), model wood chips were treated under different cooking conditions. The distribution of xylan inside these chips after 45 min of kraft pulping is displayed in Figure 4.4.

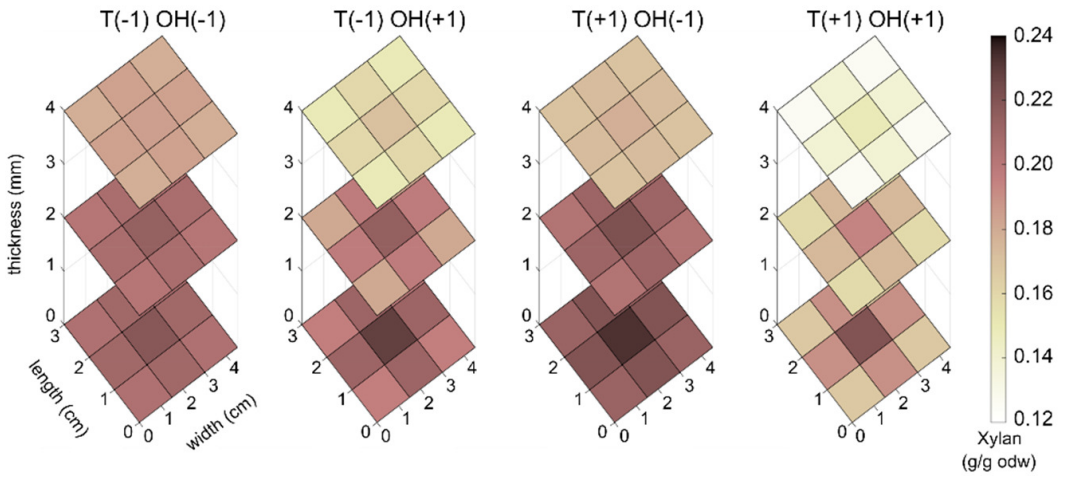

Figure 4.4. Distribution of xylan (g/g of dry wood) in model wood chips after 45 min of batch kraft cooking. Cooking conditions from left to right: 145°C & 0.25 mol OH^-/kg liq., 145°C & 0.55 mol OH^-/kg liq., 165°C & 0.25 mol OH^-/kg liq., 165°C & 0.55 mol OH^-/kg liq. Estimated error = 4%.

The measured profiles indicated a significant increase in xylan removal when using higher concentrations of hydroxide, which agrees with literature data regarding alkaline extraction of xylan (Lehuedé *et al.*, 2023). Previous tests focused on hemicellulose extraction (Jun *et al.*, 2012) also show that xylan dissolution should increase with temperature. Yet, the experiments conducted at high temperature in Figure 4.4 only led to higher xylan removal when the model chips were treated with high alkali content (0.55 mol OH^-/kg liq.).

The low removal of xylan observed when cooking at high temperature using low hydroxide concentration is explained by the balance between the

consumption (strongly promoted by the temperature increase) and the transport of hydroxide ions in the system. In the initial stages of cooking, fast alkali-consuming reactions decrease the concentration of OH^- in the cell walls and pore system of wood. As pulping advances, the local content of alkali inside the chips depends on how much hydroxide remained after the initial intake of ions, the rate of transport of OH^- from the bulk liquor towards the center of the chips, and the rate of hydroxide consumption. Thus, the use of high temperatures (and consequently high rates of alkali consumption) when cooking with low alkali (0.25 mol OH^-/kg liq.) probably led to low concentrations of hydroxide inside the model chips, reducing xylan removal.

The structure of the residual xylan was also investigated: xylan was isolated from untreated sapwood and from different layers of the cooked chips (refer to the procedures in Sections 3.8 and 3.9) and then analyzed using gel permeation chromatography and HSQC NMR. When comparing the material isolated from the cell walls of cooked samples with native xylan (isolated from birch sapwood), the latter had higher average molecular weight (around twice the value measured in the cooked samples). In addition, the acetyl groups (C2 and C3 positions) that were present in the HSQC spectra of the native material were missing in the samples of residual xylan. Therefore, the structural changes in xylan during pulping were likely a result of deacetylation and peeling.

The results also showed that the samples of residual xylan isolated from chips after 45 minutes of pulping had similar molecular weight distributions, regardless of cooking conditions. Furthermore, these samples displayed the same chemical shifts in the NMR spectra. Hence, the temperature and hydroxide concentration utilized during cooking appear to have had little impact over the structure of residual xylan in the cell walls (within the range of conditions investigated in this study).

