THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Pre-Extraction of Wood Components

Mild hydrothermal methods for a future materials biorefinery

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Abstract

Today there is an increasing urgency in discovering new, and more sustainable, replacements for fossil-based materials and chemicals. The biorefinery concept is promising in this context in that it relies on transforming biomass into a spectrum of commercial products. Wood is the primary bioresource in Scandinavia. It is mostly processed in the kraft pulping process used in the huge and important pulp and paper industry, thereby providing a suitable platform for future material biorefineries. This process allows for the efficient separation of lignin and cellulose, along with the option of adding a pretreatment step for the recovery of hemicelluloses.

Among existing pretreatments, mild hydrothermal methods, such as hot water extraction and steam explosion, are promising since they allow recovery of hemicelluloses with minimal degradation. They are based on the acidic hydrolysis (autohydrolysis) of wood components and diffusive transport through the wood tissue. In steam explosion, the latter is enhanced by the advective transport in the pore system of the wood caused by the rapid release of the pressure. The focus of this work was to explore the possibility of using steam explosion and hot water extraction on different types of forest biomass, as well as to improve understanding of the underlying mechanisms.

Performed in different experimental set ups, the work included determining the local composition of wood chips pretreated in different ways, continuous extraction of spruce and birch wood meal and a stepwise treatment of forest residues. The influence of autohydrolysis on the wood components, hemicelluloses (decreasing molar weight, deacetylation, cleavage of side groups, extraction and degradation), lignin (partial degradation and extraction) and cellulose (introduction of chain scissions) was analyzed. Moreover, the mechanism of acidification and the contribution of acetic acid were also investigated. The variations in the local composition of the wood chips obtained by steam explosion and hot water extraction were determined and related to the differences in mass transport and severity of the treatment. Finally, it was shown that the impact of steam explosion had a limited effect on refined wood residues, likely due to the relatively small size of the material.

Keywords: Biorefinery, forest residues, autohydrolysis, steam explosion, hot water extraction, pre-treatment; kraft cooking, soda cooking, spruce, birch

List of publications

This thesis is based on the following publications:

I. **Joanna Wojtasz-Mucha**, Merima Hasani and Hans Theliander,
"Hydrothermal pretreatment of wood by mild steam explosion and hot water extraction",
in *Bioresource Technology* Vol. 241, 2017, pp.120-126.

II. **Joanna Wojtasz-Mucha**, Cecilia Mattsson, Merima Hasani and Hans Theliander,
"Pretreatment and pulping of forest residues",
in *BioResources* Vol. 14, 2019, pp. 9454-9471.

III. **Joanna Wojtasz-Mucha**, Merima Hasani and Hans Theliander,
"Dissolution of wood components during hot water extraction of spruce",
Submitted

IV. **Joanna Wojtasz-Mucha**, Merima Hasani and Hans Theliander,
"Dissolution of wood components during hot water extraction of birch",
Submitted

Part of this work has been presented at:

Nordic Wood Biorefinery Conference,
20 – 22 October 2015, *Helsinki, Finland*
Chemical pretreatment of wood chips: a comparative study of mild steam explosion and hot water extraction

14$^{\text{th}}$European Workshop on Lignocellulosics and Pulp,
27 – 30 June 2016, *Grenoble, France*
Chemical pretreatment of wood chips: a comparative study of mild steam explosion and hot water
Presented by Merima Hasani

Contribution report

The author of this thesis has made the following contributions to the publications included:

I. Main author. Contributed to planning the experimental outline, planned and performed the experimental work. Analysed and interpreted the results and wrote the publication with support from the co-authors.

II. Main author. Contributed to planning the experimental outline, planned and performed part of the the experimental work. Analysed and interpreted the results and wrote the publication with support from the co-authors.

III. Main author. Contributed to planning the experimental outline, planned and performed the experimental work. Analysed and interpreted the results and wrote the publication with support from the co-authors.

IV. Main author. Contributed to planning the experimental outline, planned and performed the experimental work. Analysed and interpreted the results and wrote the publication with support from the co-authors.

List of abbreviations

AG - arabinogalactan

AGX - arabino(glucurono)xylan

ASL - acid soluble lignin

CED - copper (II) ethylenediamine

DP - degree of polymerisation

GC - gas chromatography

GGM - galacto(gluco)mannan

GM - glucomannan

GPC - gel permeation chromatography

HSQC - heteronuclear single quantum correlation

HWE - hot water extraction

HPAEC - high-performance anion exchange chromatography

LCC - lignin carbohydrate complexes

MMD - molar mass distribution

MS - mass spectrometer

NMR - nuclear magnetic resonance

PAA - peracetic acid

RI - refractive index

STEX - steam explosion

UV - ultraviolet light

The roots of education are bitter, but the fruit is sweet.
-Aristotle (and my Grandma, on multiple occasions)

Contents

1 Introduction

A biorefinery is a concept for transforming biomass into a spectrum of commercial products: biomaterials, chemicals, biofuels and energy. This concept has great potential, as there is a wide diversity of bioresources that can be converted into a range of various products. Advantages of using biomass include a virtually zero net generation of carbon dioxide, it i a renewable resource and has a relatively low cost (as, in some cases, residues from processes such as forestry, olive groves and arable farming can be used). Moreover, the economic importance of this concept is ever-increasing due to the urgency in discovering a new, and more sustainable, replacement for fossil-based materials and chemicals.

In Scandinavia, the primary choice for biomass is wood because it is highly abundant and has the promising perspective of using, at least partially, existing technologies and infrastructure.

Historically, wood has always been an important resource. It was first used for crafting tools, weapons and as fuel; in time, as a raw material in the production of furniture, paper, viscose, etc.. Today, it is still of great economic importance, with forest products being predominantly timber/lumber (e.g. for furniture and construction) and wood pulp (for paper, viscose, etc.). In the future, using wood as a raw material in biorefineries can become a key factor in the circular economy.

Further development of the wood biorefinery concept depends strongly on the knowledge and technology available. Many aspects of using wood material have

been studied in the context of biorefinery applications. In general, wood can be characterized as a complex bio-composite with a high recalcitrance towards the separation of its structural elements (i.e. cellulose, lignin and various hemicelluloses). The main structural element that is currently recovered for use in various products is cellulose: this is done using a chemical pulping process, most commonly via kraft pulping. In this process, hydroxide and hydrogen sulphide ions are employed to achieve a fairly efficient separation of cellulose by fragmentation and dissolution of the lignin, while the hemicelluloses are depolymerized and degraded to varying extents. Finally, the cooking liquor, which then contains the extracted sugars, sugar acids and lignin, is incinerated in order to recover heat and the process chemicals. The reactions involving hemicelluloses contribute to the consumption of cooking chemicals, thus adding to the operational costs. More importantly, these polysaccharides could offer a variety of structures (monomers, oligomers and polymers) that constitute attractive building blocks for bio-based materials and chemicals. It is therefore of great interest that the hemicelluloses are recovered in a pretreatment step prior to the pulping process.

Apart of the stem wood, other fractions of the tree are currently considered as being low value residues. Such left-over material can, in fact, be considered as being potential raw material for a biorefinery; forest residues could be used in e.g. the production of nanomaterials and textile fibres [Le Normand *et al.*, 2014; Li *et al.*, 2016; Moriana *et al.*, 2016]. The freedom of choosing the type of process and conditions is much greater when compared to traditional processes if the target products are nano-scale sized constituents. In this context, the fibre properties do not need to be considered, thereby opening up the possibility of using other pulping methods such as soda cooking, which is based solely on using hydroxide ions as the active chemical. This results in less efficient delignification: a longer time is required to accomplish the same extent of delignification and, consequently, the yields of hemicellulose and cellulose are lower. However, the process is relatively robust, offers the benefits of a more cost-effective chemical recovery system and generates sulphur-free lignin, which is more suitable for further conversion into materials and chemicals. As in other chemical pulping processes, hemicelluloses need to be pre-extracted from the material prior to cooking in order to ensure their suitability for broader utilization in material applications.

Along with the raw material and separation method selected, the choice of pretreatment is decisive for achieving efficient separation. Among the kinds of pretreatment available, mild hydrothermal methods, such as hot water extraction and steam explosion, allow hemicelluloses to be recovered with minimal degradation of the native structure. These methods rely on autohydrolysis: the hydrolysis of labile glycosidic bonds resulting from acidic conditions generated by release of acid groups

during heating the wood tissue. The dissolved hydrolysis products are transported out of the wood tissue, where the mass transport relies generally on diffusion of the reaction products out of the cell wall and through the pore system of the wood tissue. In steam explosion, the transport is enhanced by the advective transport in the pore system of the wood tissue caused by the rapid, disintegrating, release of pressure (an "explosion").

The choice of type, system and conditions of the pretreatment determines the range and properties of the products. It is crucial that the chemical and physical mechanisms underlying the extraction are understood so that the correct choices can be made, and should therefore be studied thoroughly.

1.1 WWSC

The work presented in this thesis was performed within the realm of the Wallenberg Wood Science Center (WWSC). The WWSC is a joint research centre at Chalmers University of Technology in Gothenburg and the Royal Institute of Technology (KTH) in Stockholm, Sweden, and was founded by The Knut and Alice Wallenberg Foundation. The research undertaken by the organisation is focused on developing new processes and material products based on the Swedish forests.

1.2 Objectives

The main objective of this work was to explore the prospects of using mild hydrothermal methods, including steam explosion and hot water extraction, for the pretreatment of different types of forest biomass by investigating their effects. Extraction from the cell wall was investigated by subjecting softwood and hardwood wood meal to hot water extraction. Local effects on the wood tissue were investigated after steam explosion and hot water extraction were applied to wood chips. Finally, the cooking experiments were performed on forest residues to evaluate how the changes rendered by steam explosion effect further processing of the material.

1.3 Outline

This work is based on four papers, which are appended. These studies used different materials and varying aspects of hydrothermal pretreatment.

- *Paper I* Mild steam explosion was compared to hot water extraction. The effects of pretreatment on the local composition of Norway spruce wood chips were investigated.
- *Paper II* Forest residues were used as the study material. The effects of the pretreatment on the composition and subsequent soda and Kraft cooking, were evaluated.
- *Paper III* Hot water extraction was performed on milled spruce in small flow-through system to improve understanding of the course of autohydrolysis of softwood.
- *Paper IV* Milled birch was subjected to hot water extraction in a small flow-through system to improve the understanding of the course of autohydrolysis of hardwood.

This thesis is organised as follows: Chapter 2 recounts the theoretical background, i.e. the basics of wood structure, along with the pulping and pretreatment processes. The experimental methods and materials used are described in Chapter 3, whilst a discussion of the results is presented in Chapter 4. Finally, the conclusion and suggestions for future work are submitted in Chapter 5.

2 Theory

2.1 Wood and pulping

There is a large variety of tree species, of which two major anatomy types can be distinguished: softwood (conifers) and hardwood (deciduous). In general, trees are higher order plants with a complicated hierarchical structure in both the longitudinal and radial directions. The main parts of a tree's macrostructure are easy to distinguish: crown, branches with leaves or needles, stem and roots. Various sections of a tree consist of different tissues and therefore have different properties and functions. Traditionally, attention is focused on the stem, which is comprised of:

- outer bark: dead tissue that provides a mechanical barrier and chemical protection
- phloem (inner bark): living cells for the transport and storage of nutrients
- vascular cambium: a layer of living cells, which produces cells towards the inside and outside of the stem
- secondary xylem: contains both living and dead cells, and serves as mechanical support to the tree and conducts water and minerals upwards. It can be divided into two parts: sapwood (both living and dead cells; tissue responsible for the transport of water and minerals, producing extractives and providing storage for nutrition)

and heartwood (dead sealed cells with no conducting function, rich in extractives). It is the xylem that shows the annual growth rings visible in the transverse cross-section of the stem [Fengel and Wegener, 1984a; Henriksson *et al.*, 2016].

The structure and composition of xylem and bark are described in detail below.

2.1.1 The structure of wood

The xylem is the part of the tree that is often considered as being "wood"; it is used for construction purposes, to make furniture and tools and in the production of pulp and paper, etc.. It is the most uniform part of the tree: around 90-95% of softwood xylem consists of tracheids, which are elongated, slender cells with flattened edges. They have a length of 2-4 mm and are arranged in radial files. Two other types of cells are present in softwood, namely parenchyma and epithelial cells. Hardwood, on the contrary, has a more complicated structure and is built up of a number of more specialized types of cells: it has different fibre (libriform fibres, fibre tracheids) and parenchyma cells (ray and longitudinal), as well as tracheids and conducting vessels. Measuring around 1mm, hardwood fibres are much shorter than softwood tracheids.

Wood cells have a rigid cell wall, comprised of a cellulose matrix scaffold encrusted with hemicelluloses and lignin. The cell wall is built up of two main layers:

- *primary cell wall*: an outer layer with randomly orientated cellulose microfibrils
- *secondary cell wall*: 2-3 layers (S1-S3) in which the cellulose microfibrils have different orientation (which is very important for physical properties).

Moreover, the space between adjacent cells is filled with a lignin-rich layer called the *middle lamella* [Daniels, 2016].

In general, wood can be considered as being a bio-composite, where cellulose is a reinforcing agent and lignin, hemicelluloses and extractives provide a barrier/stiffness, opportunities for interaction to take place and protection, respectively. However, the proportions of the components, as well as some of the characteristics of the polymers, vary not only between species but also different parts of the tree. The general compositions of both softwood and hardwood are presented in Table 2.1 [Henriksson *et al.*, 2016].

Cellulose

Cellulose is the most abundant polymer present on Earth, constituting at least 30 % of the mass of advanced plants [Kamide, 2005] and 40-45 % of wood. It is comprised of glucose units and organized in a hierarchical structure.

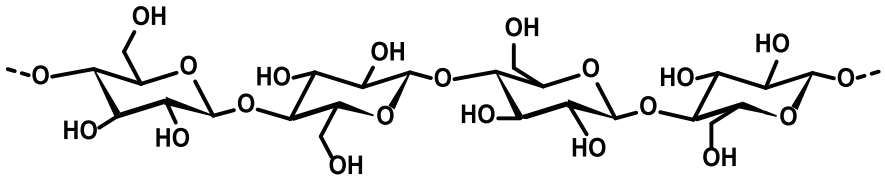

Figure 2.1 *Molecular structure of cellulose*

The **primary structure** of cellulose is a linear, unbranched chain of D-glucopyranosyl units connected by β(1-4) glucosidic bonds (Figure 2.1). The degree of polymerization is usually high, with a maximum of about 15 000 glucose units. The monomers are rotated 180° towards each other. The chains are stabilized by intramolecular (intrachain) hydrogen bonding between the C6 and C2 hydroxyl, the C5 oxygen and the C3 hydroxyl of adjacent units.

Chains are organized into semi-crystalline microfibrils, where neighbouring chains in the crystalline regions are arranged in **sheet** formations (stabilized by C2-C6 and C3-C6 interchain hydrogen bonds). The cellulose sheets are piled up into long and relatively narrow stacks (**crystalline structure**), probably due to van der Waals interactions and hydrogen bonds.

The semi-crystalline microfibrils are organised further into very long **fibrils** (at least 40 μm). The size of the fibrils differs not only between species of trees but also between the types of tissue found in them [Henriksson and Lennholm, 2016]. Cellulose fibrils are deposited in the layers of the cell wall mentioned above. The organisation of fibrils is different in each layer, being random in the primary wall and having different, but consistent, directions in the S1-S3 layers of the secondary cell wall.

Table 2.1 *Composition of softwood and hardwood [Henriksson* et al.*, 2016].*

	Composition of woof		
Type of wood	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Softwood	40-45	25-30	25-30
Hardwood	40-45	30-35	20-25

Table 2.2 *Hemicelluloses present in softwood and hardwood and their average content in the xylem.*

Type of wood	Hemicellulose	Content (%) in the xylem
Softwood	(galacto)glucomannan	5-8
	glucomannan	10-15
	(arabino)glucuronoxylan	7-15
Hardwood	glucuronoxylan	15-35
	glucomannan	2-5

Hemicelluloses

Hemicelluloses are heteropolysaccharides and have a relatively low degree of polymerization compared to cellulose. They contribute to the mechanical properties of the cell wall and regulation of its porosity, flexibility and interaction properties. The composition and quantity of hemicelluloses in the cell wall vary between plant species: the most abundant in trees are *glucomannans* and *xylans* (see Table 2.2). The content and composition of hemicelluloses can vary for different tissues in the tree [Teleman, 2016].

Glucomannan (GM) has a linear backbone of $\beta-(1\rightarrow4)-$linked D$-$mannopyranosyl and D$-$glucopyranosyl units (Table 2.2 and Figure 2.2). The proportions between glucose and mannose vary between tree species and, in the native state, glucomannan is partially $O-$acetylated on mannosyl units. Softwood also contains galactoglucomannan (GGM), which tends to have longer chains than hardwood glucomannan (90-102 vs. 60-70 monomers); moreover, some of its glucopyranosyl units are connected to a single D$-$galactopyranosyl group attached by $\alpha-(1\rightarrow6)$. Irregular acetylation and substitution with galactosyl groups prevent organisation over longer distances: if deacetylated softwood hemicelluloses may crystallize.

Partial deacetylation occurs quite easily, although the kinetics are strongly dependent on the conditions. The $\alpha-$glycosidic linkage connecting the galactosyl unit to the chain is relatively sensitive and can therefore be cleaved in both alkaline and acidic conditions. Glucomannan is particularly sensitive to alkaline conditions due to its high solubility and lack of side groups that could prevent rearrangement leading to end-wise degradation: they therefore undergo severe peeling during alkaline pulping processes. The $\beta-$D$-$mannopyranosidic linkage is relatively sensitive to acidic conditions, even more so than $\beta-$D$-$glucopyranosidic. Glucomannan can therefore

be depolymerized selectively in mild acidic conditions and the partially fragmented chains extracted thereafter [Teleman, 2016].

Xylan has a $\beta-(1\rightarrow4)-\mathrm{D}-$xylopyranosyl backbone, but there are significant differences between its structure in softwood and hardwood. Softwood generally contains arabinoglucuronoxylan (AGX), i.e. xylan with $\alpha-\mathrm{L}-$ arabinofuranose side groups; its chains have a DP of 90-120 units and are non-acetylated. Hardwood, on the other hand, contains $\mathrm{O}-$acetyl$-(4-\mathrm{O}-$methylglucurono)xylan with longer chains (100-220 units) (Table 2.2 and Figure 2.2).

Xylans are easily deacetylated in alkaline, and also to various extents in acidic conditions. In alkaline conditions xylan undergoes peeling but, thanks to the side-groups preventing rearrangements, it is less extensive compared to that of glucomannan. Moreover, 4-O-Me glucuronic acid side groups can be converted into hexenuronic acid: this acid has double bonds that consumes bleaching chemicals and causes, in turn, discolouration of the pulp. Compared to glucomannan, xylan is more sensitive to degradation in acidic conditions leading readily to the further conversion of xylose monomers into dehydrated furfural structures [Teleman, 2016].

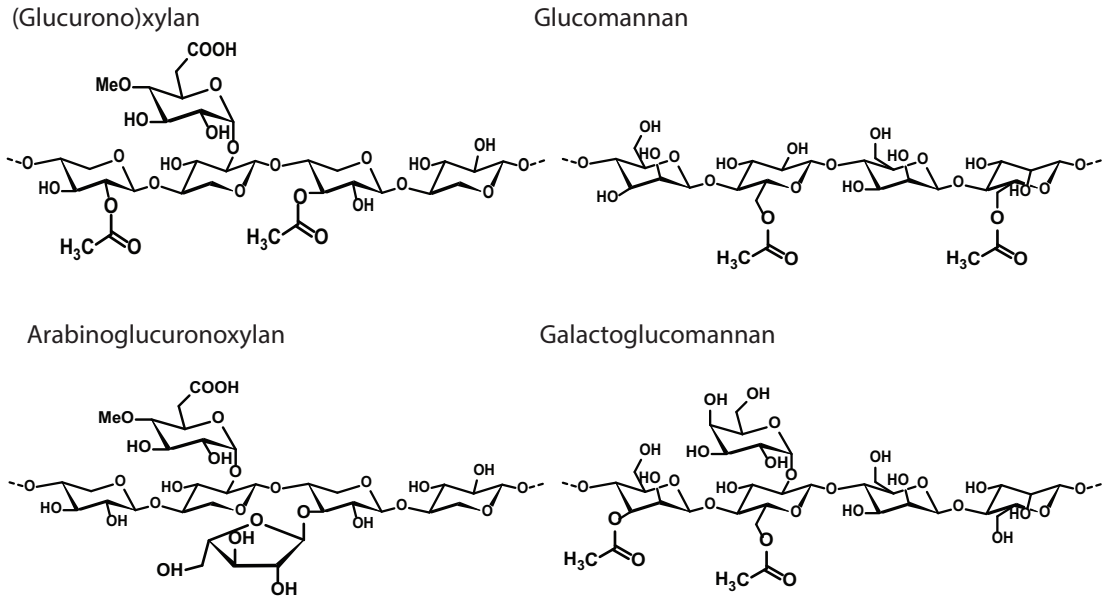

Figure 2.2 *Molecular structure of hemicelluloses.*

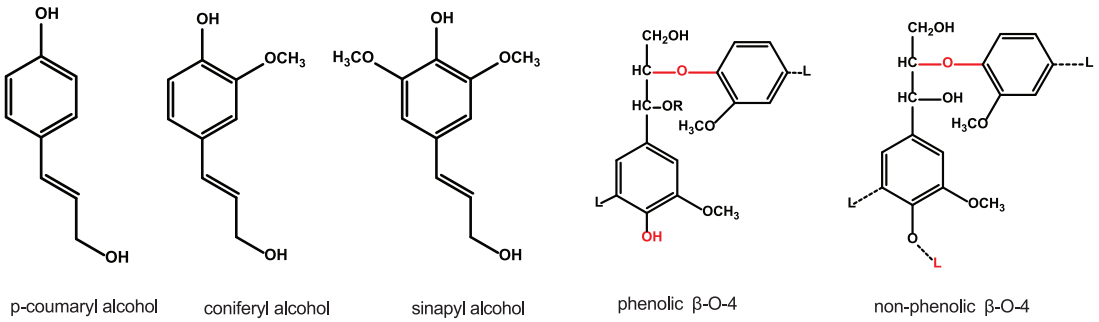

Figure 2.3 *Molecular structure of lignin monomers and the $\beta-O-4$ bond.*

Lignin

Lignin is one of the most complex biopolymers. It is a hydrophobic, amorphous polymer comprised of monomers of coniferyl alcohol, p-coumaryl alcohol and sinapyl alcohol (Figure 2.3). Unlike other biomolecules, however, it does not have an ordered structure and forms a three-dimensional web of randomly cross-linked and branched structures. Lignin synthesis via radical polymerisation is one of the last steps in the formation of the cell wall, lignin fills up the spaces between the cellulose and hemicelluloses and thereby contributes to fixating these components. It makes the cell wall hydrophobic, protects it from degradation and contributes to stiffness.

