THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Mild Steam Explosion of Norway Spruce

Investigations into a potential process step for a future biorefinery

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Forest Products and Chemical Engineering
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CHALMERS UNIVERSITY OF TECHNOLOGY

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Cover:
[Left: Norway spruce growing in Tjörn, Sweden. Right: ESEM image of wood cells in Norway spruce (magn. 500x)]

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ABSTRACT

The most common wood species in Sweden, and one of the most important renewable raw materials in Northern Europe, is Norway spruce (*Picea abies*). Today, it is utilized mainly for sawed timber and the production of pulp and paper. A modern kraft pulp mill that produces bleached pulp has a material efficiency of about 40-45%, and the final product contains mainly cellulose. The other components of the wood, e.g. hemicelluloses and lignin, are heavily degraded during the process and end up in the mill's recovery boiler, where they are burned to recover the latent energy. The biorefinery concept is an approach where more of the biomass is utilized for the production of a variety of products, e.g. new materials, chemicals and fuels.

The aim of this work is to investigate a process called "mild steam explosion" as a pre-treatment step in a biorefinery. During steam explosion, saturated steam is applied to biomass at elevated pressure, which is followed by a fast pressure release. The treatment leads to both mechanical rupture and chemical reactions, such as acid hydrolysis. The conditions of the steam explosion treatment are kept mild (approx. 140-170°C) to ensure that the degradation of the wood components is held at a minimum. The idea behind the treatment is to make the structure of wood more accessible and facilitate the extraction and isolation of the wood components, preferably those of high molecular weights. The most abundant hemicellulose component in spruce is (galacto)glucomannan and is of primary concern. This is a challenge since it is also the component that is the most sensitive to chemicals. Treatment with reducing agents, such as sodium borohydride and dithionite, are therefore used to stabilize the (galacto)glucomannan.

The findings in this thesis showed that steam explosion, even at modest conditions, made the wood structure accessible for enzymatic reactions. It was also shown that wood components from hemicelluloses and wood extractives were released into the condensed steam. Mild steam explosion was also seen to increase the rates of both extraction and delignification during subsequent treatments. The mechanical effects of the steam explosion treatment originated from steam heating, expansion during pressure release and impact. The properties of pulps after kraft cooking and oxygen delignification of steam-exploded wood chips were comparable to reference pulps. It was also found that treatment with a reducing agent stabilizes the (galacto)glucomannan during both mild steam explosion and various chemical treatments.

Keywords: biorefinery, Norway spruce, (galacto)glucomannan, kraft cooking, steam explosion.

List of publications

This thesis is based on the following papers, which can be found at the end. Reprints were made with permission from the journals.

I. **Mild steam explosion and chemical pre-treatment of Norway spruce**
Kerstin Jedvert, Anna Saltberg, Mikael E. Lindström and Hans Theliander
BioResources 7(2), 2051-2074, 2012.

II. **Mild steam explosion: A way to activate wood for enzymatic treatment, chemical pulping and biorefinery processes**
Kerstin Jedvert, Yan Wang, Anna Saltberg, Gunnar Henriksson, Mikael E. Lindström and Hans Theliander
Nordic Pulp & Paper Research Journal, 27(5), 828-835, 2012.

III. **Mild steam explosion followed by kraft cooking and oxygen delignification of spruce (*Picea abies*)**
Kerstin Jedvert, Anna Saltberg and Hans Theliander
Appita Journal, 66(4)*,* 322-330, 2013.

IV. **Structural changes in spruce wood during different steps of steam explosion pretreatment**
Muhammad Muzamal, Kerstin Jedvert, Hans Theliander and Anders Rasmuson
Accepted for publication in *Holzforschung*, 2014.