However, it is important to highlight that the results attained using isolated xylan may not be representative of all the xylan present in the samples, as the methodology utilized for isolation leads to low yields (Corradini *et al.*, 2018). Nevertheless, the trends observed in the molecular weight distributions agree with data acquired analyzing holocellulose samples (refer to Appendix – A4) and with results reported for xylan in birch kraft pulps (Westermark and Gustafsson, 1994; Rosa-Sibakov *et al.*, 2016).

Finally, to evaluate the retention and structure of xylan in different points of the cooking process, the contents of xylan in samples treated at 145°C and at 165°C were compared over time (until the samples started to defibrate). Two sections were monitored: the corner of the outer layer (region highly exposed to the cooking liquor) and the center of the inner layer (least

accessible region). Figure 4.5 shows the retention profiles and the molecular weight distribution of the residual xylan.

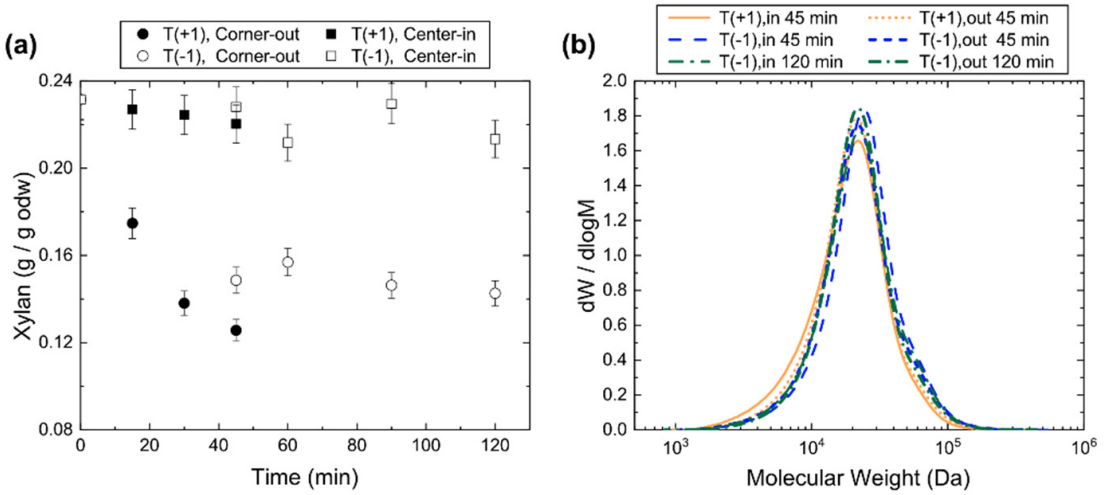

Figure 4.5. Comparison between xylan content after kraft cooking at T(-1) = 145°C and at T(+1) = 165°C (hydroxide content in both cases: 0.55 mol/kg liq.). (a) Xylan retention over time in the corner of the outer layer (Corner-out) and in the center of the inner layer (Center-in). Estimated error = 4%. (b) Molecular weight distribution of isolated xylan from the inner and outer layers of chips cooked for 45 and 120 minutes. Isolation was carried out via delignification with peracetic acid followed by extraction with DMSO (50°C/ 20 h).

The results indicated that temperature had no major impact over the uniformity of xylan removal inside the chips: samples cooked at 145°C and at 165°C displayed similar gradients of xylan when defibration started. In addition, most of the xylan lost during the pulping experiments was removed in the beginning of the process (< 45 min), which corroborates the results attained in Paper I (using industrially cut chips) and is in line with the hypothesis of xylan fractions with different accessibilities (Dammström *et al.*, 2009; Gomes *et al.*, 2020).

Moreover, the molecular weight distributions of the residual xylan were almost identical for all samples, even when comparing chips cooked for 45 and 120 minutes. This finding suggests that, after the first minutes of pulping, reactions such as peeling and alkaline hydrolysis had a negligible effect on xylan (under the cooking conditions investigated). Yet, there are results in literature (Pinto *et al.*, 2005a) in which the molecular weight of residual birch xylan was shown to decrease with pulping time. Thus, it is possible that further changes in xylan structure could occur as the fibers become more exposed to the cooking chemicals (by defibration, for example).

4.4 Effect of kraft cooking conditions: delignification of model chips

The evolution of delignification within the model wood chips was also investigated. Figure 4.6 presents the distribution of Klason lignin in samples cooked for 45 minutes under different conditions (145°C or 165°C using 0.25 or 0.55 mol OH^-/kg liq.).