The phenyl propane units constituting lignin are linked with a number of various ether and C-C linkages. The most common, and the most important with regard to processing/fragmentation in alkaline conditions, is the $\beta-$O-4 ether bond (Figure 2.3). Other important bonds are, for instance, the beta-5' and 5—5' (between the carbons in two aromatic rings). Softwood lignin is mostly comprised of coniferyl alcohol, with small amounts of p-coumaryl alcohol, whereas hardwood lignin is comprised of coniferyl and sinapyl alcohols in varying proportions. The proportion of the different monomer units and inter-unit linkages determine the properties and reactivity of lignin.

Extractives

Extractives are compounds of low molecular mass that can be extracted relatively easily from wood. Comprised of terpenes, fats, fatty acids and phenolic compounds, they serve multiple functions by acting as regulators (plant hormones etc.) and providing protection from parasites, damage, UV radiation, oxidative degradation, etc..

Their amount in wood is relatively low, but they nevertheless constitute a significant fraction of the bark and branches of trees.

Arabinogalactan

Arabinogalactan (AG) is a heavily branched pectin. It contains galactose:arabinose in a ratio of 6:1 and has a molar weight of 29-59 kDa. AG, constitutes up to 3% of birch wood [Fengel and Wegener, 1984b] and 0.2% of spruce [Willför *et al.*, 2002]. Its content is significantly higher in compression wood [Sjöström, 1993] and, in general, arabinogalactan is easily extractable.

2.1.2 Bark

Bark forms the outer layer of a tree, constituting 10-20% of the stem and 20-38% of the branches [Fengel and Wegener, 1984a]. It is an inhomogeneous tissue, consisting of two layers with different properties and functions: the inner (phloem, for liquid transport purposes) and the outer (often coarse, for protection). These layers are built up of different tissues and cells. Compared to stem wood, bark contains more lignin and extractives (e.g. a fraction of polyphenols (tannins) and suberin that are not present in the stem wood tissue, see Table 2.3) and smaller amounts of polysaccharides [Ek *et al.*, 2016]. Bark lignin and wood lignin are composed of similar basic units, although their ratio can vary. For instance, it was shown that the composition of the basic units differs to some extent because pine bark lignin has more condensed structures and more p-hydroxyphenyl than stem wood (which contains predominantly guaiacyl units). It also shows a higher molecular weight and polydispersity than stem wood lignin [Huang *et al.*, 2011]. Bark cellulose is reported to be organized in shorter chains (lower DP) and shorter fibres [Fengel and Wegener, 1984a]. Moreover, the composition of the hemicelluloses is different in this section

Table 2.3 *Typical content of extractives in bark and wood [Raisanen and Athanassiadis, 2013].*

Species of	Bark (%)	Wood (%)
Scots pine	25.2	5.0
Norway spruce	32.1	2.0
Silver birch	25.6	3.8

of the tree and there are more pectins present [Krogell *et al.*, 2012; Le Normand *et al.*, 2012; Raisanen and Athanassiadis, 2013].

Determining the exact composition of bark is challenging and requires further development of the analytical procedures commonly used for wood and pulp, as these give a poor mass balance when the amounts of extractives are high. One reason for this, among others, is that extractives precipitate with lignin and result in the values of Klason lignin being overestimated [Burkhardt *et al.*, 2013; Huang *et al.*, 2011; Kemppainen *et al.*, 2014; Krogell *et al.*, 2012]. Therefore there is a need for a more comprehensive analysis of the Klason lignin fraction and a pre-extraction and analysis of extractives. Also, expandnig carbohydrate analysis to other polysaccharides (beyond the most common ones, i.e. hemicelluloses) would be beneficial.

Finally, describing bark is made even more difficult due to seasonal variations in its composition and the various effects of the debarking and storage methods used [Bajpai, 2013; Kemppainen *et al.*, 2014]. Kemppainen *et al.* [2014] showed, for example, that industrial bark consists of up to 21% wood tissue.

2.1.3 Forest residues

Focus is traditionally placed on processing the stem of the tree, while the branches (foliage) and tops are considered as being residues. Whilst the availability of these residues together with their relatively low cost make them a promising raw material for a biorefinery, they also present a number of challenges.

Forest residues are usually comprised of a mix of wood and bark tissue from various sections and species of trees, and are consequently highly inhomogeneous in both physical and chemical properties. The presence of bark generally results in higher contents of extractives, lignin and ash compared to stem wood usually used for pulping. Determining the composition accurately is extremely difficult, mainly due to the complexity and variation of the composition but also because the methods, available (described above) designed primarily for the pulp and paper industry, are not suitable for material rich in extractives [Burkhardt *et al.*, 2013]. Compared with bark, the analysis of mixed residues is additionally more challenging due to their heterogeneity.

Moreover, the varying morphology and chemistry of the components affect the chemical reactions and mass transport of chemicals during treatments, resulting in different changes being made to different samples under the same treatment.

2.1.4 Pulping

The separation of wood components for commercial/material applications is currently performed most efficiently through pulping processes to provide access to cel-

lulose fibres. This is accomplished by different pulping processes based on chemical, mechanical or thermomechanical treatments. Selecting the most suitable process is important for obtaining the desired quality of the pulp. The dominant process for separating the constituents of wood is the kraft process, whereby fibres are liberated by the fragmentation and solubilization of lignin. Another well-established process is sulphite pulping, based on the dissolution of lignin through sulphonation. A lesser used process, that might be important from the biorefinery perspective, is soda pulping: this relies on the same principle as kraft cooking but has simpler chemistry and a lower degree of efficiency.

The active chemicals in the kraft process are sodium hydroxide and sodium sulphite. The hydroxide ions allow cleavage of the non-phenolic β-O-4 bonds while phenolic β-O-4 cleavage occurs efficiently only in the presence of hydrosulphite ions. Although this separation is fairly effective, strong alkaline conditions cause the degradation of hemicelluloses and a small fraction of the cellulose, which decreases the yield. The spent chemicals and degradation products (black liquor) are burned to recover energy and chemicals.

Soda cooking relies on a simpler chemistry, since it involves using only sodium hydroxide as the active chemical. Delignification relies on the cleavage of the non-phenolic β-O-4, while the phenolic β-O-4 bonds remain mostly unaffected. The lignin fragments are thus bigger and require more time to diffuse out of the wood tissue, making this method less efficient when compared to kraft pulping: it requires a prolonged time frame to accomplish the same degree of delignification and this, in turn, results in lower yields of both hemicellulose and cellulose. In the context of a forest biorefinery, however, this can be useful since the mechanical performance of the fibres is not of the main importance: the process is more robust with simpler chemical recovery and generates sulphur-free lignin. The degradation of hemicelluloses can be avoided by using pretreatment to recover them prior to pulping.

2.2 Biorefinery

There is a wide diversity of bioresources that can be converted into a range of marketable products in a biorefinery. *Biorefinery* is a platform for separation of chemical building blocks from biomass for utilisation in production of bioenergy, biofuels, biochemicals and biomaterials. This concept has a great potential which, however, is currently not exploited to the full due to very few processes, except pulping, being developed for the primary separation of the biomass components. In consequence, very limited feedstocks are used and only a relatively narrow range of products is produced.

2.2.1 General aspects

The objective of a biorefinery concept is to separate the chemical components of the biomass or their building blocks and convert them in a sustainable manner into a range of products. The feedstock covers chemical structures found mainly in the plant kingdom, such as cellulose, hemicellulose, lignin and fats/oils [Aresta *et al.*, 2012]. Designing a suitable process is crucial for keeping the production sustainable and profitable. There are three important prerequisites for the process:

- low environmental impact (sustainable technology: low CO_2 footprint, recovery of chemicals, non-toxic reagents)
- low energy demand
- high material efficiency.

The key to building an efficient biorefinery is adopting an interdisciplinary approach involving biochemical, chemical and thermochemical conversions.

2.2.2 Forest-based biorefinery

The history of forest biorefineries can be tracked back to the early days of sulphite pulp mills, as they used to operate as biorefineries. For instance, Domsjö Fabriker (Domsjö, Sweden) started on the path towards being a biorefinery as early as in the 1940s: apart from producing dissolving pulp, they started fermenting ethanol as a platform chemical for the chemical industry and producing cattle fodder [Domsjö-Fabriker, 2020]. Nowadays, they use cellulose from the sulphite process to make dissolving pulp for viscose production, the sugar stream is fermented into bioethanol and lignin is sold in the form of lignosulphonate. A second example of a sulphite-based, early biorefinery is Borregaard (Sarpsborg, Norway). This mill started producing chemicals alongside paper pulp as early as before the outbreak of World War II; today, it is a fully functioning biorefinery which, *"using the different components of wood, [...] produces lignin products, speciality cellulose, vanillin and bioethanol for a variety of applications in sectors such as agriculture and fisheries, construction, pharmaceuticals and cosmetics, foodstuffs, batteries and biofuels."*[1]. The sulphite process, and biorefineries based on it, became less competitive after 1940, when kraft pulping became a more efficient process for producing pulp (efficient recovery, stronger pulp, etc.) and also due to the emergence of oil-based materials and chemicals that displaced other products from sulphite mills.

[1] Information from Borregaard [2020].

Kraft pulp mills can also be considered as being basic biorefineries, since a large part of their tall oil and turpentine is separated and used in various products, e.g bio diesel, soap, solvent etc. and the residue fraction from the process, in the form of black liquor (containing lignin, hemicelluloses and spent chemicals), is burned in the recovery boiler to recover energy.

Moreover, kraft pulp mills can be considered as a platform for future forest-based biorefineries: this type of process offers an efficient separation of lignin and cellulose along with the possibility of improving material efficiency by recovering lignin and hemicellulose structures either prior to, or following, the main cellulose-lignin separation process. In fact, there is already an industrial process, known as LignoBoost, that allows lignin to be separated from black liquor Öhman *et al.* [2007]. Thus, what is actually required is a pretreatment step to recover hemicelluloses.

As the concept of the biorefinery was introduced into the scientific discussion [Levy *et al.*, 1981; Rexen *et al.*, 1988; Wyman and Goodman, 1993], forest biorefineries became of interest due to the abundance of forest biomass and the advantage of using the existing infrastructure. For several reasons pulp mills, as traditional wood processors, offer a great platform for a modern biorefinery. Wood is a promissing bioresource: it offers a variety of material building blocks, does not compete with the growing of food products in large areas and, very importantly, is independent of season. Moreover, pulp mills allow a biorefinery to become integrated systems since, they have established wood collection, handling and processing technologies, infrastructure and a skilled workforce [Bajpai, 2013; Lew *et al.*, 2012]. In an integrated system, forest residues (such as tops, branches and twigs) that nowadays are left behind in the forest or burned can be used for separating chemical building blocks and converted into value-added products. The literature already presents a number of ideas for using forest residues e.g. the pyrolysis for bio-oil and biogas [Janzon *et al.*, 2014], the separation of tannins from bark [Kemppainen *et al.*, 2014] and the production of cellulose nanocrystals [Le Normand *et al.*, 2014; Moriana *et al.*, 2015, 2016] or nanocomposites [Li *et al.*, 2016].

In the case of the pulp and paper industry, creating a biorefinery would provide an opportunity to:

- increase productivity and profitability
- increase the usage efficiency of the raw material
- continue already established production while opening up to new markets
- use side streams from conventional production [Bajpai, 2013; Lew *et al.*, 2012].

In this context, new processing steps must be included in order to separate the wood tissue more efficiently. A pretreatment step added prior to pulping could en-

able the recovery of useful chemical structures (primarily hemicelluloses, polymers and oligomers) that would otherwise be degraded, as well as lead to enhanced accessibility of the wood material for further processing and reduced consumption of cooking chemicals.

2.2.3 Pretreatment

The choice of the pre-treatment method to be employed is governed by the requirements of the subsequent processing steps (e.g. accessibility, contents of hemicellulose, etc.) as well as by the specific demands of the product (i.e. the properties of the hemicelluloses), and will be dependent on a comprehensive understanding of the method

In general, among the biomass pretreatments currently available, it appears that the hydrothermal processes, such as hot water extraction (HWE) and steam explosion (STEX), are the most promising. These are cost-effective and have low environmental impacts due to the fact that water is the only chemical that is added. Additionally, using mild conditions not only allows the recovery of hemicelluloses with minimal degradation of their native structure but also enhances the accessibility of the remaining constituents in the wood [Garrote *et al.*, 1999a; Jedvert *et al.*, 2012a; Krogell *et al.*, 2013; Rissanen *et al.*, 2014b; Wojtasz-Mucha *et al.*, 2017].

Both HWE and STEX rely on recovering partially degraded hemicelluloses from wood tissue by acid hydrolysis (i.e. autohydrolysis) mediated dissolution and the subsequent diffusive transport of the dissolved structures. In STEX, autohydrolysis is followed by a rapid, disintegrating, release of pressure (i.e. an "explosion"): apart from facilitating access to the structure, this also (through the pressure difference) gives rise to an advective mass transport of the liquid through the pore system [Jedvert *et al.*, 2012b], rapidly forcing some of the hydrolysis products out of the wood tissue.

Moreover, different products will be formed depending on the process conditions of HWE/STEX: mild conditions favour recovery of longer chain structures, whereas more severe conditions (e.g. high temperature and long treatment time) facilitate the formation of monomers and their degradation products, such as hydroxymethyl furfural, HMF (from hexoses) and furfural (from pentoses) [Abatzoglou *et al.*, 1990; Li *et al.*, 2005].

Autohydrolysis

Multiple attempts that have been made at describing [Garrote *et al.*, 1999b; Laser *et al.*, 2002; Lora and Wayman, 1978] and modelling autohydrolysis and hemicellulose extraction can be found in the literature [Conner, 2007; Liu *et al.*, 2014; Mit-

tal *et al.*, 2009; Nabarlatz *et al.*, 2004], along with many articles discussing mass transport during different pretreatments [Kvist *et al.*, 2017; Liu *et al.*, 2014; Petty, 1973]. Despite the wide array of literature available, autohydrolysis description is often overly simplified.

What is important is that, hydrogen ions do not need to be transported into the material for autohydrolysis to occur: they are already present and are released readily from wood components. The kinetics of the acid hydrolysis in wood is negligible at room temperature, but, when it is increased, the acidification progresses and hydrolysis takes place. Although the decrease in pH is often described as an effect of deacetylation, the process seems to be more complicated and involve other acids. As Bouchard *et al.* [1991] and Laser *et al.* [2002] pointed out, the extent of deacetylation is fairly limited in comparison to the total content of acetate and therefore may not be the only driving force behind hydrolysis. Thus, acidification during autohydrolysis may include the presence of other acidic moieties (e.g. glucuronic acids) and is more complex than assumed in the models based purely on acetic acid. Moreover, it is not unlikely that different acids affect various stages of the autohydrolysis process to greatly varying degrees. Garrote *et al.* [2001] suggested the following mechanism: at the beginning of the process deacetylation depends on the availability of protons, which is the rate-limiting factor and causes acetic acid to be generated. This in turn results in further acidification and an increase in the reaction rate. Finally, after prolonged treatment, the decreasing concentration of the substrates (acetylated hemicelluloses) in the solid material causes the reaction rate to decrease.

Studying autohydrolysis

Many factors influence autohydrolysis: temperature, residence time, size of the material (ranging from wood chips to wood meal), liquid/solid ratio and flow rate, for example are all important parameters (e.g. [Garrote *et al.*, 1999a; Lora and Wayman, 1978; Tarasov *et al.*, 2018]). Extraction becomes more efficient if smaller-sized materials and/or more severe conditions are used. Therefore, the equipment used for pretreatment must be considered with thoroughness and care.

First and foremost, the process conditions, which includes material size, temperature, treatment time, packing density and agitation (for batch reactors) should be controlled precisely as they were shown to be of great significance [Borrega and Sixta, 2015; Kilpeläinen *et al.*, 2014; Leppänen *et al.*, 2011; Song *et al.*, 2008; Wojtasz-Mucha *et al.*, 2020]. Secondly, the type of reactor used is of importance.

In the case of HWE (relying exclusively on autohydrolysis and diffusive mass transfer), batch reactors result in lower extraction yields compared with flow-through apparatuses [Borrega and Sixta, 2015]. Moreover, flow-through systems have the advantage of the reaction products being removed directly from the reactor, thereby

limiting degradation in the extraction liquor. Their main disadvantage is that the process dynamics at the end of the reactor is affected by the reaction products released in the front section. This effect, which can be particularly pronounced in bigger vessels, can be minimized by scaling-down the size of the apparatus in question [Leppänen *et al.*, 2011]. Many studies are also based on the accelerated solvent extractor [Ahmad *et al.*, 2018; Song *et al.*, 2008, 2012], which is a set-up of multiple, small, batch reactors that can be extracted simultaneously. Finally, a somewhat intermediate solution, using a continuous mix batch reactor was also proposed for the extraction of birch [Jara *et al.*, 2019]. Such a configuration requires large quantities of water to be used, which can make analysis more difficult. Despite the comprehensive information already available, further understanding of the process in context of the driving force, and especially so for softwood, is lacking. This gap in the knowledge of the mechanisms of acidification and deacetylation can be filled by studying them further.

The focus of this work is to increase understanding of the mechanisms underlying mild hydrothermal pretreatments, namely steam explosion and hot water extraction. The effects of autohydrolysis on the cell wall and wood tissue were investigated, as well as the local effects of steam explosion of wood chips and wood residue. The possibility of applying these methods to forest residues was also analysed by investigating the effects they have on pulping behaviour.

3 Materials and Methods

3.1 Materials

All chemicals were purchased from Sigma-Aldrich and used as received.

Paper I

Industrially cut wood chips of Norway spruce (from a Scandinavian pulp mill) were selected manually to provide the size-fraction preferred (at least 6 mm in thickness, 3 cm in width and 4 cm in length) and minimise the presence of dirt, knots and bark.

Paper II

The forest residues (branches, bark and twigs) provided by Domsjö Fabriker (Örnsköldsvik, Sweden) was comprised of a mixture of softwood (mostly spruce and pine) and hardwood (mostly birch); the material was chipped to a uniform size prior to shipping.

Papers III and IV

Spruce sawmill wood shavings and birch wood chips were kindly provided by Södra (Värö mill and Mörrum mill, Sweden). The material was air-dried, ground in a Wiley mill and sieved to a size below 0.5 mm for spruce and 1 mm for birch.

3.2 Sample preparation

3.2.1 Refining (Paper II)

A Sprout-Waldron 12-1CP 12-inch disc refiner was used to homogenize the samples of forest residues. Prior to refining, the material was heated by steaming at 125°C for 15 min. The samples were then stored at −20°C.

3.2.2 Steam explosion

Paper I

The equipment used to subject the wood chips to mild steam explosion was a modified steel autoclave (approx. 1.2 l) fitted in an insulated container (Figure 3.1). The lid of the autoclave was equipped with an inlet and outlet for steam and a temperature sensor. The valve on the outlet allows for rapid release of the pressure into a collection vessel (approx. 15 l). The autoclave was filled with 50 g of oven-dried wood chips and water corresponding to a water-to-wood ratio of 4:1 and the experimental conditions were chosen to be 150°C, and 15 and 30 min. Pretreated wood chips were washed first with approx. 5 of warm water and subsequently with cold water for one week.

Paper II

Batches of the forest residues were pre-treated in bench-scale steam explosion equipment (Figure 3.1). Each run involved 500g of forest residues being loaded into the top chamber and heated with saturated steam from the boiler until the pressure in the vessel reached 4 and 7 bar, respectively. The material was kept under pressure for 15 min and then discharged rapidly into the lower chamber of the apparatus (at atmospheric pressure).

3.2.3 Hot water extraction

Paper I

Extraction with hot water was performed in a steel autoclave (approx. 1.2 l) placed in a pre-heated PEG bath with rotation. The amount of wood and the wood-to-water ratio were the same as in the steam explosion treatment (4:1). The heating-up period was 15 minutes, whilst the temperature was kept at 150°C for treatment times of 15, 30, 60 and 90 min, respectively The washing procedure was the same as for the STEX treatment.