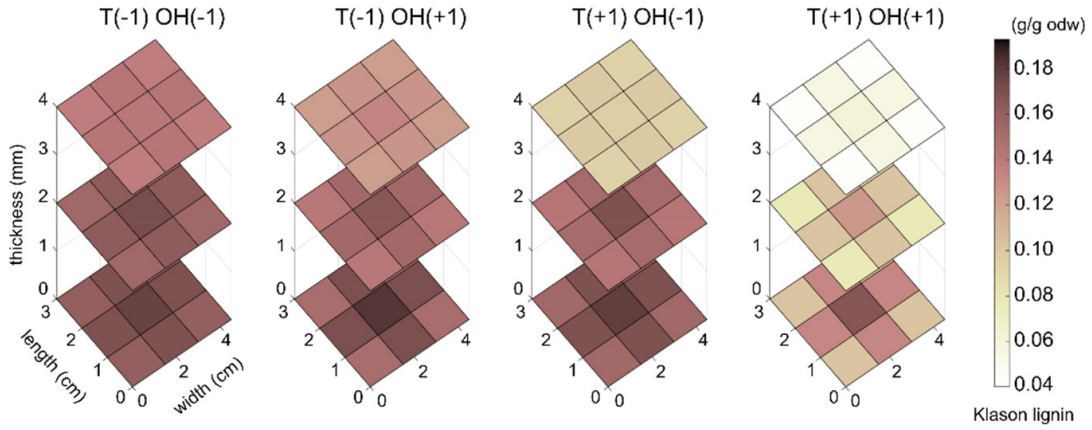

Figure 4.6. Distribution of Klason lignin (g/g of dry wood) in model wood chips after 45 min of batch kraft cooking. Cooking conditions from left to right: 145°C & 0.25 mol OH^-/kg liq., 145°C & 0.55 mol OH^-/kg liq., 165°C & 0.25 mol OH^-/kg liq., 165°C & 0.55 mol OH^-/kg liq. Estimated error = 5%.

When pulping was conducted at 145°C, the slow rates of reaction resulted in high residual lignin contents in the chips. The lignin removal was slightly more pronounced in the outer layer, which was expected given the decrease in alkali concentration in the center of the chips. The outer layer was also the most affected by the change in concentration of hydroxide ions in the cooking liquor (reinforcing the existence of a significant gradient of OH^- inside the chips).

Delignification was substantially more intense when cooking at 165°C. Still, the content of lignin in the inner layers of the sample treated with low alkali was comparable to the amounts measured when pulping occurred at 145°C. This behavior suggested that, in order to promote fast delignification of the whole chip, the increased consumption rate of OH^- (caused by the increase in the rates of reaction) must be balanced by the concentration of hydroxide ions available in the system. Such was the case with sample T(+1)OH(+1): the use of high alkali content in the white liquor probably prevented reaching extremely low concentrations of hydroxide inside the chips during cooking – leading to substantial removal of lignin even in the inner layers.

The gradient of lignin inside the samples throughout pulping was further investigated by comparing the content of Klason lignin in two different sections of the chips (the center of the inner layer and the corner of the outer layer). Figure 4.7 summarizes the results measured in samples cooked using different conditions (low vs. high hydroxide concentration and low vs. high temperature).

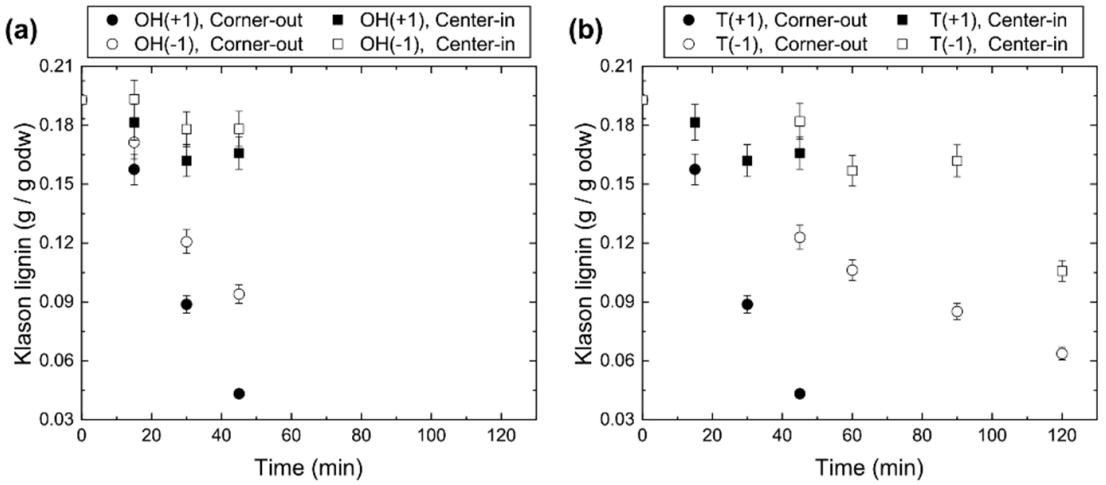

Figure 4.7. Klason lignin content over time at the corner of the outer layer (Corner-out) and at the center of the inner layer (Center-in) in chips cooked under different conditions: (a) comparison between utilizing high and low concentrations of hydroxide (165°C & 0.25 mol OH^-/kg liq. vs. 165°C & 0.55 mol OH^-/kg liq); (b) comparison between utilizing high and low temperatures (145°C & 0.55 mol OH^-/kg liq. vs. 165°C & 0.55 mol OH^-/kg liq). Estimated error = 5%.