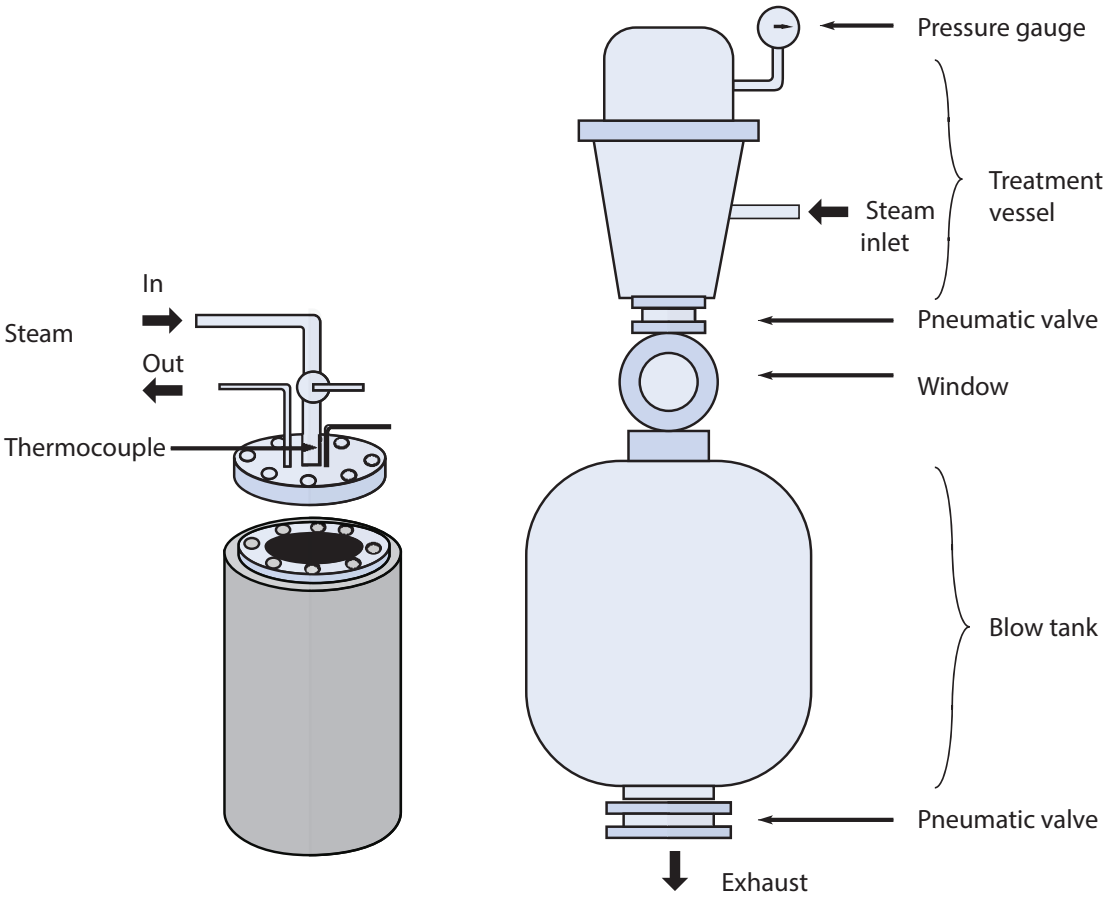

Figure 3.1 *Steam explosion autoclave(left) and bench-scale steam explosion equipment (right).*

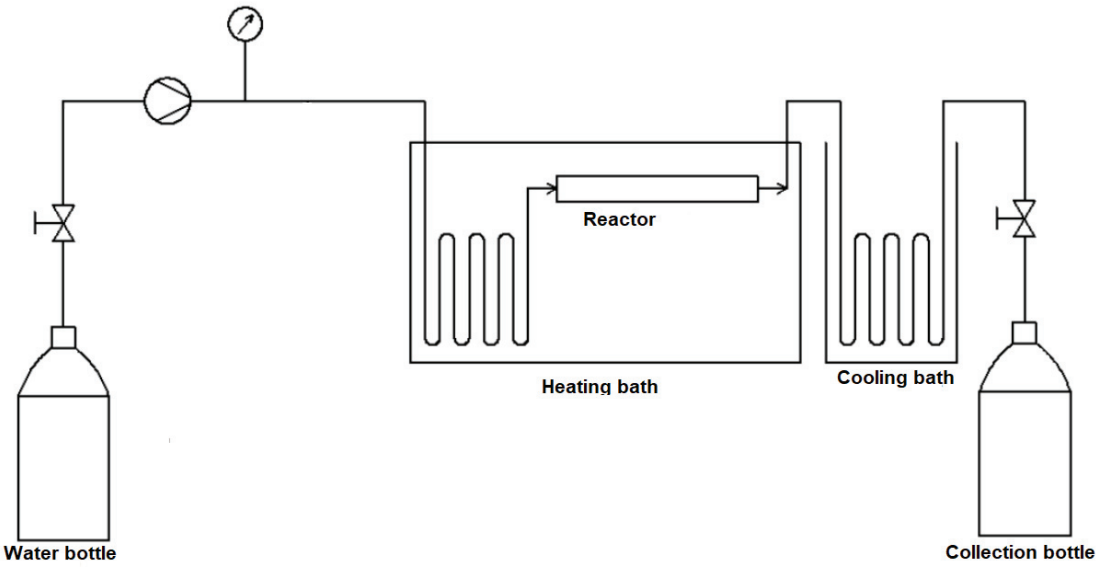

Figure 3.2 *Schematic diagram of the small-scale flow-through reactor employed.*

Papers III and IV

The experiments were conducted in a small flow-through reactor, described earlier by Bogren *et al.* [2009], consisting of a high-pressure pump, a steel column (300 x 7.8 mm), a PEG-filled heating bath and coiling in front of the column for heating and after the column for instant cooling (submerged in cold water), see Figure 3.2. The material was filled carefully inside the reactor to ensure uniform packing. The column accommodated approximately 4 g of spruce meal and 6 g of birch. Water was pumped at 5 ml/min flow (approx. 2 min residence time). The oil bath was heated to 130, 150 or 170°C, respectively, and the extraction was carried for 30, 60 or 120 min, respectively. The liquor samples were collected at time intervals of 0-10, 10-20, 20-40, 40-60, 60-80, 80-100, 100-120 min, respectively. Any further reactions were quenched by instant cooling.

The solid residues were evacuated from the column, suspended in 300 ml deionized water, dewatered on a glass filter and then washed with 700 ml water prior to being left overnight to dry at 105°C.

3.2.4 Cooking (Paper II)

Soda cooking

Cooking was carried out in 1.2 l steel autoclaves charged with liquor containing 0.37 mol NaOH/kg charged at a liquor-to-wood ratio of 9:1. The autoclaves were placed in a polyethylene glycol bath preheated to 80°C before the temperature was increased to 170°C at a rate of approximately 1°C/minute. Once the target temperature was reached, the material was cooked for 30, 60, 90, 120 or 180 min, respectively. The pulp was separated by filtering through a woven polypropylene mesh and washed with 10 l of water. The solid material thus obtained was defibrillated for 10 min in a pulp disintegrator and washed once more with 1 l of water.

Kraft cooking

The white liquor used for kraft cooking was charged at 9:1 liquor to wood ratio. The effective alkali was 29%, the sulphidity 35% and the carbonate concentration 0.1 M (corresponding to 0.37 mol OH^-/kg liquor, 0.19 mol HS^-/kg liquor hydrogen sulphide ions and 0.1 mol CO_3^{2-}/kg solution). The cooking procedure was analogous to that of soda cooking.

3.3 Characterization

3.3.1 Sample preparation

Sectioning the wood samples (Paper I)

In order to investigate how different parts of the wood chip are affected during the treatments studied, different sections of the wood chips treated with STEX (15 and 30 min) and the HWE (15, 30 and 60 min) were analysed separately. Prior to analysis, treated and washed wood chips were oven-dried and cut into sections with a saw. The parts damaged during chipping were removed, and thereafter, sections were collected from the corner, outer edge and middle of the wood chip. The fragments obtained were then sliced into layers using a microtome so that the outer and inner layers could be analysed separately (see Figure 3.3).

Homogenizing the forest residues (Paper II)

Forest residues were first refined and milled before three samples were collected for analysis, so that the overall composition of the starting material could be estimated

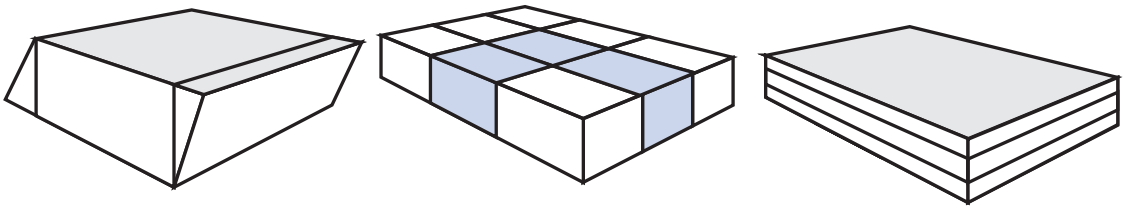

Figure 3.3 *Illustration of how a wood chip was sectioned: removal of damaged parts (left), selection of sections from the corner, edge and middle areas (middle), division of the chosen fragments into layers (right).*

accurately. As a result, the standard deviation obtained from the average values was in a similar range as that measured for stem wood.

3.3.2 Dry content and yield (Papers I-IV)

The dry content of each sample was determined by drying it overnight at 105°C. The solid fraction yield was determined as a proportion of the dry mass and the initial dry mass of the material. The cooking yield was calculated by relating the dry content of the liquors to the initial mass of the sample.

3.3.3 Severity factor (Paper I)

The effects of temperature and time of the pretreatment are often combined together into one value to give the "severity factor", which enables comparisons to be made between different time - temperature conditions. It is derived assuming first order kinetics and Arrhenius behaviour, so it should be used just as an arbitrary number representing experimental reaction conditions [Heitz *et al.*, 1991; Overend *et al.*, 1987]. The severity factor for multiple temperature stages may be determined as described in Equation 3.1:

$$\log(R_0) = \log\left[\sum_{i=1}^{n} t_i \, e^{(T_i - T_b)/\omega}\right] \tag{3.1}$$

where:

t = time (min)
T_i = temperature of the treatment stage (°C)
T_b = base temperature, here assumed $T_b = 100$ (°C)
ω = an empirical parameter, where $\omega = 14.75$ based on the activation energy [Chum *et al.*, 1990] was assumed.

Here, the severity factor was calculated for the methods selected taking the heating-up period into consideration: this calculation took into account the differences in two procedures with very different heating-up times (2 vs. 15 min).

3.3.4 Ion exchange chromatography

Samples of solid residues, pulps and freeze-dried liquors were subjected to complete acid hydrolysis using 72% sulphuric acid. After filtering off the solid residue, the hydrolysate was used to determine the total carbohydrate composition following the procedure described by Theander and Westerlund [1986].

The overall composition of carbohydrates (Papers I-IV) and monomers (Papers II and III) were determined as the amounts of monomeric sugars by using high performance anion exchange chromatography (HPAEC). The system comprises a Dionex ICS-5000 equipped with a CarboPacTM PA1 column and an electrochemical detector, using NaOH and NaOH + NaOAc as eluents. The software used was Chromeleon 7, Chromatography Data System, Version 7.1.0.898. The amounts detected were corrected to the hydrolysis yield. The hydrolysis yield was calculated from experimental data, established by performing acid hydrolysis on pure monosugar standards, as the ratio of the amount detected to the mass of the sample used: it was found to be 93.1 ± 1.9%, for arabinose, 92.9 ± 1.7% for galactose, 91.8 ± 2.0% for glucose, 78.6 ± 1.6% for xylose and 90.2 ± 0.6% for mannose.

3.3.5 Lignin quantification

The residual solid material from complete acid hydrolysis was considered to be Klason lignin, the amount of which was determined gravimetrically. The filtrate from the hydrolysis was used to determine the contents of acid soluble lignin (ASL), with its amount being calculated based on the absorbance measured with UV at a wavelength of 205 nm in a Specord 205, Analytic Jena, assuming the absorptivity constant equals 110 $dm^3g^{-1}cm^{-1}$ [Dence, 1992].

3.3.6 Gel permeation chromatography

The molecular mass was determined using size exclusion chromatography in a PL-GPX 50 plus integrated system connected with refractive index (RI) and ultraviolet (UV, 290 nm) detectors (Polymer Laboratories, Varian Inc.). The system was equipped with two columns PolarGel-M and PolarGel-M Guard (300 x 7.5 mm and 50 x 7.5 mm) with 8 μm mixed size pores; the mobile phase consisted of dimethyl sulphoxide (DMSO)/LiBr (10 mM) with a flow of 0.5 ml/min.

3.3.7 Gas chromatography

A gas chromatography system of the model Agilent 7890A, Agilent 5975C, equipped with mass spectrometer (MS) detection operating in an electron ionization mode was used to analyse the volatile compounds in the evaporated crude extractive residues. The NST MS search programme (Version 2.0) operating on the NIST/EPA/NIH Mass Spectral Database 2011 (NIST 11) was used to perform the spectral interpretations.

Paper II

A 10 mg sample of residue was dissolved using 0.6 ml ethyl acetate before 15-25 mg of the internal standard heptadecanoic acid methyl ester (15-20 mg/0.9-1.1 g ethyl acetate) was added. The solution was derivatized with 0.1 ml BSTFA (99:1; N, O-bis(trimethylsilyl)trifluoroacetamide: chlorotrimethylsilane) and trimethylsilyl reagent. After 30 minutes has passed, 1 μl of the solution was injected into the gas chromatographic system via an autosampler. The analytes were then separated in chromatographic columns (HP-5MS, 30 m in length, 0.25 mm in internal diameter and 0.25 μm in stationary phase thickness) using helium as the carrier gas at 1 ml/min for the MS column. The temperatures of the system were set to 300°C for the injector and 50°C for the GC oven for 2.25 minutes before being raised to 300°C at a rate of 2°C/minute; the temperature of the GC oven was set thereafter at 300°C for 30 minutes. The MS source and the quadruple temperature were set to 250 and 150°C, respectively.

Papers III and IV

Prior to analysis, the freeze-dried liquor samples (4 mg) were methanolysed using a reagent consisting of 2.8 ml Ac-Cl in 17.2 ml anhydrous methanol and then left to react at 80°C for three hours. After evaporation, pyridine was used as a solvent and an internal sorbitol standard was added. The solvent in the samples was then evaporated before the samples were silylated, using 150 μl hexamethyldisilazane and 80 μl chlorotrimethylsilane as the silylation reagents. The solvent was evaporated again and 2 ml of diethyl ether was added to the dry samples, which was then filtered before 1 μl sample of the solution was introduced into the GC system via an autosampler. The analytes were separated in the chromatographic columns (HP-5MS, 30 m in length, 0.25 mm in internal diameter and 0.25 μm in stationary phase thickness) using helium as the carrier gas at 1 ml/min for the MS column. The temperatures in the system were set to 300°C for the injector and 28°C for the GC oven for 2 minutes before being raised to 140°C at a rate of 20°C/minute, followed by an increase to 200°C at a rate of 4°C/minute and to 300°C at a rate of 15°C/minute; the

temperature of the GC oven was held at 300°C for 5 minutes. The MS source and the quadruple temperature were set to 250 and 150°C, respectively.

3.3.8 Quantification of non-volatile extractives

The amount of solvent-soluble, non-volatile material in the forest residues was quantified using the Tappi T204 cm-07 standard method with acetone as the solvent. The final extractive content was then calculated as a fraction of the original sample of dry forest residue.

3.3.9 Acetic acid and acetyl group analysis

The amounts of acetic acid and acetyl groups in the samples were determined using a KACET kit from Megazyme (Bray, Ireland). The acetyl groups were quantified after deacetylation by 1-hour alkaline hydrolysis in 1M NaOH(aq) at 70°C.

3.3.10 DP on cellulose

A 4g sample of solid residue was weighed for peracetic acid (PAA) delignification. The reaction was performed according to the procedure described by Kumar *et al.* [2013], with a 5% dry content and 5.5 g of PAA. After 53 hours had passed, the remaining material was washed until neutral pH was reached and then stored frozen. Samples were air-dried overnight prior to viscosity measurements being made. SCAN-C 15:62 [Immergut *et al.*, 1953] was used to evaluate the intrinsic viscosity in 0.5 M CED (copper (II) ethylenediamine), see Equation 3.2.

$$\mathrm{DP}_\eta = 0.75[\eta] \tag{3.2}$$

where:

DP_η = degree of polymerisation
η = intrinsic viscosity in CED

Only a relative comparison of the DP can be made because it is likely that PAA delignification contributes to cleavage of the glycosidic bonds.

3.3.11 Nuclear magnetic resonance

$^{1}\mathrm{H}^{13}\mathrm{C}$ HSQC NMR spectra were recorded for precipitated lignin (ca. 100 mg in 0.75 ml of DMSO-d6, Paper II), freeze-dried spruce (ca. 30 mg in 0.5 ml of DMSO-d6/NaOH in $\mathrm{D_2O}$, Paper III) and birch (ca. 30 mg in 0.5 ml of DMSO-d6, Paper IV)

extraction liquors . The phase-sensitive qualitative $^{1}H^{13}C$ HSQC NMR spectra were all recorded at 25°C in a Bruker Avance III HD 18.8 T NMR spectrometer (Rheinstetten, Germany) equipped with a 5 mm TCI Cryoprobe (cold 1H and 13C channels) operating at a frequency of 800 MHz for ^{1}H and 201 MHz for ^{13}C. The ^{1}H spectra were recorded at a 30° pulse angle, 6 s pulse delay, 1024 scans and 2.04 s acquisition time. The phase-sensitive qualitative HSQC spectra were recorded at a standard Bruker pulse sequence "hsqcedetgpsisp 2.3" with a 0.25 s ^{1}H acquisition time, 5.3 μs ^{13}C acquisition time, 3 s interscan delay and ^{1}JC–H coupling constant of 145 Hz for a total of eight scans; each spectrum was recorded for 4 h.

The NMR spectra were processed and analysed using the default processing template of MestReNova Vers.10.0.0 software, along with automatic phase and baseline correction.

4 Results and Discussion

This chapter describes the main results obtained in the different studies. They are presented here as a coherent report together with a discussion. The papers in which the results are reported are indicated for each section.

4.1 Pretreatment of wood (Paper I)

The effects of mild hydrothermal pretreatments on wood chips were investigated by performing hot water extraction or steam explosion followed by analysis of the composition of the remaining wood tissue. A series of treatments were performed for both methods at the same temperature but different residence times. The conditions, final pH, mass balances and composition of the native and treated wood as well as the extraction liquors are presented in Table 4.1.

4.1.1 Steam explosion and hot water extraction

A mass balance based on the weight of the initial material (wood chips), the residual solids (treated wood chips) and the dissolved solids (recovered from the extraction liquors) showed that 96-100 % of the material was recovered after steam explosion. Based on the chemical analysis performed, 93-97% of the solid and 55-66% of

Table 4.1 *Conditions, yields and compositional analysis of the treatments applied to the wood chips.*

	Native	*Hot water extraction*			*Steam explosion*	
Treatment conditions:						
Temperature (°C)		150			150	
Heating-up time (min)		15			2	
Treatment time (min)		15	30	60	15	30
Severity factor		2.9	3.1	3.5	2.7	3.0
Extract pH		3.6	3.5	3.3	4.3	4.1
Mass balance based on yield (%):						
Solid fraction yield		94.9	97.9	92.3	95.6	95.9
Extraction yield		1.4	2.3	4.3	1.7	1.9
Total solid yield		96.3	100.2	96.6	97.3	97.8
Mass balance based on compositional analysis (%):						
A. On wood:						
Cellulose	41.9	42.7	45.6	47.7	42.7	41.4
GGM	21.4	17.4	16.7	13.0	18.8	16.5
AGX	5.1	6.0	4.5	4.4	5.8	6.1
Klason	25.2	29.5	28.9	31.5	27.4	30.1
Total in wood	93.6	95.6	95.7	96.6	94.7	94.1
B. Based on extracts:						
Cellulose		0*	0*	0*	0*	0*
GGM		26.4	39.7	37.1	15.7	18.2
AGX		12.3	15.2	13.8	12.6	13.9
Klason		15.5	11.1	9.6	32.8	26.8
ASL		0.2	0.2	0.1	0.1	0.2
Total in extract		53.8	65.5	59.7	61.2	58.8

*acc. to the calculations all the glucose detected originates from galactoglucomannan

Figure 4.1 *Morphological changes induced by the treatments. From left: native wood, STEX-treated and HWE-treated (residence times as stated).*

the dissolved fractions were quantified and assigned to different wood components: the remaining, unidentified part of the dissolved content may contain fatty acids as well as degradation products from sugar monomers [Abatzoglou *et al.*, 1990; Jedvert *et al.*, 2014]).

The wood chips that were subjected to STEX or HWE at different degrees of severity are shown in Figure 4.1. Their appearance shows that the wood was clearly affected differently by the pretreatments. This change in colour may be related to the chemical alterations that occur in the hemicelluloses and lignin, leading to the formation of new chromophoric groups [Zhang and Cai, 2006].

In Figure 4.2, the yields obtained for both STEX and HWE are plotted against the severity factor (describing the combined effect of temperature and time, including both the heating-up step and the treatment itself). The HWE treatments generally had higher severity factors and higher extraction yields (with the exception of one with the shortest treatment time); there is, moreover, a more or less linear correlation between extraction yield and severity for hot water extraction (see also Table 4.1).

The pH decreased with increasing treatment time for both HWE and STEX and thus the final pH is strongly correlated to the severity of the treatments (see Table 4.1). The time dependence was more pronounced for HWE performed in a rotating autoclave with a longer heating-up period (and therefore a longer autohydrolysis time) than for STEX performed in a fixed autoclave with a relatively short heating-up time. For STEX the reaction time for autohydrolysis is relatively short, but, due to the effective advective mass transport (due to the explosion step), a large amount of dissolved components is transported out to the surrounding liquid. The mass transport of the dissolved substances in HWE relies purely on diffusion and may therefore be the limiting step of the extraction operation.

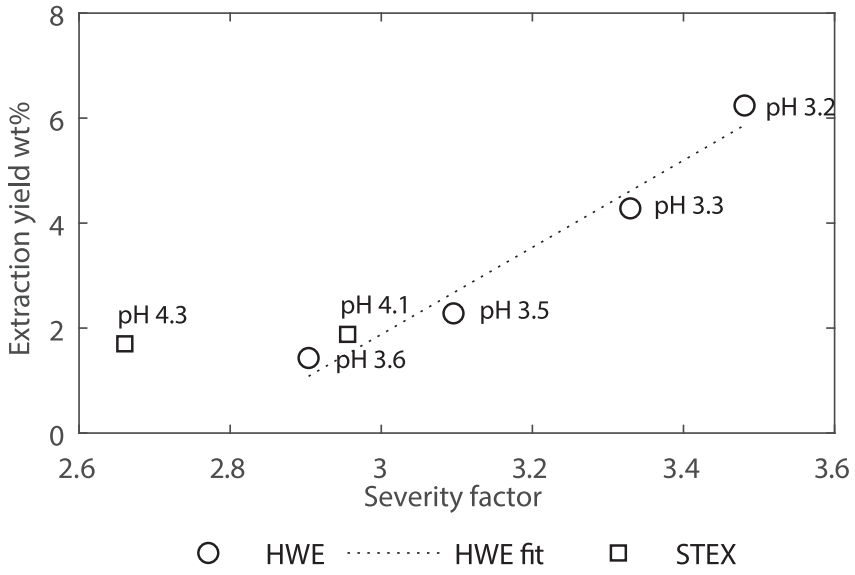

Figure 4.2 *Extraction yield versus severity factor.*

As the severity factor combines the treatment conditions such as temperature, heating-up and treatment time, it allows comparison of the data: e.g. the shortest HWE treatment time corresponds to $\log R_0 = 2.9$ and is relatively close to that of the longest treatment time of STEX ($\log R_0 = 3.0$). At the exemplified conditions STEX yielded slightly higher extraction. The difference in the yield may originate in the explosion step: during both STEX and HWE, the polymers undergo autohydrolysis and reaction products are transported out of the wood tissue by diffusion. Additionally, the rapid pressure release in STEX creates a pressure gradient across the wood tissue: this, pushes the liquid out through the pore system and thereby facilitates removal of the dissolved material. The combined effect of these phenomena determines both the amount and properties of the wood components extracted indicating that, at similar severity, more material is extracted from steam-exploded material.

The extraction of hemicelluloses is enhanced with time during both treatment methods, resulting in an increase in the content of hemicelluloses in the liquid and its decrease in the solid fraction. The removal of acid-sensitive GGM increases with time in both treatments, whilst the AGX follows this trend only in HWE (at longer overall treatment times). A possible explanation here is that the lower pH applied during HWE at long times provides conditions sufficient to accomplish the degradation and extraction of the AGX. However, these trends were not fully reflected in the composition of the liquors. In fact, the relative amounts of GGM and AGX in the

liquid fractions decreased at long treatment times (HWE 60 min), possibly due to the degradation of the monomer that occurs during the more severe HWE treatments. These observations are consistent with previous studies showing monomer degradation at comparable severity, typically at the severity factor corresponding to HWE 60 min (3.5) and above [Garrote *et al.*, 2004; Li *et al.*, 2010].

Table 4.1 shows that as the content of hemicellulose in the wood samples decreased, the relative amounts of lignin and cellulose increased. This change was more pronounced for the HWE pretreated wood, and very clear upon longer treatment: this, is in line with the findings presented above.

Figure 4.3 shows the result of the molar mass measurements of the extracted components. The UV signals reflect the molecules having aromatic structures (lignin, in this case), while the RI signal is more general and includes both carbohydrates and lignin. The chromatograms obtained for the material analysed show a considerable overlap of the UV and RI signals, which could indicate the presence of lignin-carbohydrate complexes [Tunc *et al.*, 2010; Lawoko and van Heiningen, 2011].

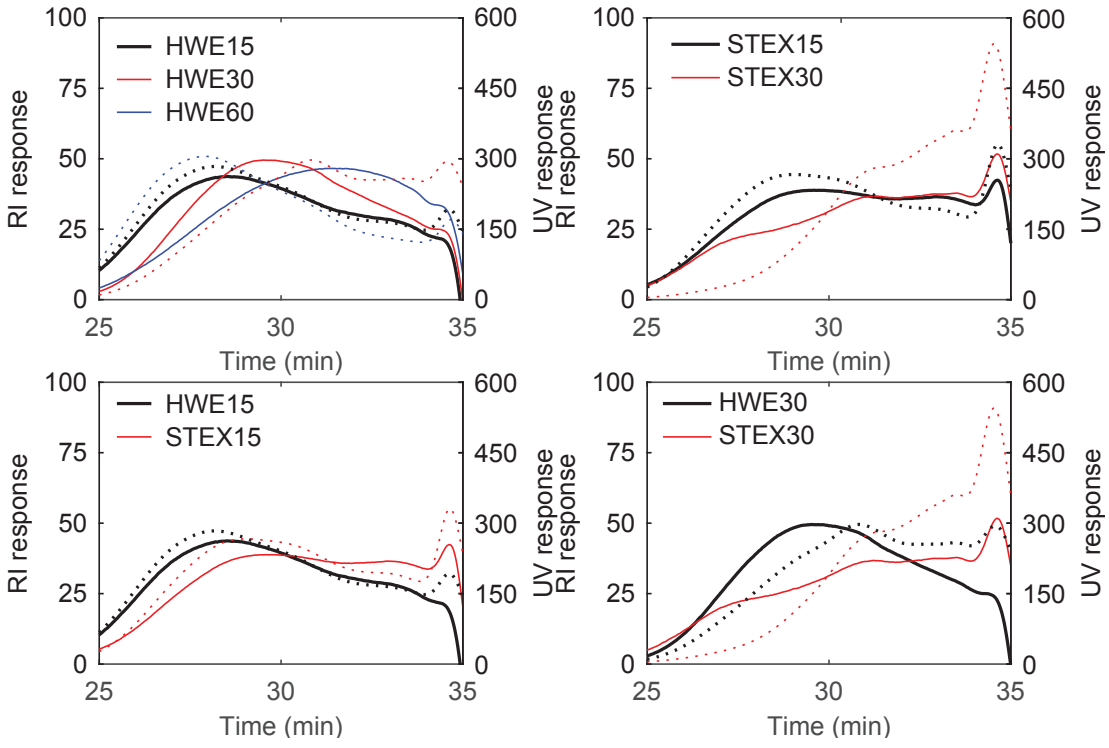

Figure 4.3 *The GPC results obtained for extracts from STEX and HWE. RI response: solid lines; UV: dotted lines.*

The onset of the RI response for prolonged HWE treatment shifts towards a lower molar mass, suggesting that the carbohydrates undergo a higher degree of hydrolysis. For STEX liquors, the response for both treatment times shows a substantial overlap and thus no time-related change. The fractions of higher molar mass observed for the STEX samples are in agreement with data in the literature on the extraction of hemicellulose from systems with a lower mass transport resistance (small-size wood chips, milled wood, etc.) [Rissanen *et al.*, 2014b; DeMartini *et al.*, 2015].

The UV signals for both HWE and STEX treatments, show a shift from a relatively high molar mass fraction for liquors from the sample treated for 15 min towards a lower molar mass after 30 min of treatment. This change can indicate either the degradation of the extracted lignin into smaller fragments or the hydrolysis of carbohydrate chains in lignin-carbohydrate complexes upon prolonged treatment [Li *et al.*, 2005; Martin-Sampedro *et al.*, 2014]. It is interesting to note that the prolonged HWE treatment (60 min) results in an increased molar mass, possibly because of either the partial condensation of lignin structures or the extraction of larger structures that require a longer time to be transported out of the wood tissue.

4.1.2 Local effects of HWE and STEX on wood

The contents of carbohydrate and lignin in different sections of the treated wood chips were studied in order to investigate local effects caused by the treatments studied. The central section of each chip (the inner layer of the middle section, see Figure 4.4) was used as a reference because its composition was assumed to be the least affected. Deviations from this reference were calculated by subtracting its contents of carbohydrate and lignin from the values determined for the individual sections.

In Figure 4.4 it can be observed that all treated sections show differences in composition depending on their location in the chip. They are more pronounced in the HWE samples, and mostly in the form of variations in the content of hemicelluloses between the outer and inner layers: the edge, corner and outer layer fragments of the HWE treated chips contain less hemicelluloses than the middle fragment for all treatment times. A profile is visible in the local composition already after 15 min of HWE treatment and becomes more pronounced after 30 min as the autohydrolysis proceeds: these variations are most probably a consequence of mass transport within the treated wood chip [Krogell *et al.*, 2016; Rissanen *et al.*, 2016, 2014a,b]. Diffusion is relatively efficient in the outer parts whereas in the inner fragment, the reaction products accumulate partially: diffusion is slower in this section, and its rate is lower than the reaction rate. Also, despite the high mobility of the hydrogen ions, local variations in acidity are possible due to local variations in the content of acetyl groups.

STEX results in fewer compositional variations (Figure 4.4). The most probable explanation for this is that the rapid pressure discharge that facilitates advective mass transport within the material, help to release any accumulated reaction products from the wood tissue. Moreover, the residence time is shorter compared to HWE, so autohydrolysis is less extensive, and accumulation of its products may be limited.

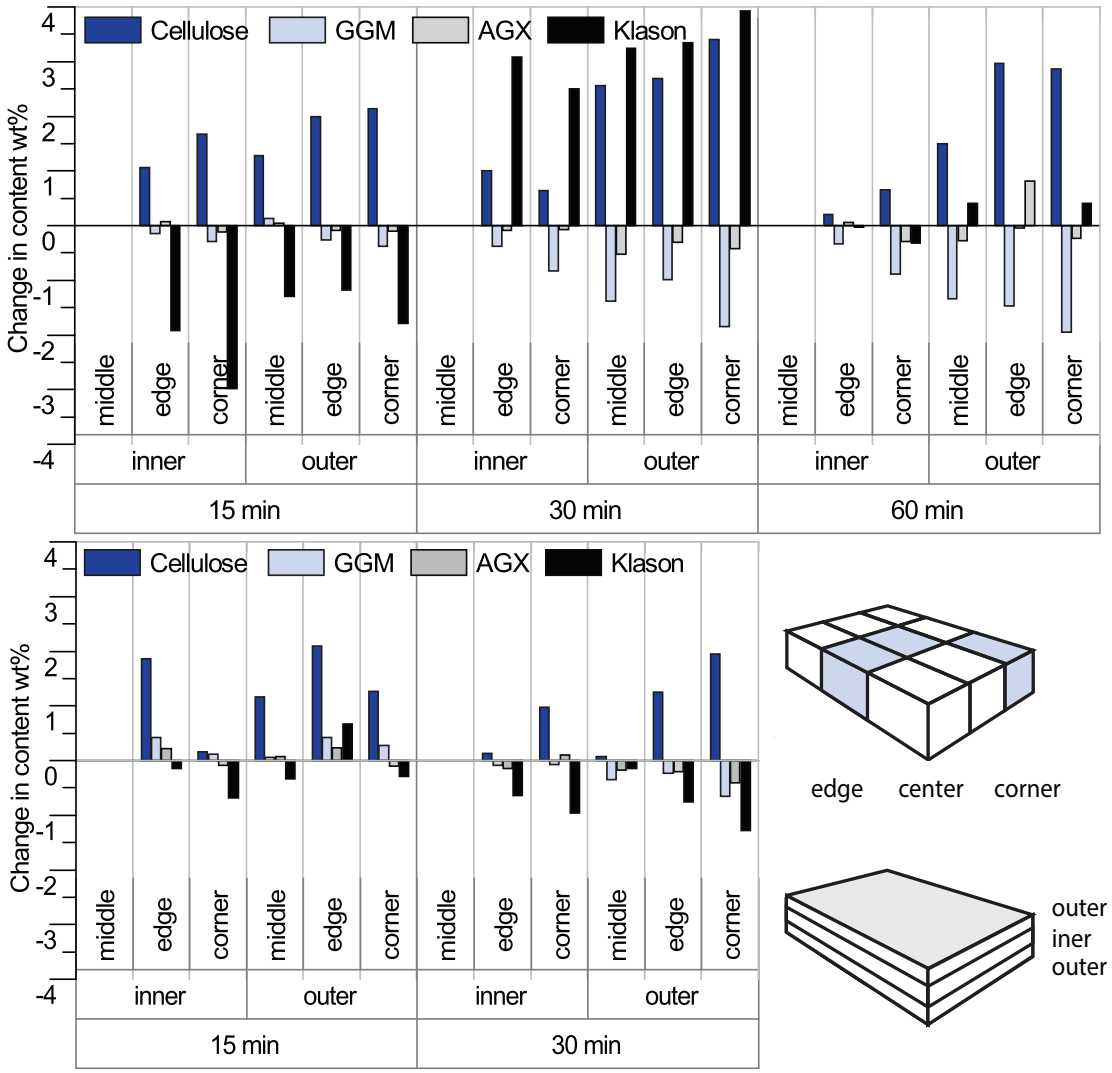

Figure 4.4 *Variations in the local composition of HWE (upper panel) and STEX (lower panel) treated wood. The schematic illustration shows the wood sections selected.*

Interestingly enough, a short STEX treatment time (15 min) induced an apparent increase in the content of hemicellulose and a decrease in Klason lignin in the external pieces, which actually indicates changes in the inner, i.e. reference, section. This consists with previous findings showing that the internal parts of steam-exploded chips are affected the most by the disintegrating effect of the treatment [Jedvert *et al.*, 2012b]. A possible explanation for this may be that STEX causes advective transport, which gives rise to the removal of hemicellulose from the more disintegrated internal morphology of the wood chip, and results in a relative increase in the content of hemicellulose in the more outer fragments. The effect is strongest in the middle since it relies on a pressure difference. A weak profile was observed after 30 min of pretreatment as the hemicelluloses are removed from the external part of the wood chips (due to autohydrolysis coupled with a more efficient mass transport from the outer sections, as discussed above). DeMartini *et al.* [2015] presented similar concentration profiles for hemicellulose subjected to short, but more severe, STEX-treatment.

4.2 Autohydrolysis (Papers III and IV)

The hydrothermal treatment relies on autohydrolysis and mass transport in the cell wall and pore system, respectively, as shown above. It would be beneficial for improved understanding to study these processes separately. The mechanism of autohydrolysis, and how it differs in differs types of wood (i.e. softwood and hardwood) was therefore chosen for further investigation.

The focus of Papers III and IV was on increasing understanding of the mechanisms of autohydrolysis, combined with mass transport in the cell wall, to reveal the structural changes it causes in hemicelluloses and cellulose. Softwood and hardwood wood meal therefore underwent hot water extraction in a small flow- through reactor. The material was milled to a size below 1 mm to limit the mass transfer resistance to the cell wall (level). Moreover, to ensure that the degradation of the reaction products was minimized, as further reactions were quenched by removal of the liquors from the reactor followed by instantaneous cooling.

4.2.1 Extraction yield

Hot water extraction of wood meal in the small flow through reactor resulted in virtually no mass losses (since total dry masses recovered after extraction are close to 100%) and high extraction yields (described by the total dissolved solid fraction of wood (TDS)), as reported in Table 4.2. It is known that the extraction yield of wood is dependent on both the size of the wood and the pretreatment system/conditions

Table 4.2 *Total dissolved solids (TDS) and yield of the flow in hot water extraction of spruce and birch in relation to time and temperature.*

Temp.	Time	TDS	Yield	TDS	Yield
°C	min	%		%	
		Spruce		Birch	
	30	2.8	100.8	2.6	101.1
130	60	6.5	102.3	4.5	96.2
	120	14.9	99.8	10.8	99.6
	30	11.2	106.2	9.6	95.9
150	60	17.7	89.5	29.8	100.7
	120	26.9	100.7	33.7	98.2
	30	26.5	103.3	36.3	106.0
170	60	30.6	105.7	43.9	101.8
	120	32.5	104.3	43.8	98.6

employed [Borrega and Sixta, 2015]. Here, the increased extraction yield obtained was most likely due to improved mass transport conditions (smaller dimensions and larger mass transfer area) and an enhanced driving force (the flow of fresh water). Moreover, it can be noticed that the TDS is dependent on the severity of the extraction, namely treatment time and temperature (and, consequently, pH), with more material being released at more severe conditions. Also, the extraction of hardwood generally had higher extraction efficiency. In order to follow the progress of the autohydrolysis in hardwood and softwood, the composition of the solid residue, as well as the extraction liquors, were analysed; the results are presented below.

4.2.2 Acidification

Wood has a slightly acidic character at room temperature, but elevated temperatures are nevertheless required if the acids present in the cell wall are to initiate degradation of the surrounding matrix. The mechanism of further acidification of wood tissue is

often assumed to be related directly to the deacetylation of hemicelluloses. This mechanism was tested by analysing the extracted liquors and residual solid samples for total acetyl group and free acetic acid content, see Table 4.3.

Solid residues obtained from the extraction of hardwood contained more acetyl groups compared to softwood. There are two reasons behind this dependency: firstly, the original birch tissue contained much more acetyl groups than spruce and, secondly, spruce wood seems to be more susceptible to removal of acetyl groups because these are attached to the easily extracted GGM (the extraction is triggered already at 130°C).

Generally speaking, the extraction of acetic acid is efficient for both spruce and birch wood. However, most of the acetyl groups remain bound to the hemicelluloses even after their extraction out of the tissue, resulting in the extracts having relatively low contents of free acetic acid. This indicates that deacetylation is relatively slow which, in turn, suggests that acetic acid may not be making the only/main contribution to acidification. This idea was investigated by estimating the pH based only on the released acetic acid (assuming its pKa to be 4.74 at 25°C) and compared with the pH values measured (see Figure 4.5). For both types of samples, the estimated pH values were higher than those measured, thereby indicating that there are other sources of protons.

Slow deacetylation and limited proton generation suggest that the release of acetic acid is, of course, contributing to the decrease in pH but also that the initial acidification and autohydrolysis are driven by other organic acids present in the cell wall: acids that can originate from both hemicelluloses and pectins (as discussed below).

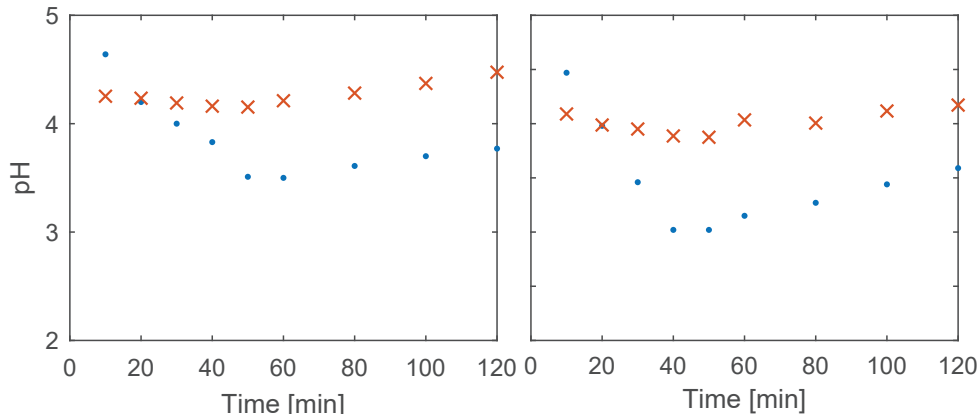

Figure 4.5 *Estimated pH values based on the amount of free acetic acid present (x), measured (.) at 150°C in the spruce (left) and birch (right) samples.*

Table 4.3 *Concentration of acetyl groups in the native wood, the solid residues and released in form of acetic acid after extraction in different conditions.*

Temp.	Time	Solid	Liquid		Total	Solid	Liquid		Total
(°C)	(min)	(mg/g wood)	(mg/g wood)		(%)	(mg/g wood)	(mg/g wood)		(%)
			bound	released			bound	released	
			Spruce				Birch		
Original wood		27.16	-	-	100	38.62	-	-	100
	30	22.9	1.9	0.1	92	37.3	0.7	0.2	99
130	60	26.4	3.4	0.3	111	35.4	1.9	0.5	98
	120	19.5	6.9	0.7	100	33.1	7.6	2.1	111
	30	19.1	3.7	0.4	85	30.7	10.4	0.9	109
150	60	9.1	10.1	1.0	74	15.6	32.6	2.3	131
	120	6.9	16.2	1.5	90	11.0	40.6	3.6	143
	30	6.0	14.1	0.8	77	9.7	30.5	2.8	111
170	60	3.6	15.3	1.0	73	2.9	33.1	3.3	102
	120	2.0	17.5	1.1	76	5.9	33.4	4.5	114

4.2.3 Extraction

Acidification at elevated temperatures triggers the acidic hydrolysis of different chemical bonds in the wood matrix, which leads to the partial depolymerisation, deacetylation and further degradation of hemicelluloses and lignin.

Table 4.4 exemplifies the changes in the composition of the solid fraction obtained after treatment at 150°C. Extraction of both spruce and birch leads to significant decreases in the amount of hemicelluloses, showed by reduced contents of arabinose, galactose, xylose and mannose. The lignin and “other” fractions also decreased (to a higher degree in hardwood); the glucose content decreased slightly.

Table 4.4 *Total dissolved solids (TDS), yield and composition of the residue solid compared to the original spruce and birch wood.*

Time	Ara	Gal	Glu	Xyl	Man	Klason	Other
min	mg/g wood						
	Spruce						
Original wood	10.1	19.4	422.8	44.5	99.7	293.0	110.6
30	b.d.	11.6	416.3	31.0	74.7	268.9	147.1
60	b.d.	4.0	371.6	18.5	36.2	217.8	69.8
120	b.d.	1.4	413.3	17.5	26.3	230.0	48.8
	Birch						
Original wood	2.9	9.5	409.9	197.7	19.8	230.9	126.8
30	0.1	6.1	389.0	172.0	17.6	178.3	97.7
60	b.d.	2.0	372.1	92.5	11.7	148.8	80.6
120	b.d.	0.7	380.3	69.2	9.9	122.9	62.7

b.d. = below detection limit

Hemicelluloses

The hemicelluloses most abundant in spruce are galacto(gluco)mannan and arabino-(glucurono)xylan and, in birch, glucuronoxylan and glucomannan. The extraction of the wood meal resulted in significant decreases in their content in the solid residues: decreases were most pronounced at prolonged treatment times and at higher extraction temperatures. Table 4.4 shows the changes in solid residues at 150°C, and Tables 4.7 and 4.8 present the composition of the liquors from spruce and birch treatments at different temperatures.

The influence of temperature on extraction was significant, e.g. 120 min of treatment led to a reduction in the GGM content of the wood to 12.9% at 130°C followed by 4.8% and 0.6% at 150°C and 170°C, respectively. The extraction behaviour in relation to time differed for temperatures studied, which is likely a result of a rather complex kinetics influenced not only by temperature but also by local pH, local com-

position and structure of the cell wall. For instance, the extraction rate increased slowly at 130°C but, at 170°C it was the highest at beginning of the treatment and decreased when the hemicelluloses were depleted from the solid material. The latter is, of course, the result of the fact that almost all of the glucomannans has already been extracted.

Mannose constituted 10% of the spruce tissue but only 2% of the birch. In spruce, it is removed very efficiently: 74% of the initial mannose is removed after 2h treatment at 150°C and 97% during the prolonged extraction at 170°C. In birch, on the other hand, where the initial concentration is much lower, mannose seems to be more resilient: only 76% was removed after 120 min at 170°C.

Birch glucomannan constitutes only 4% of the native tissue and is comprised of mannose and glucose at a ratio of approx. 1:1-2:1 [Timell, 1969; Fengel and Wegener, 1984b]. Since it is possible that there may be other sources of glucose in the samples analysed, trends in the GM content were evaluated based on the concentration of mannose that can be found only in GM. It is interesting to note that the biggest decrease in mannan content is noted in the first 10-20 minutes of the extraction, and that the reaction rate increases with temperature. Glucomannan is most probably released in the oligomeric form, since no free monomeric mannose was detected.

Galactose, being the side group of spruce GGM, was removed completely from spruce already after 30 min at 150°C. The original sample of birch contained less than 1% galactose, but it was also extracted easily.

In the case of spruce, the total Man:Glu ratio in the extracts obtained at 130 and 150°C was higher than 3.5:1, which is the proportion found in native spruce wood, thu indicating that all of the glucose detected originated from dissolved GGM. The glucose fraction increased at 170°C demonstrating its release from other sources, i.e. starch and cellulose.

Xylose, being the main constituent of xylan, constitutes 4% of the spruce and as much as 20% of the native birch. It is relatively more difficult to remove it completely: 23% of the original xylan remained in the spruce and 20% in the birch after prolonged treatment at 170°C.