The lignin profiles indicated that temperature played an important role in promoting uniform delignification of the chips. The same was not observed when analyzing the effects of changes in hydroxide concentration: when the alkali content was increased, a decrease in residual lignin was measured both in the inner and in the outer layers of the chips (as the available alkali content increased in both regions). Hence, while high hydroxide content increased the extent of delignification, the gradient of lignin inside the chips remained substantial (when defibration began the lignin content in the center was about four times higher than in the surface).

When low temperature was used during pulping, the difference between the rates of reaction and transport inside the chips decreased, likely reducing the gradient of active ions and dissolved lignin within the pore system, culminating in a more uniform removal of lignin from the chip. Therefore, the data showed the potential of utilizing low kraft cooking temperatures to reduce the incidence of shives and rejects during pulping.

4.5 Effect of the anisotropic nature of wood on kraft pulping

Another aspect studied during the experiments with model wood chips involved the influence of wood structure on kraft pulping. The complex microstructure that originates the wood tissue comprises different kinds of cells, with distinct characteristics and distribution, resulting in a material with non-uniform properties. Figure 4.8 displays the microstructure of the wood used to make the model wood chips.

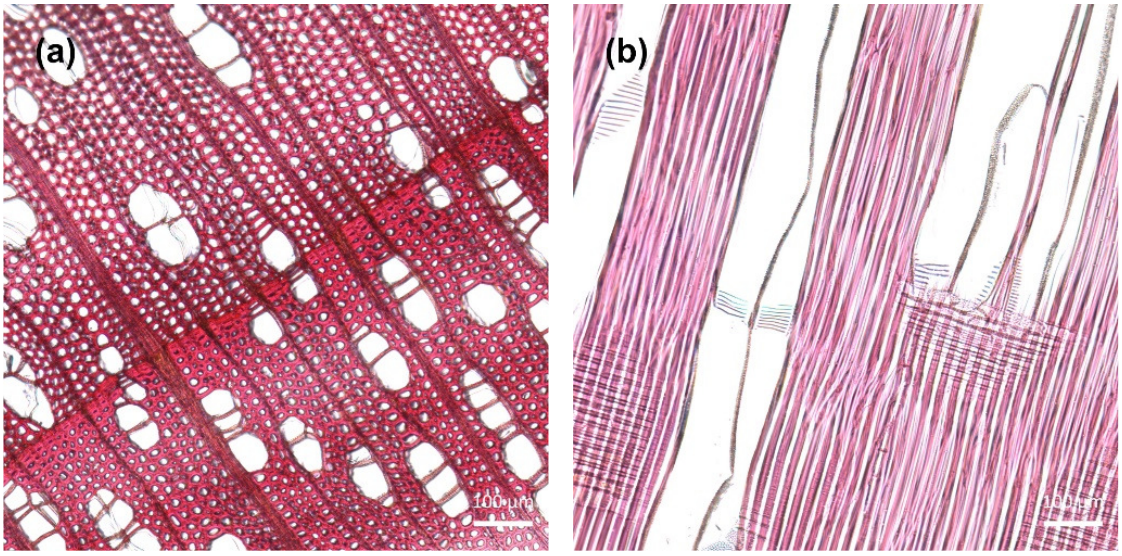

Figure 4.8. Transverse (a) and radial (b) sections of birch sapwood photographed under white light. The wood can be classified as diffuse-porous. The vessels are small (< 80 µm) and occur isolated or arranged in multiples. The radial section highlights the differences between the purple-red cell walls in fibers (high S/G ratio) and the brown walls of vessels (low S/G ratio).

The impact of wood morphology during pulping was evaluated by comparing delignification and xylan removal in different lateral sections of the chips. The first section (Side I) had one transverse surface exposed to the bulk liquor (i.e., the main concentration gradients occurred longitudinally), the second one (Side II) had a tangential surface exposed to the bulk (i.e., main gradients occurred tangentially) – more details about the sectioning procedure are explained in Figure 3.3.
Figure 4.9 presents the composition of these sections in terms of residual Klason lignin and xylan.