Spruce arabinose, present as a side group on xylan as well as a component in pectins and arabinogalactan, is easily depleted from the solid material whilst the xylan backbone is more resilient. Moreover, xylose and arabinose were also found in both oligomers and free monomers. The fraction of xylan released as monomers is scarce and increases slowly with treatment temperature, while a considerable release of monomeric arabinose could be detected even at short treatment times. The presence of arabinose in the spruce extracts after short extraction times, where the xylose fraction is not significant, together with the fact that only xylan is left in the residual wood after severe extraction, indicates early cleavage of the AGX side chains,

which is in agreement with the fact that the furanosic side chains are more prone to be hydrolysed off [Sjöström, 1993]. Moreover, considerable amounts of arabinose were detected in the birch extraction liquors indicating that there are other sources of arabinose.

An interesting finding is, that the quantity of arabinose detected as free monomers was higher in some samples than the total amount of arabinose detected in the liquor after hydrolysis. The reason for this might lay in the acidic character of the sample. The freeze dried material was subjected to acid hydrolysis and the amount of carbohydrates detected in the hydrolysate was corrected using the hydrolysis yield. However, since the sample was acidic from the start, degradation of the monomers was likely to be more significant than for the neutral sugar monomers used as standards for acid hydrolysis yield determination. Therefore, in future experiments, the liquors should be neutralized prior to freeze-drying.

GX extraction from birch was strongly related to the treatment temperature, as 11% of the original xylan was removed after 120 min at 130°C, 65% at 150°C and 80% at 170°C respectively. It can be concluded that it was extracted in oligomeric form, as hardly any xylan was detected in form of monomers, even after the most severe treatment.

Approximately 23% of the original GX remained in the wood despite prolonged extraction at 170°C, which might be related to the topochemistry of the hardwood cell wall. Some publications suggest that there may be two different fractions of xylan with different degrees of extractability [Conner, 2007; Jara *et al.*, 2019; Dammström *et al.*, 2009; Ruel *et al.*, 2006]: one located close to the cellulose fibres and the other possibly bound to lignin.

Arabinose and galactose were present in birch extraction liquors, both as oligomers and monomers. It is interesting that some quantities of arabinose and galactose were also found in the spruce extracts prior to other monomers, originating from AGX and GGM, being released. The origin of these arabinose and galactose fractions is probably arabinogalactan. The presence of free arabinose and galactose monomers might suggest that the AG side chains are hydrolysed, while the amount of galactan found later in the extraction of birch might suggest that the remaining backbone is extracted in oligomeric form.

In order to obtain the total composition of carbohydrates (including the sugar acids, such as glucuronic and galacturonic acids), the freeze-dried liquors were subjected to methanolysis and silylation followed by GC-MS analysis. The results, which are summarized in Table 4.5, correspond in general, to the results obtained from ion chromatography. Additionally, glucuronic acid and galacturonic acids were found in both spruce and birch extracts. The former originated from arabinoglucuronoxylan in spruce and glucuronoxylan in birch, whilst the latter is an indication

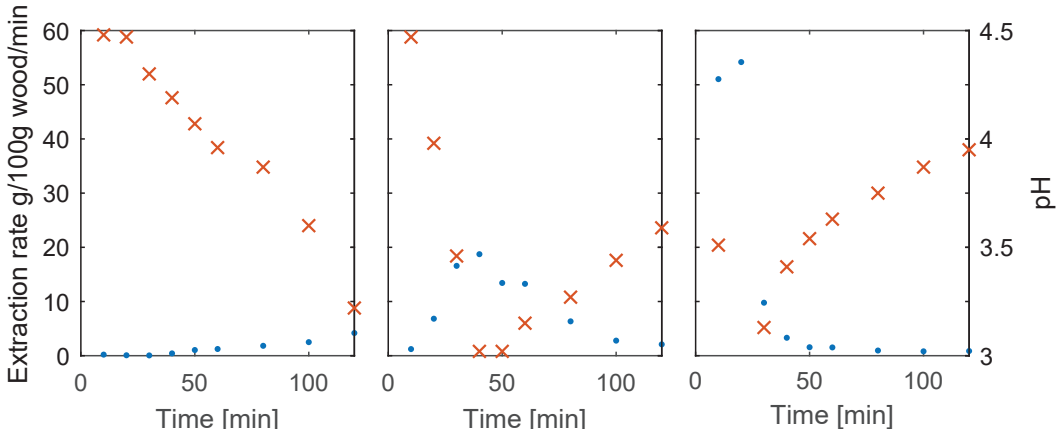

Figure 4.6 *The extraction rate of oligomeric xylose from birch (expressed in g/100g/min) (dots) versus pH (x) for temperatures 130, 150 and 170 °C (left to right).*

of the presence of pectins. No traces of volatile wood extractives were found; in the case of fatty acids, only a peak assigned to octadecanoic acid was present.

The concentration of glucuronic acid was lower than expected, based on the concentration of xylose. In despite of this, an estimation of its concentration suggests that it is present in higher concentrations than free acetic acid in corresponding samples, thereby confirming the acidification mechanism discussed above (see 4.4.2).

In general, the carbohydrate composition of the liquors has a strong temperature and pH dependence, which is especially clear for xylan (Figure 4.6). Possible explanations for this trend include the depolymerisation of hemicelluloses being accelerated by the low pH (the local concentrations of protons and hemicelluloses are expected to be significantly higher in the cell wall than in the extraction liquor), which promotes hydrolysis in the tissue prior to extraction, and the extraction of xylans, with their glucuronic acid side groups that influence the pH of the extraction liquor.

4.2.4 Lignin

It is important to bear in mind that lignin extraction was performed on wood meal: a fraction of lignin in the middle lamella was therefore revealed and could be extracted more easily.

Klason lignin is removed to a significant extent from the treated wood. At a temperature of 150°C, 22% of the lignin was removed from spruce and 47% from birch. This increased further for the latter up to 59%, at 170°C. The decrease in lignin content in the residual solids corresponds to the fact that Klason lignin constitutes

Table 4.5 *The carbohydrate compounds identified (using the probability values from the mass spectral data base) in the GC-MS analysis of the freeze-dried liquors extracted at 150°C. The data is presented as relative percentages, with the sugar compounds identified summed up to a total of 100%.*

	10min	20min	30min	40min	50min	60min
Spruce						
Galactose	3.2	3.0	3.3	3.9	5.4	4.4
Glucose	14.5	10.7	10.6	11.6	14.3	14.0
Mannose	38.7	39.4	41.9	47.8	54.5	52.1
Xylan	3.0	9.7	16.4	13.9	13.8	13.5
Arabinose	19.8	14.8	7.6	1.3	0.0	0.0
Glucuronic acid	12.4	13.5	9.7	11.3	0.0	4.1
Galacturonic acid	8.6	8.8	10.6	10.2	11.9	11.8
Birch						
Galactose	1.8	0.0	0.0	0.0	0.0	0.0
Glucose	28.0	8.3	2.8	0.0	3.2	4.1
Mannose	27.2	5.7	3.1	3.1	3.3	4.3
Xylose	19.2	61.1	84.8	89.3	86.1	27.4
Arabinose	14.7	13.6	5.0	3.9	4.4	57.9
Glucuronic acid	1.7	4.1	1.6	2.3	3.0	3.7
Galacturonic acid	9.5	9.3	2.8	1.5	0.0	2.6

a relatively large fraction of the dissolved solids. It consists of an acid-insoluble material comprising not only lignin but also precipitated extractives, fatty acids and sugar degradation products. The latter may, however, increase at more severe acid conditions.

For both types of wood, the dissolution of lignin seems to be extremely significant at the beginning of the treatment; similar observations were made by Jara *et al.* [2019] for maple wood meal. The presence of lignin in the liquors may indicate that there is an easily extractable fraction of lignin, even though this might originate from the increased accessibility of the middle lamella due to the fact that wood powder was used in the experiments.

In the case of spruce, the extraction of lignin seems to be slightly less efficient than for birch. It may be related to structural difference between softwood and hardwood lignin, with the spruce lignin being more prone to condensation at high temperatures. Moreover, the sum of the Klason fraction found in the solid residue and liquors exceeds the amount of lignin present in native spruce wood, which is not the situation for birch. This is most probably the result of carbohydrate degradation products (e.g. HMF) either condensing together with lignin during pretreatment, or precipitating along with the extracted lignin, to form an insoluble fraction in acid hydrolysis [Overend *et al.*, 1987; Borrega *et al.*, 2011].

In the case of birch, it was observed that the extraction pattern of lignin follows the behaviour of xylan; their amounts found extracted in the liquors indicate that they are partially co-extracted. This, in turn, implies the possible presence of lignin carbohydrate complexes (LCC): these, may contribute to the high overall removal rate of lignin at 170°C and 120 min, when up to 60% of the original lignin and 80% of the original xylan were removed.

A fraction of the lignin remained nevertheless in the solid residue, even after the most extensive extraction. The presence of a fraction that is very difficult to remove, together with indications of easily extractable lignin, suggests the existence of other types of lignin. Although this possibility was discussed in earlier studies [Ruel *et al.*, 2006; Jara *et al.*, 2019], there is a lack of knowledge regarding the differences in both the structure and origin of these other lignin structures. Here, it might be related to the uncovered middle lamella.

Molar mass of the extracted components

The results obtained from the molar mass analysis are presented in Figure 4.7. In general, GPC analysis of the extracted material shows variations in the molar mass of the samples extracted at different temperatures and after different extraction times. The UV and RI spectra overlap partially at all conditions, thus indicating the possible presence of LCC bonds. Moreover, the comparison of the chromatograms obtained

for liquors extracted at different conditions show, yet again, that the temperature had a big impact on the molar mass of the extracted material.

The RI spectra of both birch and spruce extracts indicate the presence of structures with a Mw above 10 kDa, while the UV response in this region is limited. It is therefore probable that this fraction contains mainly carbohydrates. The area of the RI peaks discussed follows the same trend as the amounts of arabinose and galactose detected in the extracted liquors (see Tables 4.7 and 4.8) and may therefore correspond to the presence of high molar arabinogalactan in the samples. The AG probably needs to undergo some hydrolysis prior to extraction as, in its native state, it has an average molar mass of 22 kDa [Willför *et al.*, 2002]. Other explanations for the presence of such high molar mass fraction include the extraction of starch (high molar starch in hardwood is removed in hot water extraction, as shown earlier by Tunc and van Heiningen [2011]) and the release of pectins from the middle lamella and primary cell walls that are exposed as a result of grinding.

The peak onset for spruce seems to shift towards a lower molar mass with prolonged treatment time whilst for birch, the variation is comparably modest for longer extraction times (approx. longer than 30 min). There is also larger variation with time for carbohydrates extracted from spruce at moderate temperatures (130 and 150°C): this indicates that the hydrolysis process is probably faster in birch, which correlates with the lower pH of the extracted liquid. Moreover, the influence of temperature is also visible in the spectra: higher temperatures resulted in narrower peaks with an onset at lower Mw. Therefore, it can be assumed that lower extraction temperatures led to a greater variation in the size of the polysaccharides extracted and a longer average chain length.

Following the spectra towards the lower molar mass, a peak is observed corresponding to Mw of 5.4 kDa visible in the RI chromatograms. It appears in liquors extracted at 150°C or 170°C and at residence times exceeding 20 min. As the UV response in this region is limited, it can be assumed that this may be a fraction of high molar mass xylan along with very small amounts of lignin. This is in agreement with earlier findings by Kilpeläinen *et al.* [2014] who suggested the presence of a xylan fraction with a molar mass of 2-8 kDa. Some degree of hydrolysis is probably necessary to extract this fraction since it is not found at 130 °C.

Another peak, with a top at around 1.5 kDa, is visible in all birch chromatograms recorded in both RI and UV. It might correspond to a xylan fraction of Mw ca. 1 kDa identified in other attempts of hot water extraction [Chen *et al.*, 2010; Borrega *et al.*, 2011; Kilpeläinen *et al.*, 2014]. Response from both detectors might indicate the presence of LCC structures; moreover, a peak at 380 Da may also correspond to LCC.

Two peaks were associated with the elution of xylan, thereby indicating the presence of two xylan fractions in birch. This might be explained by two mechanisms

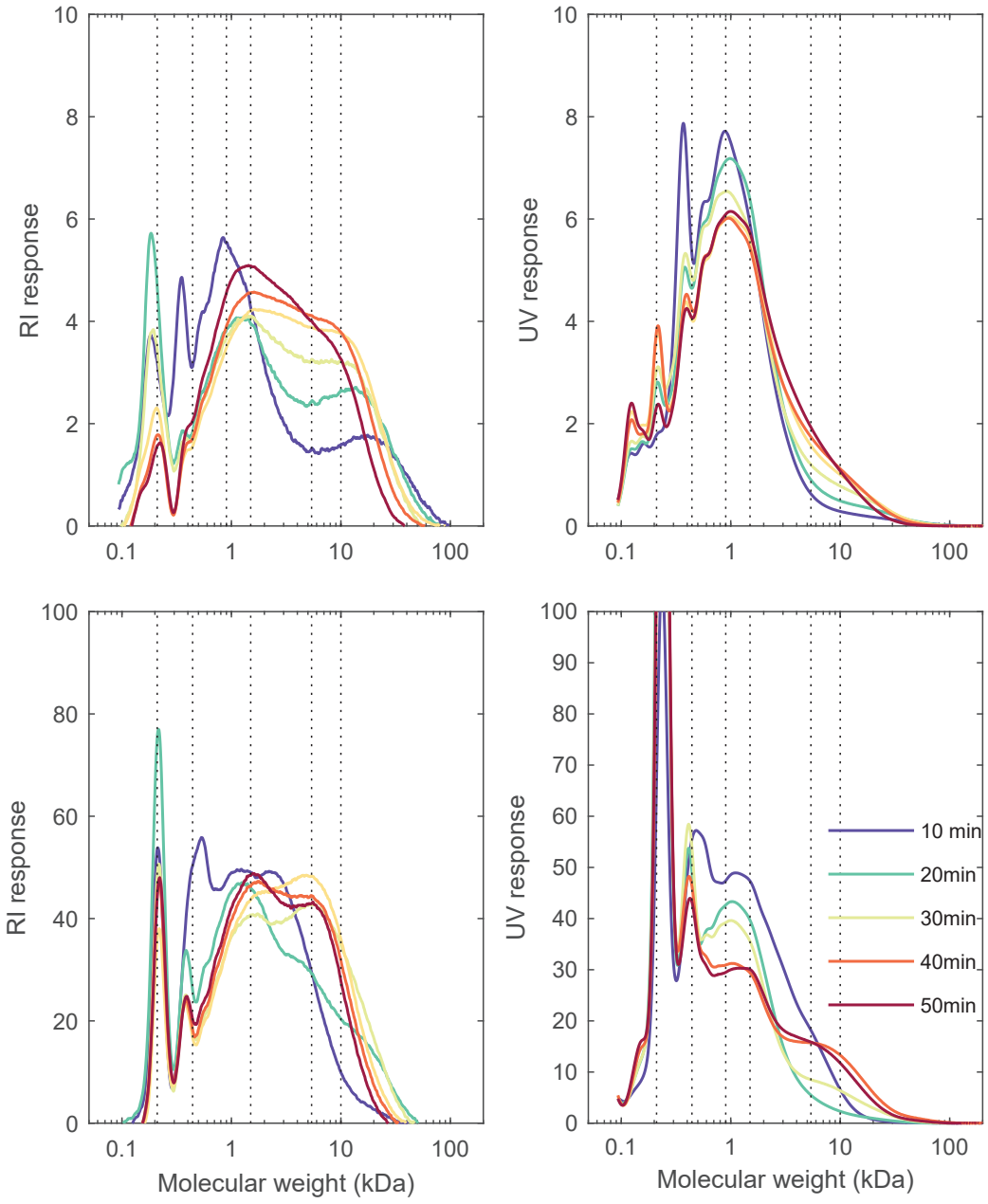

Figure 4.7 *GPC chromatograms of spruce (top) and birch (bottom) extraction liquors obtained at 150 °C.*

leading to extraction, namely depolymerisation into shorter fragments and hydrolysis of the LC bonds. As they are expected to be random, both may be of importance in leading to the decrease in molar mass and the presence of lignin-free fractions.

The spruce spectra contain a peak at 0.9 kDa visible in both UV and RI.

The lowest Mw peak in RI appears for birch at a retention time corresponding to approx. 210 Da. Being visible both in RI and UV, it cannot simply be associated with sugar monomers because a pentose unit has a Mw of 150 Da. The UV chromatograms also have an even lower peak that might indicate the presence of some degradation products. Another possibility, since it is mostly visible in liquors subjected to the mildest treatment, is that it may correspond to some extractives and/or their degradation products.

The lowest Mw signal in RI for spruce corresponds to 180 Da; the size of the peak seems to correlate with the amount of monomers released, although it could also be caused by uronic acids.

It is interesting that the UV peak in the spruce liquors' spectra related to the lowest molar mass is in the region of the molar mass of hydroxymethylfurfural (HMF), which has a Mw of 126 g/mol and is a degradation product of hexoses. Assuming the presence of HMF, and taking into consideration that the fraction of Klason lignin in the liquors is high, it is reasonable to assume that sugar degradation occurs during the course of autohydrolysis. The "HMF peak" occurs relatively early in the extraction process and is more pronounced at higher temperatures, so it can be assumed that a fraction of the sugars released start to degrade early on. No peak corresponding to furfural, a product of the degradation of pentoses could be detected, as its molecular mass (96 g/mol) is below the detection range of the instrument.

NMR

The freeze- dried liquor samples were analysed with HSQC NMR in order to study the changes in the structures of the hemicellulose and lignin. For spruce samples, the analysis was performed for liquors extracted at different temperatures whilst for birch, the effects of different extraction times at 150°C were investigated.

Spruce

The NMR spectra obtained for spruce samples confirmed the presence of both lignin and carbohydrates. Furthermore, signals originating from acetylated hemicellulose residues and free acetic acid were also found, which is in agreement with the discussion above. No signals characteristic of LCC coupling were found. However, the absence of these signals does not exclude the possibility that LCC were present: it can, instead, be due to the fact that they are less common than other bonds in the wood structure, thus giving a signal too weak to be observed in a sample rich in

carbohydrates. The effect of different extraction temperatures could not be observed clearly using this method

Birch

NMR spectra obtained for birch extracted at 150 °C also indicate the presence of lignin and carbohydrates. However, xylan was the only hemicellulose that was identified clearly, probably due to its high concentration in comparison to the other components.

The signals identified confirm the acetylation discussed earlier and indicate both C3 and C2 acetylation on xylose. The signals seem to increase in intensity with extraction time, which is in agreement with the data obtained for the quantification of acetic acid. Moreover, the spectra of extracts obtained after 40 min of extraction show an increased intensity in the signals assigned to the reducing end of xylose, suggesting increasing depolymerization of xylan with time (and therefore more chain ends). The gradual increase in the content of short-chained xylan and lignin-carbohydrate bonds may suggests that there are two xylan fractions: high molecular lignin-free xylan and short xylan chains connected to lignin. However, there may also be other explanations, as it may also be a result of random depolymerisation of the cell wall matrix that leads to a gradual release of smaller structures of limited solubility: high molecular xylan fragments probably require less depolymerisation to be extracted compared to LCC fragments, since the complicated, aromatic, network structure of lignin make solubilisation more difficult.

Also, some differences in the structure of lignin were observed. These were related mostly to the appearance and strength of signals corresponding to LC bonds and guaiacyl and syringyl units. However, it is not possible to conclude whether this corresponds to different types of lignin present in the tissue, as suggested by Ruel *et al.* [2006], as the removal of lignin is not complete in the conditions used in this work.

4.2.5 Cellulose

The extraction of glucose from the wood samples is limited, but that does not imply that the cellulose structure remains unaffected. As reported in Table 1.6, the intrinsic viscosity in CED, degree of depolymerisation and average chain scissions number are affected to different extents by the treatments. Different types of wood were delignified separately and they therefore cannot be compared with each other in terms of viscosity and DP. However, it is possible to make a relative comparison within one type of sample treated in the same way, which allows the average chain scission number (CSN) to be estimated and thus provide information of the extent to which autohydrolysis affected the cellulose. It can be seen that the CSN increases with the

Table 4.6 *The intrinsic viscosity in CED, degree of polymerization and number of chain scissions of cellulose from PAA-delignified residual solids and the original wood.*

Temp.	Time	Visc.	DP	CSN	Visc.	DP	CSN
°C	min	cm^3/g	-	-	cm^3/g	-	-
			Spruce			Birch	
Original wood		683	478	-	810	608	-
	30	627	439	0.1	676	507	0.2
130	60	674	472	0.0	741	556	0.1
	120	614	430	0.1	754	566	0.1
	30	664	465	0.0	794	596	0.0
150	60	528	370	0.3	773	580	0.0
	120	490	343	0.4	668	501	0.2
	30	425	298	0.6	667	500	0.2
170	60	376	263	0.8	575	431	0.4
	120	306	214	1.2	519	389	0.6

treatment severity for both types of samples, even though softwood cellulose is more affected overall. This can be related to the extractability of hemicelluloses located close to cellulose fibres in native wood tissue. Softwood cellulose is in contact with glucomannan, whereas glucuronoxylan surrounds hardwood cellulose. The former is easily extractable whilst the latter is more resistant and therefore may, to a certain extent, have a protective function.

Table 4.7 *Total composition of the freeze-dried spruce extracts and the free monomers detected.*

		Total composition								Free monomers					
Temperature (°C)	Time (min)	Ara	Gal	Glu	Xyl	Man	Klason	ASL	Other	Ara	Gal	Glu	Xyl	Man	Sum
		(mg/g wood)								(mg/g wood)					
130	10	0.68	0.60	0.59	b.d.	1.58	11.90	0.18	10.87	0.50	0.02	0.01	0.03	b.d.	0.55
	20	0.52	0.15	0.08	b.d.	0.34	2.65	0.02	5.70	0.79	0.02	b.d.	0.01	0	0.82
	30	1.34	0.43	0.24	0.04	1.03	5.79	0.04	7.02	1.77	0.05	b.d.	0.04	0	1.85
	40	0.52	0.21	0.11	0.06	0.52	2.14	0.02	3.79	0.85	0.03	b.d.	0.03	0	0.90
	50	0.77	0.40	0.20	0.17	1.03	3.53	0.02	6.71	1.23	0.05	b.d.	0.05	0	1.32
	60	0.58	0.40	0.19	0.20	1.01	2.97	0.03	8.77	1.10	0.06	b.d.	0.06	0	1.22
	80	0.73	0.53	0.31	0.48	1.66	3.73	0.03	11.22	1.25	0.10	b.d.	0.12	0	1.47
	100	0.66	0.87	0.61	0.94	2.78	4.85	0.04	11.80	1.05	0.16	b.d.	0.21	0	1.43
	120	0.40	0.95	0.66	1.09	3.11	3.06	0.04	15.30	0.71	0.21	b.d.	0.28	0.01	1.21
150	10	1.27	0.64	0.60	b.d.	2.20	16.22	0.10	17.80	2.06	0.07	b.d.	0.04	0	2.17
	20	1.24	0.75	0.58	0.52	2.67	8.13	0.05	18.58	2.24	0.15	b.d.	0.15	0	2.53
	30	0.70	1.25	1.03	1.18	4.52	5.03	0.05	20.64	1.17	0.22	b.d.	0.27	0	1.66
	40	0.39	1.68	1.39	1.63	5.79	5.47	0.05	25.36	0.53	0.32	b.d.	0.41	0.01	1.27
	50	0.16	1.67	1.48	1.44	5.74	4.11	0.06	24.71	0.30	0.48	0.02	0.70	0.11	1.61
	60	0.04	0.95	0.90	0.93	3.36	3.16	0.04	14.73	0.10	0.32	0.02	0.49	0.11	1.04
	80	0.02	0.88	0.90	1.30	3.70	3.23	b.d.	17.42	0.06	0.21	0.02	0.37	0.08	0.75
	100	b.d.	0.49	0.56	0.91	2.24	3.15	b.d.	10.33	0.03	0.13	0.02	0.26	0.08	0.52
	120	b.d.	0.31	0.38	0.62	1.48	2.13	b.d.	8.47	0.02	0.10	0.03	0.22	0.09	0.46
170	10	3.28	2.93	5.12	4.58	17.44	28.54	0.25	73.29	7.12	0.49	b.d.	0.57	b.d.	8.18
	20	0.55	3.52	4.78	5.90	16.03	14.43	0.17	62.33	1.15	1.26	0.12	1.95	0.43	4.91
	30	0.05	0.54	1.00	0.55	2.27	4.96	0.04	11.21	0.08	0.33	0.08	0.67	0.22	1.38
	40	0.02	0.18	0.45	0.59	1.03	2.58	0.02	4.76	0.03	0.13	0.06	0.32	0.12	0.66
	50	0.03	0.19	0.60	0.83	1.22	3.88	0.03	5.96	0.03	0.16	0.10	0.42	0.17	0.88
	60	0.02	0.12	0.45	0.60	0.83	3.51	0.02	3.83	0.02	0.12	0.10	0.36	0.15	0.74
	80	b.d.	0.14	0.51	0.71	0.90	3.70	0.03	7.96	0.02	0.14	0.15	0.47	0.18	0.96
	100	b.d.	0.04	0.19	0.26	0.27	2.88	0.03	4.50	0.01	0.07	0.10	0.25	0.10	0.54
	120	b.d.	0.01	0.07	0.08	0.08	0.59	0.01	0.85	b.d.	0.01	0.03	0.06	0.02	0.13

Table 4.8 *Total composition of the freeze-dried birch extracts and the free monomers detected.*

		Total composition								Free monomers					
Temperature (°C)	Time (min)	Ara	Gal	Glu	Xyl	Man	Klason	ASL	Other	Ara	Gal	Glu	Xyl	Man	Sum
		(mg/g wood)								(mg/g wood)					
	10	0.30	0.40	2.90	0.20	1.10	9.70	1.20	3.00	0.06	0.01	0.04	0.03	b.d.	0.14
	20	0.30	0.20	0.50	0.10	0.10	3.10	0.40	1.10	0.06	0.01	0.01	0.02	b.d.	0.09
	30	0.10	b.d.	0.10	0.10	b.d.	0.40	0.10	0.30	b.d.	b.d.	b.d.	b.d.	b.d.	0.01
	40	0.30	0.20	0.30	0.40	b.d.	2.00	0.40	1.20	0.08	0.01	0.01	0.02	b.d.	0.12
130	50	0.40	0.30	0.40	1.00	b.d.	3.00	0.70	1.70	0.11	0.02	0.01	0.03	b.d.	0.17
	60	0.40	0.20	0.30	1.20	b.d.	2.50	0.60	1.70	0.09	0.02	0.01	0.03	b.d.	0.15
	80	0.50	0.50	0.70	3.60	0.20	5.50	1.10	5.30	0.03	0.05	0.02	0.11	b.d.	0.21
	100	0.40	0.50	0.60	5.00	0.20	4.80	1.20	6.50	0.15	0.05	0.02	0.12	b.d.	0.34
	120	0.30	0.70	0.60	8.40	0.20	6.00	1.60	8.70	0.01	0.05	0.01	0.13	b.d.	0.2
	10	0.80	0.60	3.50	1.20	1.60	11.60	1.00	3.00	0.30	0.03	0.08	0.09	b.d.	0.52
	20	1.00	0.80	1.50	6.80	0.70	9.40	1.40	7.80	0.46	0.09	0.04	0.21	b.d.	0.8
	30	0.70	1.10	1.30	16.60	0.60	10.40	2.30	16.30	0.27	0.12	0.11	0.36	b.d.	0.86
	40	0.20	1.00	1.10	18.70	0.60	10.60	2.80	21.70	b.d.	0.12	0.02	0.01	0.66	0.8
150	50	b.d.	0.60	0.60	13.40	b.d.	12.00	2.00	28.40	b.d.	0.07	0.03	b.d.	0.96	1.06
	60	b.d.	0.60	0.90	13.30	0.40	9.60	2.10	13.20	0.07	0.12	0.09	0.58	b.d.	0.86
	80	b.d.	0.50	1.10	12.70	0.60	11.60	2.10	11.70	0.06	0.10	0.04	0.48	b.d.	0.67
	100	b.d.	0.30	0.60	5.60	0.30	5.90	1.10	6.00	0.02	0.05	0.03	0.34	b.d.	0.45
	120	b.d.	0.20	0.60	4.20	0.20	4.80	0.90	4.80	0.02	0.05	0.04	0.34	0.01	0.45
	10	3.40	3.70	10.0.	0.85	5.80	40.30	6.50	58.80	0.59	0.22	0.24	0.79	b.d.	1.85
	20	0.10	2.40	3.70	54.20	1.80	33.30	7.70	58.50	0.13	0.26	0.09	1.24	b.d.	1.71
	30	b.d.	0.50	1.10	9.80	0.60	12.00	2.20	9.90	0.05	0.14	0.15	0.69	b.d.	1.03
	40	b.d.	0.10	0.70	3.30	0.30	5.10	0.80	3.50	0.02	0.05	0.07	0.30	b.d.	0.44
170	50	b.d.	0.10	0.40	1.60	0.10	2.70	0.50	1.60	0.01	0.02	0.03	0.15	b.d.	0.21
	60	b.d.	b.d.	0.40	1.50	0.10	3.60	0.50	0.60	0.01	0.02	0.04	0.15	b.d.	0.22
	80	b.d.	0.10	0.70	1.90	0.20	3.50	0.60	2.10	0.01	0.02	0.06	0.20	b.d.	0.3
	100	b.d.	b.d.	0.70	1.60	0.20	3.20	0.60	1.50	0.01	0.01	0.06	0.18	b.d.	0.27
	120	b.d.	b.d.	0.80	1.70	0.10	3.10	0.70	1.40	0.01	0.01	0.07	0.19	0.01	0.29

4.3 Pretreatment of forest residues (Paper II)

This section presents the results of the experiments performed in order to evaluate the possibility of using the hydrothermal pretreatment in a biorefinery process utilizing forest residues. The material was subjected to soda and kraft cooking after steam explosion to investigate the changes rendered by pretreatment affect further processing of the material.

4.3.1 Characterisation of the raw material

The material used in this study was constituted of a highly inhomogeneous mixture of xylem and bark tissues from various parts of different species of tree (Figure 4.8). Analysing the original material was relatively difficult, due to its inherent homogeneity and high content of extractives. In order to understand the composition of the material and its variations to a greater degree, some fraction of wood chips (possibly originating from stem wood and bigger branches), small branches and bark were handpicked and analysed separately, see Table 4.9.

The results showed the content of lignin to be relatively high in the bark and branches. This is related not only to an inherently high content of lignin in the material but also to the high content of extractives that affects the analysis, thereby resulting in an overestimation of the content of Klason lignin (some extractives co-precipitate with the lignin during acid hydrolysis). Also, the "other" fraction (i.e. undetected compounds, comprising mostly extractives and ash) was relatively large in these sections when compared to wood.

The wood tissue had a significantly higher content of carbohydrates than the other samples that were analysed. Although the arabinose content is higher in bark and branches than in wood tissue (possibly due to the presence of arabinans and pectins in bark), the galactose fraction is highest in branches and may be related to the presence of reaction wood. The content of lignin (30.4%) and low mannose fraction (5.5%), together with a high content of xylose (14.2%), suggest a large fraction of hardwood in the material [Raisanen and Athanassiadis, 2013; Teleman, 2016].

Extraction of the refined forest residues with acetone allowed the content of extractives to be measured (Table 4.10). The detection of acetone soluble extractives typical of softwood (such as isopimaric, dehydroabietic and abietic acids), as well as those more commonly found in hardwood (such as β-sitosterol and botulin) [Fengel and Wegener, 1984a], are other indications that the material is a mixture of softwood and hardwood. The overall content of extractives in the material was 16.9 %. It is worth mentioning, however, that this value may have been even higher in the original material, as it is possible that some of the extractives were vaporised during steaming and refining.

Table 4.9 *The amount of anhydrosugars present in the sample, expressed as a weight fraction.*

Sample	Ara (%)	Gal (%)	Glu (%)	Xyl (%)	Man (%)	Klason (%)	Other (%)
Bark fraction	3.9	1.9	18.1	5.7	1.7	47.0	21.7
Branches fraction	2.3	3.8	26.3	10.1	4.4	36.4	16.8
Wood fraction	0.5	0.9	37.8	22.4	3.5	22.4	12.6
Refined material	1.2	1.6	36.7	14.2	5.5	30.4	10.3
*	(0.1)	(0.1)	(1.3)	(1.6)	(0.3)	(1.1)	(1.7)

**The deviation found from 3 samples.*

4.3.2 Steam explosion

Some of the changes rendered by steam explosion such as the fragmentation of the material and the change in its colour, could be observed with the naked eye (Figure 4.8), some were identified by means of chemical analysis (Table 4.11) and others were seen as effects of the further delignification of the forest residues (and will be discussed later).

Table 4.10 *The contents of extractives in the forest residues, expressed as a weight fraction.*

Extractive	Amount(%)
Linoleic acid	2.8
Octadecanoic acid	2.9
Isopimaric acid	1.3
Dehydroabietic acid	3.7
Abietic acid	2.0
β-Sitostero	1.6
Betulin	2.6
Total	16.9

Figure 4.8 *The material analysed. Top panel: untreated, steam-exploded and refined forest residues. bottom panel: separated fractions of wood chips, bark and branches.*

Table 4.11 presents the changes in the composition of the material after steam explosion at 4 or 7 bar (the amounts of the components are given as weight percent of the sample, and not of the original material). The most striking change is the decrease of the "other fraction" (from 10.5 to 8.7 and 6.1 wt% for 4 and 7 bar treatment, respectively). A possible explanation for this is the evaporation and dissolution of extractives during steam pretreatment, although some of the extractives may react with the lignin during the treatment or precipitate with it when subjected to the acid treatment that forms a part of the Klason analysis procedure [Burkhardt *et al.*, 2013]. The changes in the content of extractives through both solubilisation/evaporation and co-precipitation with lignin result in a small increase of the Klason fraction.

The amounts of the components are presented as fractions of the material, and thus they show apparent changes in the composition: a significant decrease in one fraction may imply an increase of a more stable component. In this case, the "other fraction" decreases, which is probably the reason for an apparent increase in the glucose content, as cellulose (being the main source of glucose) ought to remain stable during this treatment. In the same way, the relatively stable content of carbohydrates originating from hemicelluloses indicates the partial extraction of hemicelluloses: if

Table 4.11 *Composition of the refined untreated and steam exploded material, in wt% on refined wood residues*

STEX (bar)	Ara (%)	Rha (%)	Gal (%)	Glu (%)	Xyl (%)	Man (%)	Klason (%)	Other (%)
-	1.2	1.6	1.1	36.7	14.2	5.5	30.4	10.5
4	1.0	1.6	1.1	38.0	15.7	5.7	29.1	8.7
7	0.7	1.6	1.1	39.6	15.6	5.3	31.1	6.1

this were completely stable, a relative increase in the hemicellulose content would have been observed.

Compared to what was found for wood tissue, the removal of hemicelluloses seems to be limited: in particular, there seems to be no effect on xylan, while the mannan content decreases only at 7 bar treatment.

The effect of steam explosion on the composition of the material was somewhat limited compared to the results obtained for softwood in Paper I. It may be related to the properties of the forest residues, such as the contents and characteristics of the hemicelluloses, lignin and extractives. Apart from having different origins, the sizes of the treated material differed: steam explosion was performed on refined material and, in Paper I, on whole wood chips. The literature on steam explosion shows that the size of the wood pieces being treated influences the effect of the treatment significantly: the impact is greater on larger chips [Ballesteros *et al.*, 2000; DeMartini *et al.*, 2015; Jedvert *et al.*, 2012b]. The material here was refined and was therefore relatively small in size; consequently, the pressure difference had a very limited influence on the solid material when the pressure dropped suddenly in the whole sample (i.e. the explosion effect). The limited effects of the explosion reduce the advective transport and thus explain the rather modest removal of solubilised structures from refined forest residues upon STEX.

4.3.3 Pulping forest residues

The steam-exploded forest residues were cooked for 60 and 180 min in kraft and soda liquors; an additional series of cooks were performed on untreated material for reference purposes, with the same hydroxide ion concentration and cooking times of 30 to 180 min (60 or 180 min for pretreated samples). The kraft cooks had higher total ion concentrations due to the addition of hydrogen sulphide and carbonate ions.

The material, obtained in the form of pulps, was analysed for composition as was the precipitated black liquor lignin; the results are given in Table 4.12.

Comparison between soda and kraft pulping

Analysis of the pulps shows that the cooking yields were relatively diverse for the samples and treatment conditions selected. In general terms, soda cooking was found to result in higher total yields (due to the greater amount of residual lignin present after cooking). The delignification and removal of carbohydrates seem to be rapid initially (i.e. during heating-up and 30 min cooking) and slower later on in the treatment: this is similar to the cooking behaviour known for xylem (with differences in the cooking efficiency). As expected, the removal of lignin was more efficient when kraft cooking was applied, since the cleavage of phenolic β-O-4 bonds in lignin is mediated by hydrogen sulphide ions. When compared to kraft [Jedvert *et al.*, 2013] and soda cooks of softwood [Wigell *et al.*, 2007], pulps based on forest residues contain less lignin, which can be related to the different composition (mixed hardwood and softwood) and morphology of the initial material.

Hemicelluloses undergo rapid dissolution and peeling during the heating-up stage (GGM), and at the beginning of the cooking process (both GGM and AGX); this is followed by alkaline hydrolysis of the glycosidic bonds and secondary peeling later on in the cook. As a result, kraft cooking of softwood causes degradation of ca. 75% of the galactoglucomannan and 40% of the arabinoglucuronoxylan present [Sjostrom, 1977]. It seems that arabinose, galactose and mannose follow this trend when forest residues are pulped.

Nonetheless, the xylan fraction declines rapidly only at the beginning of the cook: the remaining (roughly half of the initial value) is relatively stable throughout the rest of the cook, showing a minimal increase with time (Figure 4.9). The behavior of xylan follows the changes in the content of Klason lignin in the precipitate obtained from the black liquor. The content of xylan and lignin in pulp is known to show a linear relation for wood subjected to soda [Wigell *et al.*, 2007] or kraft cooking [Matthews, 1974]. This trend was not found for forest residues.

A possible explanation for the behaviour found for xylan may be an increase in its re-adsorption on cellulose fibres: this is commonly associated with the chemical pulping of wood, and depends on the cooking conditions and concentration of xylan in the liquor [Hansson and Hartler, 1969; Hansson, 1970; Meller, 1965]. It is possible that, the somewhat different structure of the cellulose in the material promotes increased re-adsorption, or that different types/structures of xylan are present. Thus, the re-adsorption properties contributing to the resorption may be different from those of stem wood.

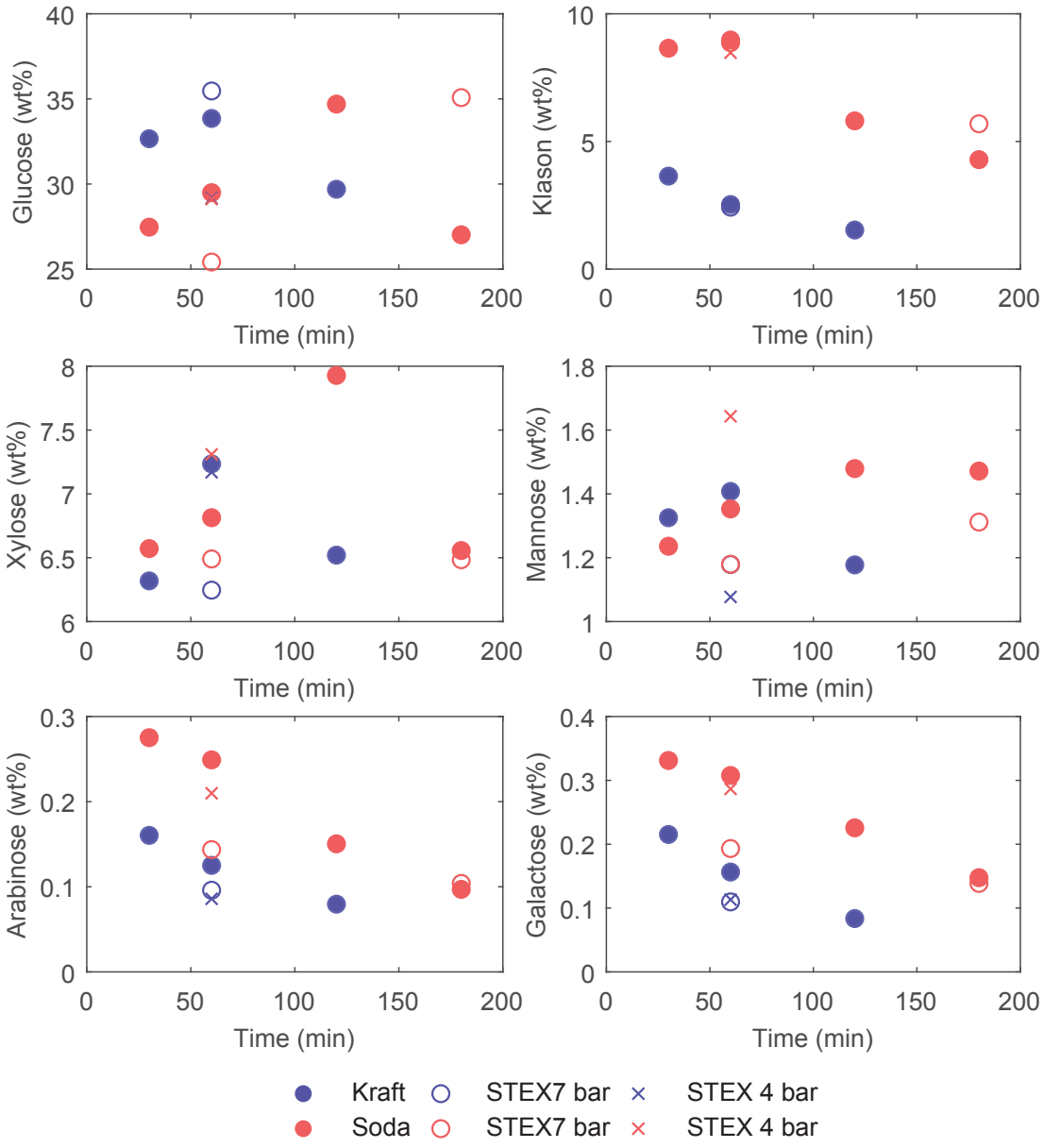

Figure 4.9 *Changes in the composition of sugar and lignin upon cooking for 30 to 180 min (as wt% of the initial material).*

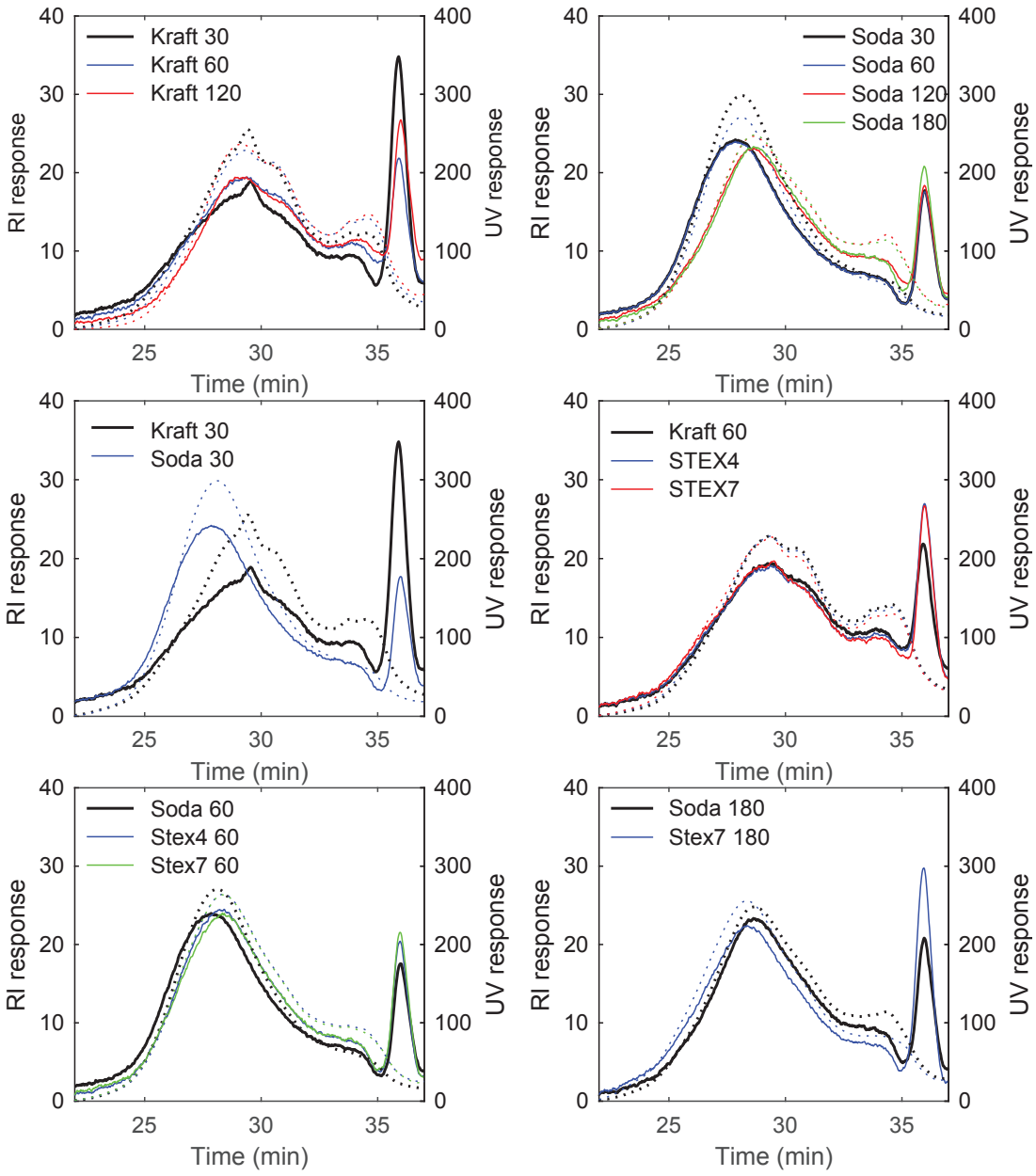

Figure 4.10 *The GPC chromatograms obtained for lignin precipitate from the black liquor, where RI response is represented by solid lines, while UV by dotted lines.*

It is interesting that the content of xylan is generally higher in soda pulps. As the hydroxide ion concentration was the same in both treatments, the difference should lay in the presence of hydrosulphide ions. Thus, a possible explanation is that the degradation of lignin and the removal/release of xylan from lignin carbohydrate complexes are more efficient during the course of kraft cooking.