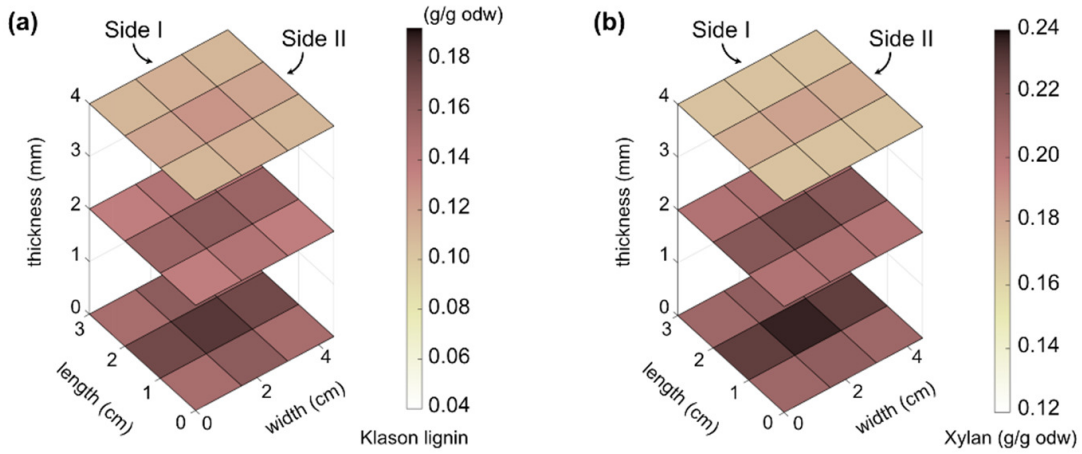

Figure 4.9. Average chemical composition (g/g of dry wood) of the wood chip sections attained after kraft cooking experiments performed in triplicates. Cooking conditions: 45 minutes of pulping at 155°C & 0.40 mol OH^-/kg liq. (a) Lignin content, error = 5%. (b) Xylan content, error = 4%.

The results exposed a significant difference in the composition of the lateral sections, with the extents of delignification and xylan removal in Side I being comparable to the ones in the corners of the chips. This behavior was probably a result of the lower resistance to mass transport in the longitudinal direction when compared to the tangential one, as diffusion and liquor penetration are aided by the vessels and the lumen of fibers (as opposed to relying solely on the pits of the cell walls). Thus, besides illustrating the impact of the morphological features of wood during pulping, the results also reinforced how mass transport can influence the local extent of lignin and xylan removal inside the wood chips.

5 Conclusion

5.1 Concluding remarks

The experiments conducted in this work investigated the mechanisms affecting delignification and xylan removal during kraft pulping of birch. In Paper I, the initial advection of liquor (and, consequently, hydroxide and bisulfide ions) during impregnation was shown to improve delignification during cooking, demonstrating how the availability of cooking chemicals inside the chips influences the pulping process. In addition, the results indicated that xylan removal was faster than delignification, but unlike lignin, the xylan content in the pulp samples was only mildly affected after the first stages of pulping, suggesting that a fraction of xylan was or became difficult to remove under the studied cooking conditions.

Still in Paper I, differences in the content of dissolved wood components were observed between bulk and pore black liquor samples. The concentrations of lignin and xylan in the liquor collected from the pore system were always higher than the ones measured in the bulk, moreover, their changes over time were distinct: while the concentrations of lignin and xylan increased continuously with time in the bulk liquor, they reached a maximum in the pore system and then decreased with time. Thus, the data showed the existence of a significant mass transfer resistance inside the wood chips (both in the cell walls and pore system), which was reinforced by the increased concentration of high molecular weight material in the pore liquor compared to the bulk.

As Paper I evidenced the existence of distinct characteristics between bulk and pore liquor during kraft cooking of industrially cut wood chips, Paper II focused on assessing how these local differences affected the progress of pulping in different regions of the chips. The experiments with model chips suggested that the availability of hydroxide ions inside the pore system was a key factor determining the extent of delignification and xylan removal, which depended on the hydroxide content in the bulk liquor and the cooking temperature. In addition, wood morphology was shown to influence the local removal of lignin and xylan, with higher rates of removal being measured along the longitudinal direction of the chips than in the radial and tangential directions.

Paper II also pointed out that changes in the native structure of xylan occurred early in the process (mostly due to deacetylation and a certain extent of peeling), with negligible alterations being detected as cooking progressed. Thus, the different temperatures and hydroxide contents utilized in the

experiments eventually led to the same extent of structural changes in xylan. Furthermore, the study revealed that the removal of xylan decreased with time, corroborating the findings from Paper I.
When considering the delignification behavior, the experiments revealed that lower cooking temperatures can potentially lead to less rejects and shives, as lignin was more evenly distributed inside the chips when cooking was carried out at 145°C.