The glucose originates mostly from cellulose and its content in the solid residue is therefore affected to just a limited extent. The content of glucose decreases only slightly upon cooking, possibly due to the peeling of glucomannan and cellulose as well as to the degradation of starch (Table 4.12). The variations detected in the glucose content can also be a reflection of the aforementioned inhomogeneity of the material. Kraft cooking seems to yield a higher glucose content than soda cooking, as is commonly observed in wood pulping operations.

The lignin extracted from the material during cooking was analysed (after precipitation from the black liquors) for molar mass by GPC; the results are shown in Figure 4.10. As expected, the RI and UV signals overlap but the RI responses of all the samples show a peak indicative of low molar non-aromatic compounds. These molecules may originate from extractives and are possibly degradation products.

A comparison of the results obtained from different pulping experiments shows that lignin from soda cooking generally has a higher molar mass, which concurs with observations for wood: a possible explanation is that it is extracted without cleaving phenolic $\beta-\mathrm{O}-4$. In the case of soda pulping, plots obtained for the 30 and 60 min cooks overlap: the prolonged treatment induced a shift towards a lower molar mass, suggesting degradation of the lignin present in the liquor.

Effects of pretreatment on pulping

Comparing the steam-exploded material prior (Table 4.11) and post kraft and soda cooking (Table 4.12), it can be ascertained that the "other fraction" in pulps is much smaller and most likely indicates the dissolution of extractives: the mass balance improved as the extractives were removed. A comparison between steam-exploded and untreated material cooked for 60 min shows that the pulps have similar contents of Klason lignin, which is in contrast to the behaviour observed previously for wood, where the pretreatments enhanced delignification [Jedvert *et al.*, 2013]. The difference can be related to the aforementioned effect of the size of the material being treated reducing the effect of the steam explosion. It is interesting to note that although the content of Klason lignin was the same in the pulps, it was found to be higher in the precipitates of black liquor taken from the pretreated samples. This discrepancy can be a result of the generation of pseudo-lignin (lignin condensed with degradation products from hemicelluloses) during the treatment [Heitz *et al.*, 1991].

Steam explosion affected the carbohydrate composition of the pulps. It resulted in a lower xylan content after 60 min of cooking, independent of cooking method; when performed at 7 bar, the xylan content in the lignin precipitate from the black liquor was also found to be lower. The latter can be a result of the partial extraction and degradation of xylose into furfural in acidic conditions during steam pretreatment [Abatzoglou *et al.*, 1990; Janzon *et al.*, 2014; Li *et al.*, 2005]. Last, but not least, the glucose content was somewhat decreased in the pulps, possibly as a consequence of the removal of hemicelluloses and pectins during pretreatment.

Table 4.12 *Composition of the pulps and the precipitate obtained from acidified black liquor.*

Pretreatment	Cooking Conditions			Composition of Pulp								Composition of Precipitate			
STEX	Type	Time	Yield	Ara	Gal	Glu	Xyl	Man	Klason	ASL	Other	Ara	Gal	Xyl	Klason
(bar)		(min)	(wt %)	(wt% on initial material)								(wt% on lignin)			
-	-	-	-	1.16	1.60	36.69	14.17	5.50	30.37	0.12	10,27				
-	Kraft	30	42.2	0.16	0.22	32.66	6.32	1.33	3.64	0.41	-2.52	0.6	0.4	7.3	71.2
-	Kraft	60	44.7	0.13	0.16	33.85	7.24	1.41	2.53	0.31	-0.93	0.6	0.6	6.3	71.9
-	Kraft	120	39.8	0.08	0.08	29.69	6.52	1.18	1.53	0.39	0.31	0.4	0.4	2.3	66.1
4	Kraft	60	39.0	0.09	0.11	29.17	7.17	1.08	2.53	0.38	-1.49	0.5	0.5	5.0	73.5
7	Kraft	60	45.7	0.10	0.11	35.47	6.25	1.18	2.43	0.27	0.17	0.2	0.2	1.2	78.7
-	Soda	30	46.6	0.28	0.33	27.47	6.57	1.24	8.65	0.66	1.46	0.7	0.4	13.8	70.8
-	Soda	60	48.8	0.25	0.31	29.49	6.81	1.35	8.97	0.63	1.02	0.7	0.5	10.2	72.9
-	Soda	120	50.1	0.15	0.23	34.69	7.93	1.48	5.81	0.56	-0.70	0.5	0.5	5.3	76.1
-	Soda	180	42.2	0.10	0.15	27.02	6.56	1.47	4.29	0.00	2.59	0.4	0.5	3.3	76.0
4	Soda	60	51.2	0.21	0.29	29.11	7.31	1.64	8.48	0.54	3.60	0.5	0.4	8.8	73.2
7	Soda	60	49.5	0.14	0.19	25.42	6.49	1.18	8.87	0.50	6.75	0.3	0.2	2.6	82.3
7	Soda	180	49.5	0.10	0.14	35.08	6.48	1.31	5.69	0.25	0.70	0.1	0.1	0.2	77.0

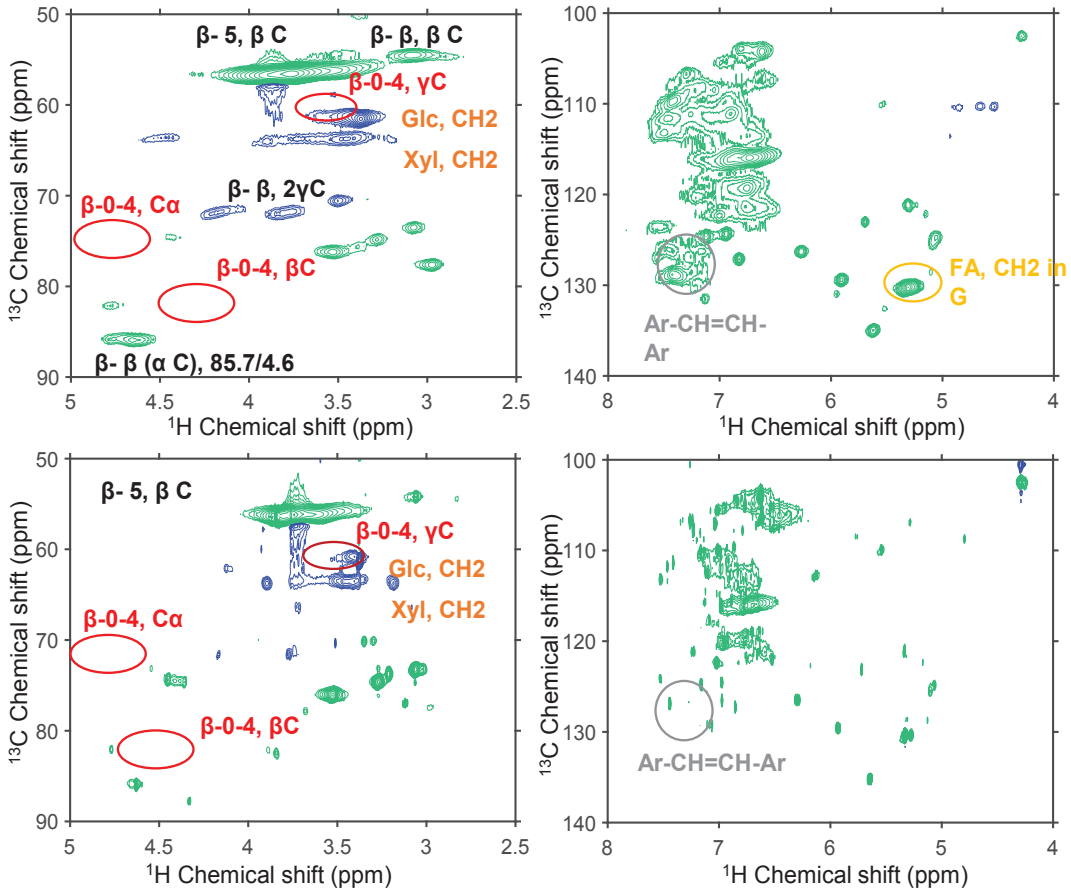

Figure 4.11 *The NMR-spectra of the lignin rich fractions extracted in 180 min soda cook of steam exploded (top) and non-pretreated forest residues (bottom)*

The GPC results reflect the molar mass distribution (MMD) after pretreatment and cooking, making it difficult to evaluate the effect of the steam explosion itself. The plots for kraft lignin, shown in Figure 4.10, for untreated and steam-exploded material overlap fully. For the 60 min soda cook, steam explosion caused a minimal shift towards longer elution times, suggesting a decrease of the molar mass. However, when the cook was prolonged to 180 min, the lignin MMD of the pretreated sample was shifted towards the higher molar mass region. Depending on which lignin fractions are extracted from the material, the effects of pretreatment will not necessarily be detectable for all the pulping times. Steam explosion has an effect mostly on large lignin fragments (due to condensation), which will not be visible until these fragments have been transported out from the material, i.e. only after long delignification times.

The $^{1}H^{13}C$ HSQC NMR-spectra of the lignin rich fractions extracted in the soda cooks of the forest residues reveal the presence of both S- (106.6/7.32 and 103.8/6.7 ppm) and G-lignin along with considerable amounts of extractives, mainly fatty acids: CH_2-COOH (34.2/2.2 ppm), CH_2 (25/1.5, 29.1/1.1, 31.8/1.2 ppm), CH_3 (15/0.7 ppm). The latter concurs with the low molar mass signal observed in the MMD chromatograms.

No signals of $\beta - O - 4$ could be found after soda cooks were completed (Figure 4.11). It may be noted that lignin extracts from soda cooks preceded with the STEX treatment (7 bar) gave rise to signals that were absent/vague in the samples cooked without pretreatment, namely signals indicating:

- the increased presence of β - β linkages:αC (85.7/4.6), βC (54.1/3.1) and 2γC (71.4/4.1 and 74.1-3.8)
- the presence of stilbene structures, ArCH=CHAr (126-129.7/7.0-7.4)
- the enhanced presence of xylane and hexose residues: Xyl (CH_2, 62.1-64.3/3.3-3.8) and Glc ($CH_2$2 60/3.-3.8)
- an enhanced presence of fatty acids such as: CH=CH (128.2/5.3 130.1/5.3).

These findings indicate an increase in the mass transport of material (carbohydrates, extractives and conjugated/condensed lignin structures) during soda cooking upon STEX pretreatment. They also call attention to the possibility of lignin condensing during STEX, implied by the occurrence of $\beta - \beta$ linkages along with a shift to higher molar mass in the GPC chromatograms. However, it should be kept in mind that these structures are also present, to a varying extent, in native lignin; the relatively strong $\beta - \beta$ NMR signals observed could simply be a result of the enhanced extraction of these structures due to STEX combined with a relatively long delignification time (180 min).

5

Conclusions and Future Work

5.1 Conclusions

The aim of this work was to increase the understanding of the mechanisms underlying two mild hydrothermal pretreatments. The initial experiments included determining the local composition of the steam-exploded wood chips, and were followed by the continuous extraction of spruce and birch wood meal and a stepwise treatment of forest residues. The multiple analyses performed within these projects provided an insight into the autohydrolysis and mass transport of different sizes and types of material from the perspective of hot water extraction and steam explosion. Analysis of the results gathered allowed the following conclusions to be drawn:

- During autohydrolysis, acidification is driven by the deprotonation and release of organic acids in the cell wall, which leads to deacetylation. The release of free acetic acid contributes, to some extent, to the decrease in pH and drives the autohydrolysis further. However, as the rate of deacetylation is slow, most of the acetates remain bound to the hemicelluloses even after their extraction from the wood tissue.

- The extraction liquors obtained during hot water extraction of wood meal at 150-170°C contain a proportion of monomers, that most likely originate from side groups of hemicelluloses.

- Along with glucomannan and xylan, arabinogalactan (in the high Mw form and as free monomers) is extracted from wood meal samples.

- Cellulose is, to a certain extent, affected by autohydrolysis during hot water extraction at temperatures between 130 and 170°C. The effect on the degree of polymerization is limited under mild conditions, and becomes more significant at 170°C. The CSN observed for birch and spruce was different as spruce cellulose is subjected to a higher number of chains scissions (probably due to the hard-to-extract xylan localised close to the cellulose fibres in hardwood, which are thus, to some extent, protected).

- Hot water extraction applied to wood chips for a short time introduces significant variations in the local composition of the treated wood, leaving the composition of the inner part of the tissue less affected. Prolonged treatment, on the other hand, leads to more severe overall hydrolysis.

- Steam explosion achieves a comparably greater removal of hemicelluloses from wood chips than hot water extraction, since the compositional effects are influenced strongly by the rapid pressure-release step. During prolonged treatment, however, autohydrolysis, coupled with mass transport resistance, becomes important in the development of different composition profiles within the wood chips.

- The impact of steam explosion was found to be limited when material of smaller size was pretreated, such as refined wood residues.

- There are some indications that pseudo-lignin forms in the pulps when the pulping is preceded by a STEX of forest residues, although this is possibly connected to the presence of extractives.

- The delignification rate of forest residues is faster than that of wood.

- The behaviour of xylan during delignification differs for forest residues when compared to wood: rapid initial extraction followed by a rather stable content during the rest of the pulping process, along with no commonly-observed co-extraction of xylan-lignin, indicates the possibility of enhanced re-adsorption on celluloses and/or the occurrence of different types of xylan.

5.2 Future work

It is suggested that future work should focus on more detailed investigation of the molecular weight and structure of the hemicelluloses recovered in relation to the pretreatment conditions and raw material. It should also involve investigating structural changes that are possibly induced in lignin upon steam explosion pretreatment. In a broader perspective, the prospect of integrating hydrothermal pretreatments with existing pulping processes, and applying them in combination with other pretreatments, is a topic of interest.

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A

Appendix A

Carbohydrates quantification

The polymeric content of wood and liquor samples was calculated based on the detected monosugar amount using the following procedure:

The amounts detected were corrected to the given hydrolysis yields and the amount of anhydro sugars was calculated from the sugar monomers (by the withdraw of water: detected values were multiplied by 0.88 for pentosans and 0.90 in case of hexosans).

The GGM content was calculated as sum of measured galactan, mannan and part of glucan (assumed ratio of mannose and glucose was 3.5:1 Meier [1958]).

AGX was calculated as sum of detected arabinan and xylan. Cellulose content was calculated as the detected glucan amount after withdraw of glucan included in GGM.

$$\text{Cellulose} = \text{glucose} - (1/3.5)*\text{mannose}$$

$$\text{GGM} = \text{galactose} + (1+ (1/3.5))*\text{mannose}$$

$$\text{AGX} = \text{xylose} + \text{arabinose}$$

B
AppendixB

NMR characterization

The evaluation of the NMR spectra was based on earlier work by Giummarella *et al.* [2016]; Berglund *et al.* [2019]; Hannuksela and Hervé du Penhoat [2004]; Yuan *et al.* [2011]. Figures B.1 to B.6 present the results obtained for freeze-dried spruce liquors dissolved in DMSO or H202 and figures B.7 to B.14 represent birch liquors obtained at 150°C.