5.2 Future work

Papers I and II indicated how the local chemical environment in the pore system of wood chips can affect delignification and xylan removal during kraft pulping and highlighted the importance of the transport of ions and dissolved wood components between cell walls and bulk liquor. Therefore, there are many research questions that can be further explored in order to complement and expand the work developed so far. For instance, to support the discussion developed in Papers I and II, it would be interesting to measure the alkali content in the pore liquor, or even the hydroxide concentration profile, in the case of the model chips.
Alternatively, in order to expand the study, a more extensive set of experiments regarding the impact of ionic strength on pulping could be developed to broaden the results discussed in Paper I, for example by considering different salts and a wide range of concentrations, not only in the impregnation liquor, but in the cooking liquor as well. Another option is expanding the analysis developed in Paper II regarding the impact of wood morphology: a more thorough examination can be conducted by comparing wood species with distinct anatomical features (e.g., different size of vessels). Also, instead of focusing solely on the composition of the chips, experiments assessing the microstructural changes within wood during pulping could provide a more detailed picture of how delignification progresses inside the chips.

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Appendix

A.1 Repeatability of cooking experiments

In order to estimate the repeatability of the cooking experiments with model chips, three independent cooks were performed using center point conditions (155°C and 0.40 mol OH^-/kg liq. for 45 min). The detailed results, including the averages ($\bar{x}$) ± deviations (σ) and residual standard deviations (RSD) are presented in Tables A.1 and A.2.

Table A.1. Average distribution of lignin in model chips subjected to the same cooking conditions (center point). Results based on measurements conducted in three independent samples.[1]

Section	Klason lignin		ASL	
	$\bar{x} \pm \sigma$ (g/g odw)	RSD (%)	$\bar{x} \pm \sigma$ (g/g odw)	RSD (%)
C-C	0.181 ± 0.004	2.19	0.054 ± 0.002	3.60
C-I	0.164 ± 0.008	4.88	0.047 ± 0.002	4.84
C-O	0.126 ± 0.002	1.69	0.036 ± 0.002	4.80
S II-C	0.173 ± 0.003	2.02	0.051 ± 0.003	6.32
S II-I	0.156 ± 0.003	2.18	0.045 ± 0.002	5.28
S II-O	0.119 ± 0.003	2.72	0.035 ± 0.002	5.21
S I-C	0.163 ± 0.003	1.83	0.044 ± 0.003	7.14
S I-I	0.145 ± 0.005	3.74	0.041 ± 0.003	8.08
S I-O	0.114 ± 0.004	3.44	0.032 ± 0.002	5.80
Cor-C	0.150 ± 0.004	2.57	0.045 ± 0.003	7.60
Cor-I	0.137 ± 0.002	1.78	0.041 ± 0.002	5.96
Cor-O	0.110 ± 0.002	1.46	0.034 ± 0.002	5.05

[1]The results are presented for each of the following sections: center piece - inner layer (C-C), center piece -intermediate layer (C-I), center piece - outer layer (C-O), side piece II - inner layer (S II-C), side piece II - intermediate layer (S II-I), side piece II - outer layer (S II-O), side piece I - inner layer (S I-C), side piece I - intermediate layer (S I-I), side piece I - outer layer (S I-O), corner piece - inner layer (Cor-C), corner piece - intermediate layer (Cor-I), corner piece - outer layer (Cor-O).

Table A.2. Average distribution of glucan and xylan in model chips subjected to the same cooking conditions (center point). Results based on measurements conducted in three independent samples.[1]

Section	Glucan		Xylan	
	$\bar{x} \pm \sigma$ (%, w/w)	RSD (%)	$\bar{x} \pm \sigma$ (g/g odw)	RSD (%)
C-C	40.30 ± 0.24	0.60	0.236 ± 0.003	1.24
C-I	43.77 ± 1.71	3.91	0.225 ± 0.004	1.97
C-O	48.61 ± 2.30	4.74	0.182 ± 0.005	2.50
S II-C	42.07 ± 0.58	1.37	0.229 ± 0.003	1.22
S II-I	44.73 ± 0.53	1.18	0.218 ± 0.002	0.97
S II-O	51.41 ± 0.58	1.14	0.179 ± 0.001	0.64
S I-C	44.16 ± 0.70	1.60	0.216 ± 0.004	2.01
S I-I	46.89 ± 1.19	2.53	0.205 ± 0.004	1.76
S I-O	52.89 ± 0.73	1.39	0.170 ± 0.001	0.72
Cor-C	45.57 ± 0.42	0.92	0.210 ± 0.003	1.67
Cor-I	47.60 ± 0.49	1.02	0.203 ± 0.003	1.37
Cor-O	53.36 ± 0.47	0.88	0.170 ± 0.007	3.85

[1]The results are presented for each of the following sections: center piece - inner layer (C-C), center piece -intermediate layer (C-I), center piece - outer layer (C-O), side piece II - inner layer (S II-C), side piece II - intermediate layer (S II-I), side piece II - outer layer (S II-O), side piece I - inner layer (S I-C), side piece I - intermediate layer (S I-I), side piece I - outer layer (S I-O), corner piece - inner layer (Cor-C), corner piece - intermediate layer (Cor-I), corner piece - outer layer (Cor-O).

To estimate the repeatability during the determination of each component in the cooked chips, the highest RSD value in Tables A.1 and A.2 was considered. Hence, the estimated errors are: 5% for Klason lignin measurements, 8% for acid-soluble lignin, 5% for glucan and 4% for xylan.

A.2 Determination of extractives in pulp

A preliminary test was conducted with different sections of cooked model chips in order to assess the impact of not removing extractives prior to the acidic hydrolysis step in the Klason lignin procedure. To simulate the most critical scenario, heartwood chips with 16 mm of thickness were used (which should lead to steep gradients in extractives between the different layers of the chip). Cooking was carried out at 155°C, using a liquor:wood ratio of 22. The hydroxide and hydrogen sulfide concentrations were 0.4 mol/kg liquor and 0.1 mol/kg liquor, respectively.

Extraction procedure:
Pulp samples from different parts of model chips (Cor-O, corner of outer layer, and C-C, center of inner layer) were ground (particle size < 1 mm) and dried in an oven (105°C) overnight. Then, Soxhlet extraction was performed with acetone (0,5-1,0 g of dry sample / 160 mL acetone). The temperature was set to achieve 24 extractions / 4,5 h. The solid residue was dried at 40°C for 24 h and the mass was determined.
Afterwards the material was subjected to Klason lignin and carbohydrates quantification. The results were compared to the ones attained with cooked samples without prior extraction.

Results:
Table A.3 displays the content of extractives in the two different sections of the model cooked chips and Tables A.4 and A.5 show the measured composition of the samples in comparison to the results attained without performing extraction.

Table A.3. Content of extractives in different sections of cooked model chips.

Section[1]	Extractives in the section (%, w/w)
Cor-O	1.02%
C-C	1.88%

[1]Sections: Cor-O = corner piece - outer layer, C-C = center piece - inner layer.

Table A.4. Comparison between the Klason lignin content in cooked samples with and without prior extraction with acetone.[1]

Section[2]	μ (%, w/w)	σ (%, w/w)	RSD (%)
Cor-O, Extracted	16.14	0.34	2.13
Cor-O	16.89	0.06	0.38
C-C, Extracted	19.13	0.12	0.60
C-C	20.36	0.32	1.56

[1]μ = average content, σ = standard deviation between duplicates, RSD = residual standard deviation. [2]Sections: Cor-O = corner piece - outer layer, C-C = center piece - inner layer.

Table A.5. Comparison between the content of carbohydrates (%, w) in cooked samples with and without prior extraction with acetone. Measurement error: 0.05% < RSD (%) < 1.50%.

Section[1]	Arabinan (%)	Galactan (%)	Glucan (%)	Xylan (%)	Mannan (%)
Cor-O, Extracted	0.28	0.43	49.27	23.15	-
Cor-O	0.29	0.45	49.69	23.52	-
C-C, Extracted	0.34	0.66	39.64	25.02	1.40
C-C	0.34	0.66	39.32	24.79	1.35

[1]Sections: Cor-O = corner piece - outer layer, C-C = center piece - inner layer.

The concentration of extractives in the outer layer of the cooked samples is lower than in the inner layer by approximately 46%, which is expected given the higher exposure to the cooking liquor. Moreover, the measured values agree with the reported content of extractives in birch pulp.

When focusing on the Klason lignin measurements, the results attained after extraction were 4.5% lower for the sample taken from the outer layer and 6.0% lower for the sample from the inner layer (when compared to samples not subjected to extraction). Thus, the results seem to be affected to similar extents throughout the whole wood chip. Therefore, not performing the extraction procedure will likely result in an overestimation of the Klason lignin content, but it does not seem to compromise the comparison between the different sections / layers.

In the carbohydrates measurements (Table A.5), no major trends were observed regarding the impact of removing the extractives prior to the analysis. For the sample taken from the outer layer, the extracted material

had slightly lower content of sugars, but the same was not seen with the sample from the inner layer. Thus, it seems like the effect of the extractives (if present) is overshadowed by the experimental error.

Conclusion:
It appears that not performing the extraction prior to the Klason / carbohydrates analyses will result in deceptively high Klason lignin values, however, the extent of the effect seems to be fairly similar for different sections of the wood chip. No clear impact was seen regarding the quantification of sugars. Hence, for the purposes of the present study, the gain in accuracy provided by the extraction step is eclipsed by the time it demands.