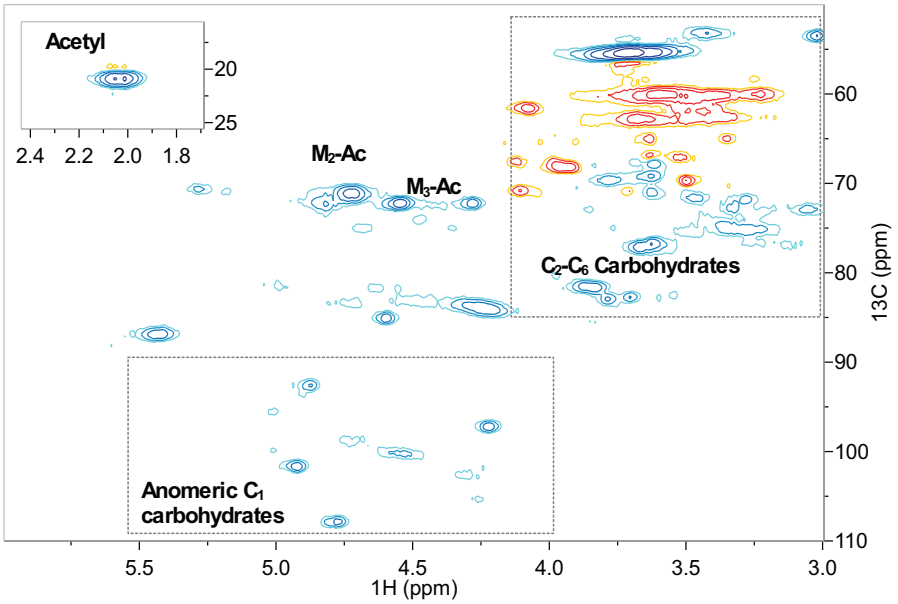

Figure B.1 *HSQC spectra of spruce liquor extracted at 130°C dissolved in DMSO.*

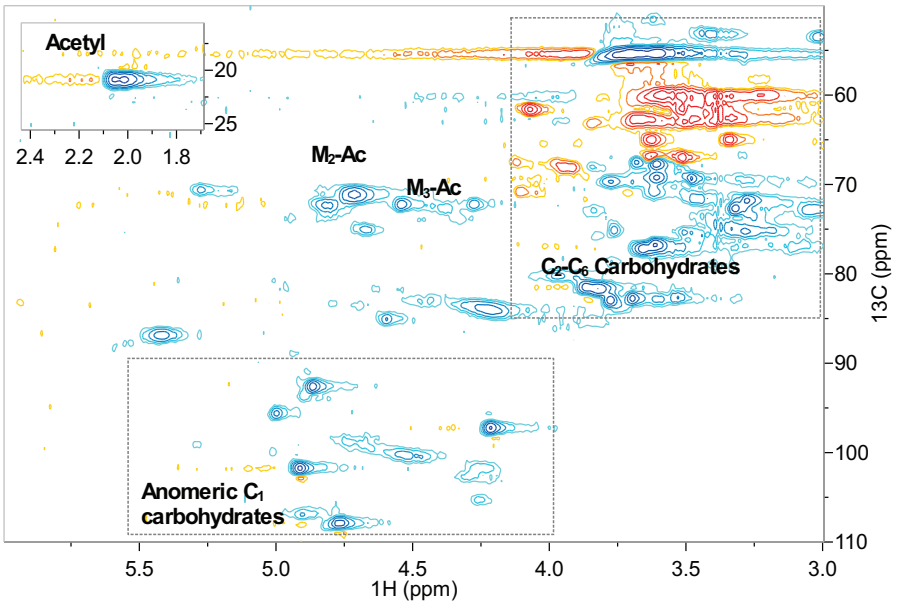

Figure B.2 *HSQC spectra of spruce liquor extracted at 150°C dissolved in DMSO.*

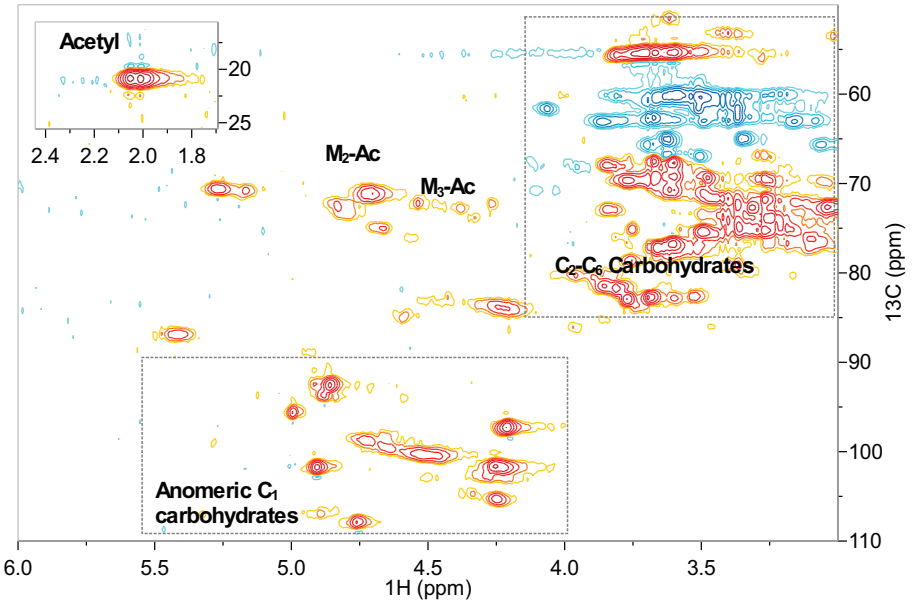

Figure B.3 *HSQC spectra of spruce liquor extracted at 170°C dissolved in DMSO.*

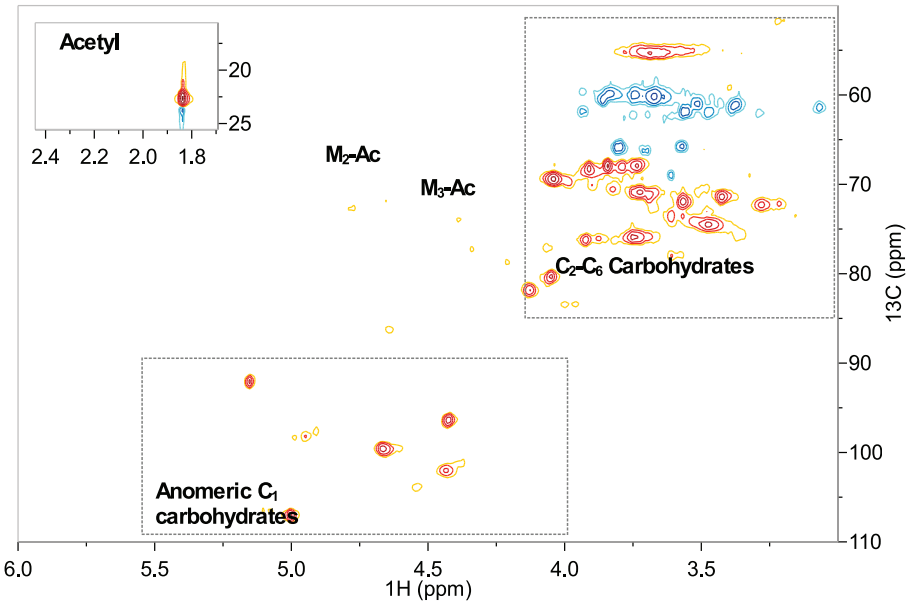

Figure B.4 *HSQC spectra of spruce liquor extracted at 130°C dissolved in D20.*

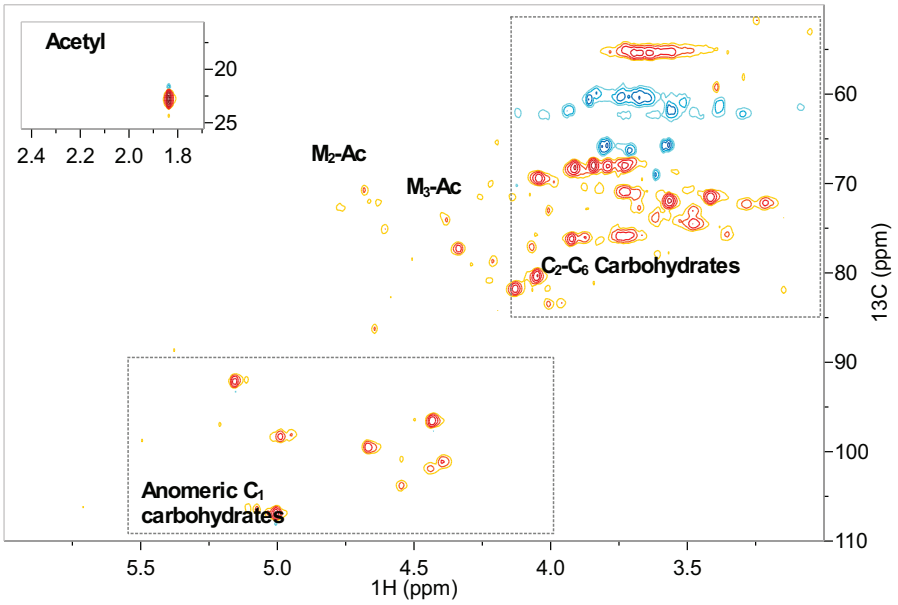

Figure B.5 *HSQC spectra of spruce liquor extracted at 150°C dissolved in D20.*

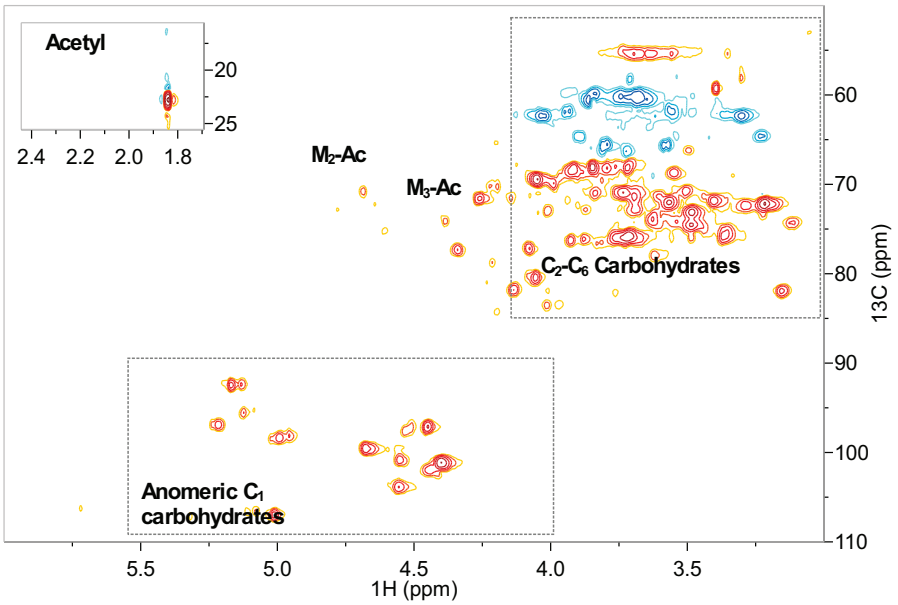

Figure B.6 *HSQC spectra of spruce liquor extracted at 170°C dissolved in D20.*

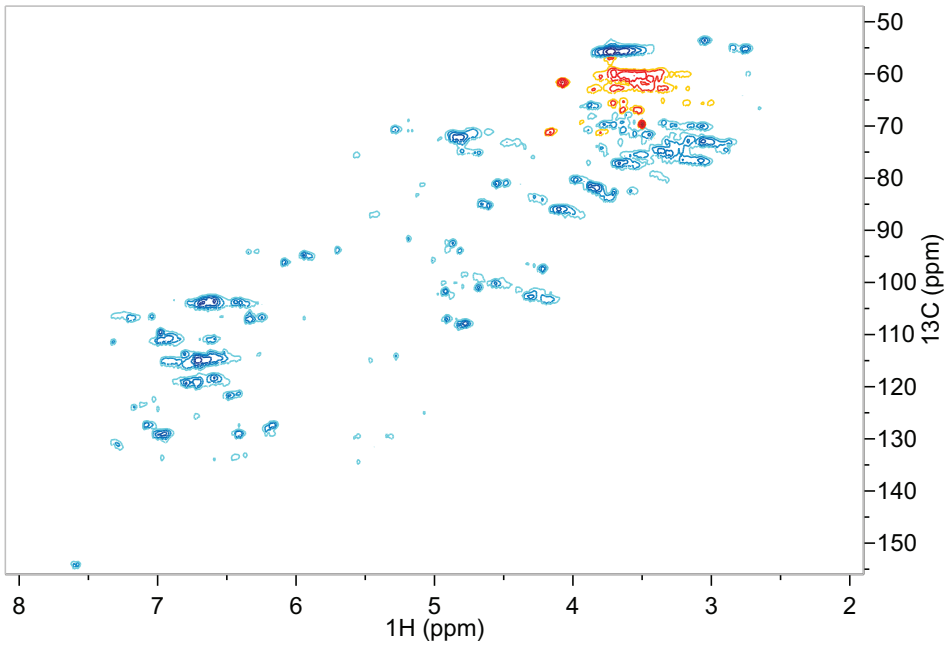

Figure B.7 *HSQC spectra of birch liquor samples extracted at 150 °C at 10 min.*

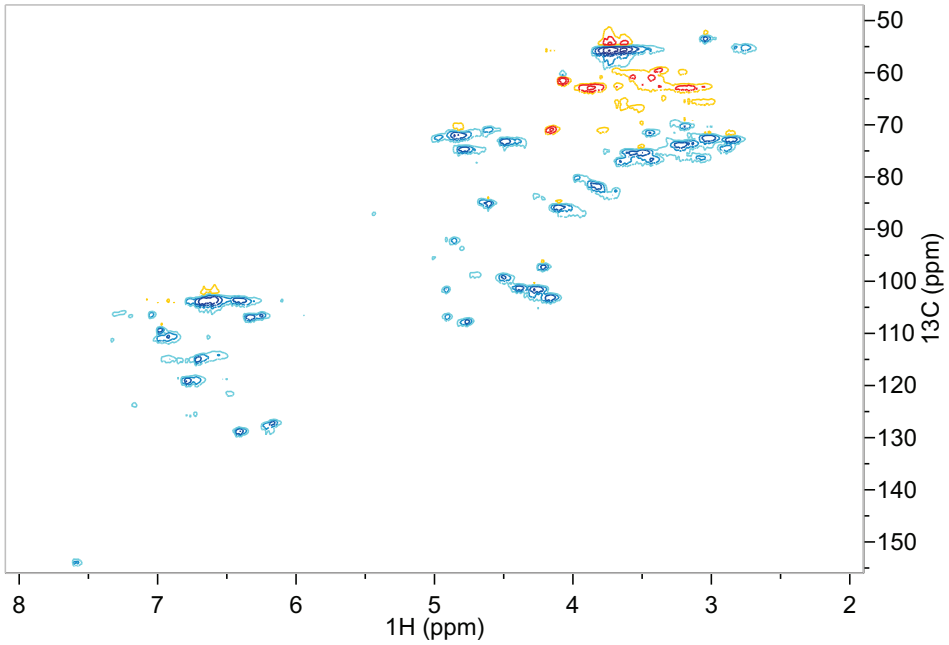

Figure B.8 *HSQC spectra of birch liquor samples extracted at 150 °C at 20 min.*

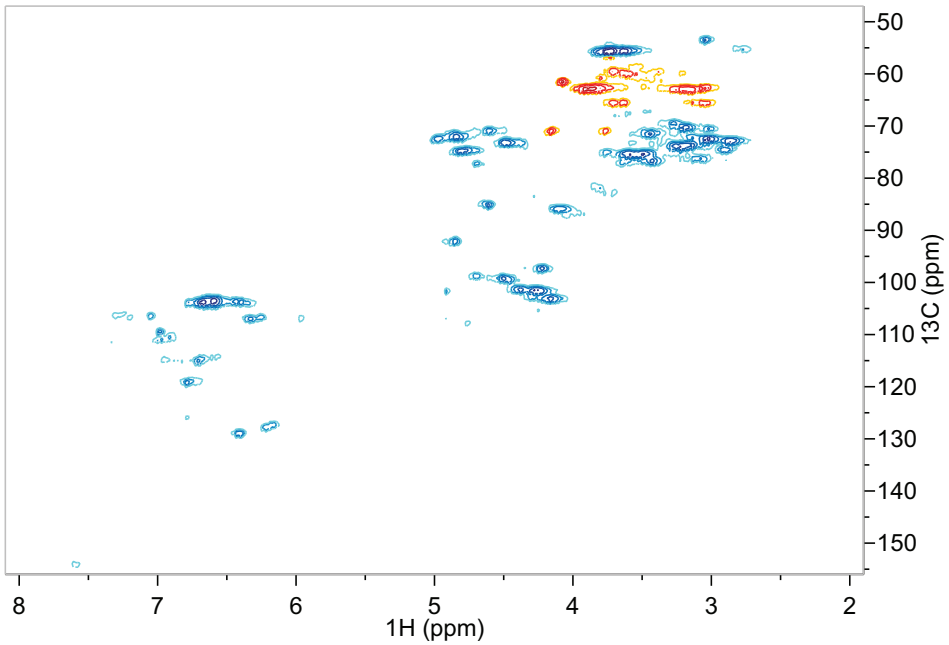

Figure B.9 *HSQC spectra of birch liquor samples extracted at 150 °C at 30 min.*

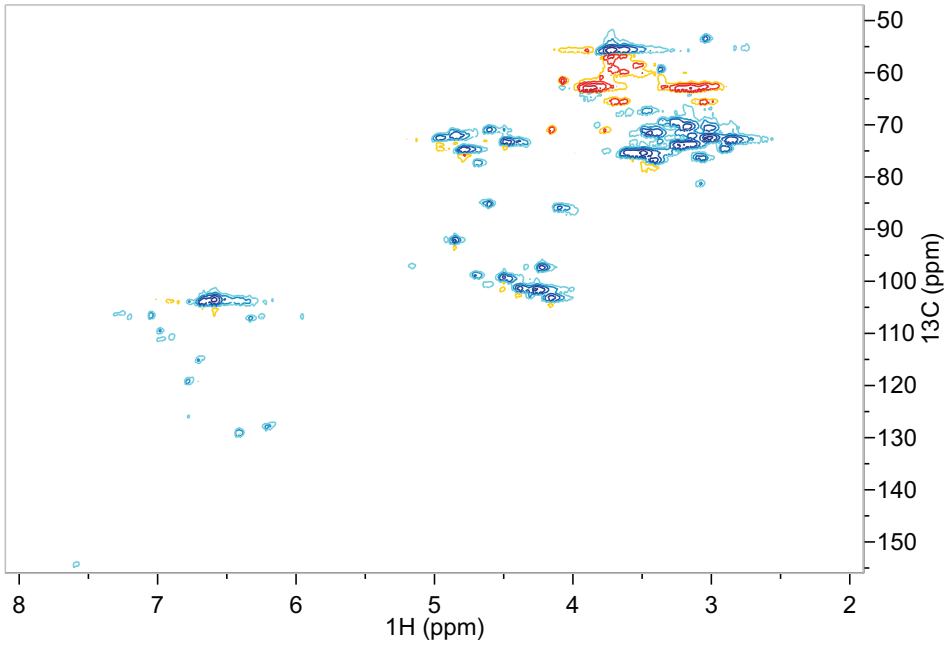

Figure B.10 *HSQC spectra of birch liquor samples extracted at 150 °C at 40 min.*

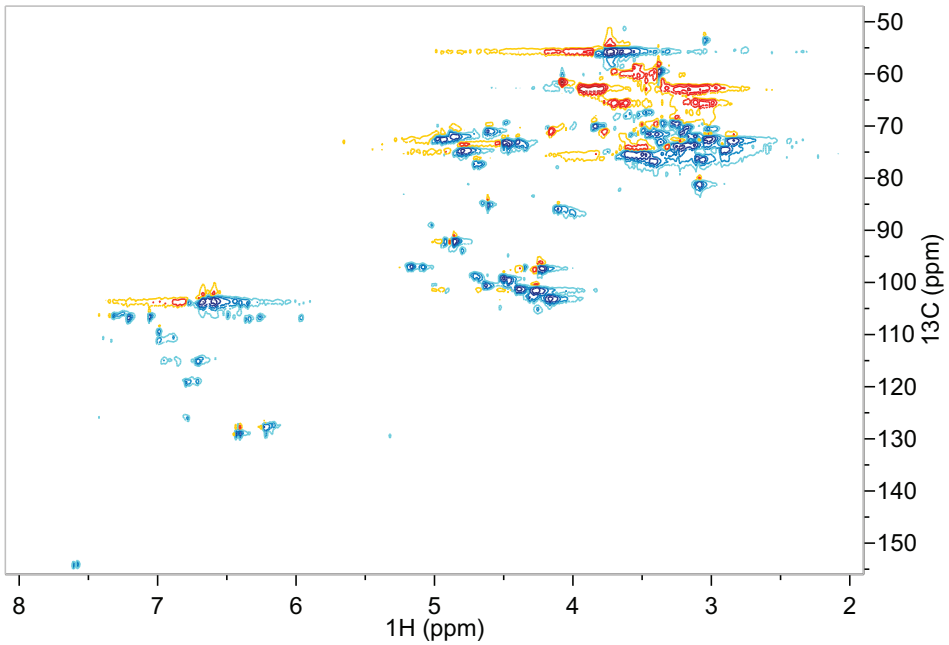

Figure B.11 *HSQC spectra of birch liquor samples extracted at 150 °C at 60 min.*

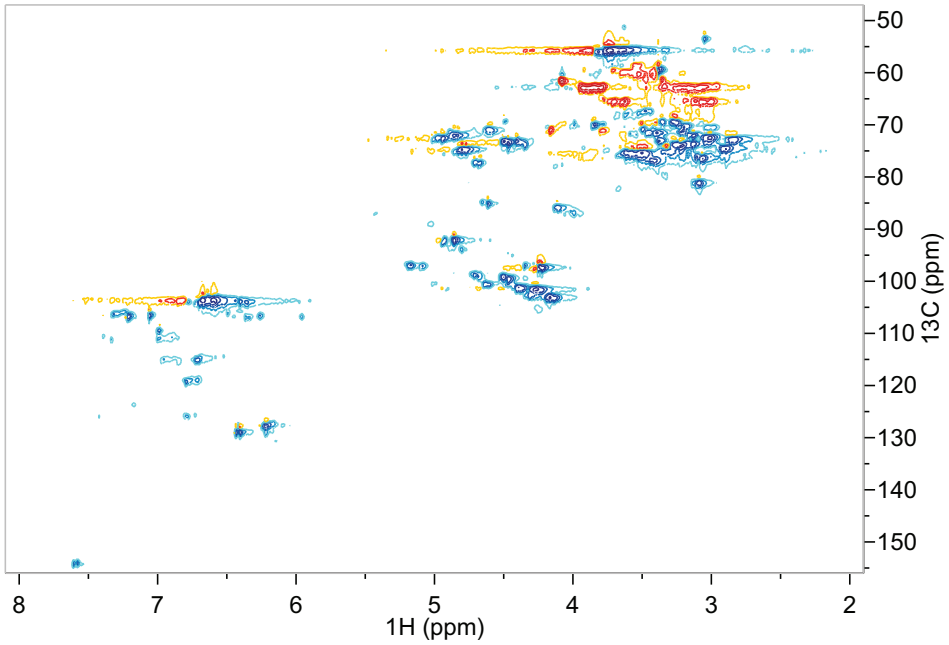

Figure B.12 *HSQC spectra of birch liquor samples extracted at 150 °C at 80 min.*

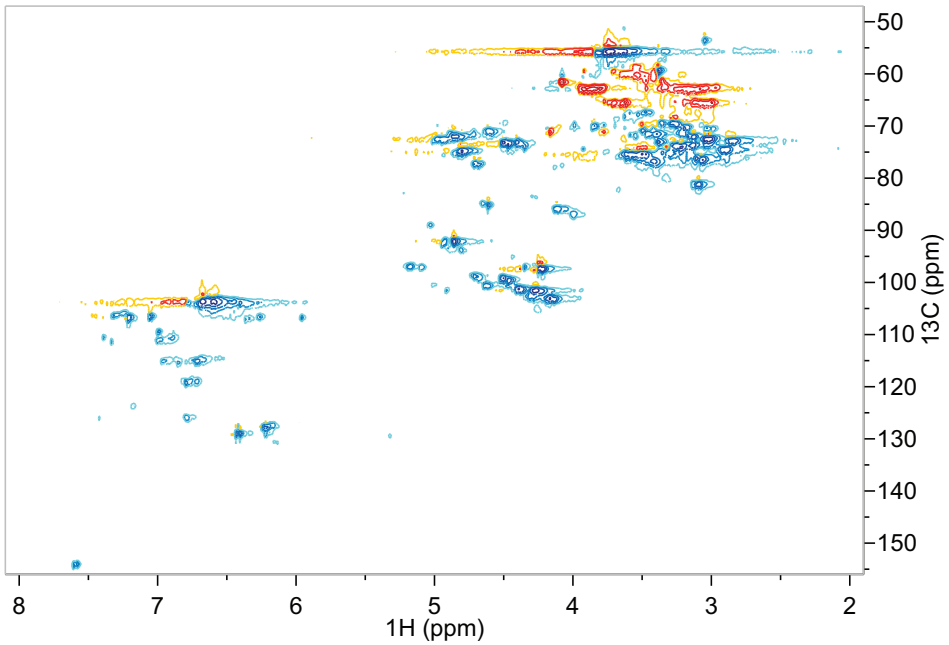

Figure B.13 *HSQC spectra of birch liquor samples extracted at 150 °C at 100 min.*

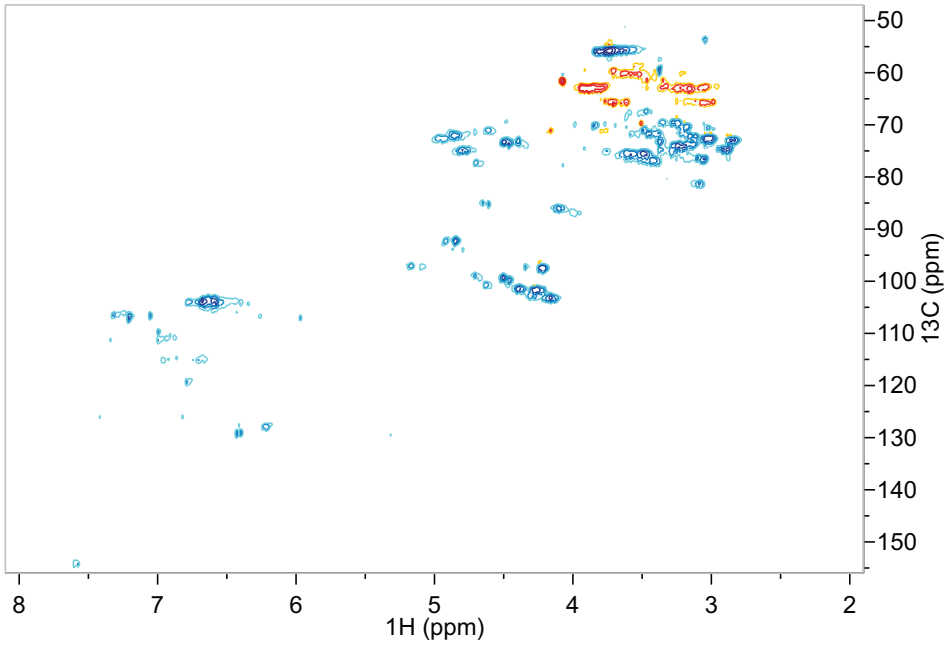

Figure B.14 *HSQC spectra of birch liquor samples extracted at 150 °C at 120 min.*