A.3 Xylan isolation via alkaline extraction

Methodology: In the experiments conducted in Paper II, after the holocellulose samples were extracted with DMSO, the solid residue was washed with distilled water and collected for further extraction with sodium hydroxide. The procedure was based on the methodology described by Corradine *et al*. (2018). In short, 30 mL of NaOH 1 M were added to the washed solid. Then, the system was left at 25°C under agitation (300 rpm) for 20 h. The extract was separated from the solid residue by vacuum filtration in a glass microfiber filter (Whatman GF/A 55 mm, Cytiva). About 25 mL of distilled water were used to wash the solid retained in the filter.
To precipitate xylan, ethanol was added to the filtrate (2:1, v) together with enough acetic acid to reach pH 5. Next, the system was kept at 4°C overnight, followed by centrifugation at 4500 rpm / 30 min / 4°C (Heraeus Megafuge 40R, Thermo Scientific). The isolated xylan was freeze-dried (FreeZone Triad benchtop freeze dryer, Labconco) and stored in a desiccator. Xylan recovery (based on samples from holocellulose of untreated sapwood): 40%.

Limitation: despite the good xylan recovery achieved via alkaline extraction, the samples displayed poor solubility in DMSO and water, which compromised the analysis of molecular weight distribution.

A.4 Molecular weight distribution (MWD) of holocellulose samples

The MWD measurements of holocellulose samples aimed to assess if cellulose was significantly depolymerized/degraded during the experiments. In addition, this analysis served as a complementary test to the MWD measurements of isolated xylan. Figure A.1. presents the results.

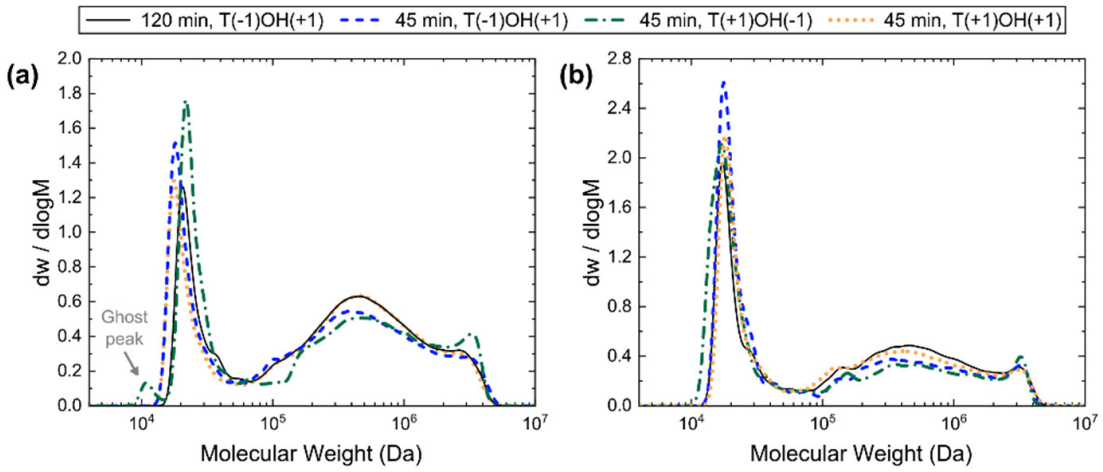

Figure A.1. Molecular weight distribution of holocellulose samples from pulps treated at 145°C & 0.55 mol OH^-/kg liq. (for 45 and 120 min.), at 165°C & 0.25 mol OH^-/kg liq. (for 45 min.) and at 165°C & 0.55 mol OH^-/kg liq. (for 45 min.). All samples were subjected to peracetic acid delignification (25°C/ 48 h) to remove the residual lignin. (a) Results observed in the outer layer of the chips, with all three regions (corner, side and center) combined. (b) Results observed in the inner layer of the chips, with all three regions (corner, side and center) combined.

The two fairly resolved distributions present in the MWD plots can be associated to xylan (lower molecular weight range) and cellulose (higher molecular weight range). Still, no attempt to quantify/compare the average molecular weights is made, especially because the dn/dc value used for the measurements (0.136 mL/g) is only suitable for pure cellulose. Instead, the results are used as qualitative trends regarding the characteristics of the different holocellulose samples. In general, similar MWD was observed in samples treated under different pulping conditions. In addition, no major differences were seen between the behavior of samples taken from inner and outer layers of the chips. Both results agree with the overall trends observed when analyzing isolated xylan. Furthermore, the data suggests no significant differences in the cellulose chains, even when comparing samples cooked for different times (145°C for 45 or 120 min).