V. **Analyses of wood components in mild steam explosion liquors from spruce**
Kerstin Jedvert, Merima Hasani, Tyrone Wells Jr. and Hans Theliander
Submitted

VI. **Dithionite impregnation combined with mild steam explosion of spruce wood – an improved version of kraft pulping**
Yan Wang, Kerstin Jedvert, Mikael E. Lindström, Hans Theliander and Gunnar Henriksson
Manuscript

Results relating to this work have also been presented at the following conferences:

i. Chemical pre-treatment in combination with steam explosion for wood component separation
Kerstin Jedvert, Anna Saltberg and Hans Theliander
(poster presentation)
In: Conference proceedings. 11th European Workshop on Lignocellulosics and Pulp, Hamburg, Germany, August 16-19, 2010, pp 219-222.

ii. Characterization of spruce wood chips after mild steam explosion
Anna Saltberg, Kerstin Jedvert and Hans Theliander
(poster presentation, presented by Anna Saltberg)
In: Conference proceedings. 65th Appita Annual Conference & Exhibition, Rotorua, New Zealand, April 10-13, 2011, pp 359-362

iii. Mild steam explosion as a new step in wood component separation?
Kerstin Jedvert
(oral presentation)
SPCI convention, Stockholm, Sweden, May 17-19, 2011

iv. Extraction of hemicelluloses after chemical pre-treatment combined with mild steam explosion
Kerstin Jedvert, Anna Saltberg, Mikael E. Lindström and Hans Theliander
(poster presentation)
In: Conference proceedings. 16th International Symposium on Wood, Fibre and Pulping Chemistry, Tianjin, China, June 8-10, 2011, pp 867-871

v. Mild ångexplosion – ett steg för separation av vedkomponenter?
Kerstin Jedvert
(oral presentation)
Ekmandagarna, Stockholm, Sweden, January 24-25, 2012

vi. Post-processing and characterization of mild steam-exploded spruce (*Picea abies*) wood chips
Kerstin Jedvert, Anna Saltberg and Hans Theliander
(poster presentation)
In: Conference proceedings. 17th International Symposium on Wood, Fibre and Pulping Chemistry, Vancouver BC, Canada, June 11-14, 2013, published online (http://epaccontrol.com/online/iswfpc2013/)

vii. Mild steam explosion of spruce
Kerstin Jedvert
(oral presentation)
In: Book of abstracts, 3rd Avancell conference – Creating Value from the Swedish Forest Resources, Gothenburg, October 8-9, 2013, pp 23-25

Contribution report

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

I. Main author. Active in planning the experimental outline, performing the experimental work and interpreting the results.

II. Main author. Active in planning the experimental outline, performing the experimental work and interpreting the results.

III. Main author. Active in planning the experimental outline, performing the experimental work and interpreting the results.

IV. Active in planning the experimental outline and performing the experimental work together with the first author. Active in discussions of the results. Joint effort made in writing the paper.

V. Main author. Active in planning the experimental outline, performing the experimental work and interpreting the results. Did not perform the HSQC-NMR analysis.

VI. Active in planning the experimental outline and performing the pre-impregnation and mild steam explosion, as well as some of the analyses, together with the first author. Did not perform the kraft cooks. Active in discussions of the results. Joint effort made in writing of paper.

List of abbreviations

Ac – acetyl

AGX – arabinoglucuronoxylan

ASL – acid soluble lignin

DMSO – dimethyl sulphoxide

DNS – dinitrosalicylic

DP – degree of polymerization

EA – effective alkali

ECU – endo/exocellulase unit

ESEM – environmental scanning electron microscopy

GC-MS – gas chromatography – mass spectroscopy

GGM – (galacto)glucomannan

GPC – gel permeation chromatography

HMDS – hexamethyldisilazane

HPAEC – high-performance anion exchange chromatography

HSQC – heteronuclear single quantum coherence/correlation

IC – ion chromatography

LCC – lignin-carbohydrate complexes

LCF – lignocellulose feedstock

Mw – weight average molecular weight

NIR – near infrared

NMR – nuclear magnetic resonance

o.d. – oven dry

PCA – principal component analysis

PD – polydispersity

PLS – partial least squares

RI – refractive index

RT – room temperature

SEC – size exclusion chromatography

STEX – steam explosion

TMCS – trimethylchlorosilane

Happy is he who gets to know the reasons for things.

- Virgil (70-19 BC), Roman poet.

Contents

1. Introduction

Wood has been used by people for millennia not only to construct houses, ships and furniture and the suchlike but also as a source of fuel. Research related to wood has also been performed for a long time, and especially since wood was introduced as the raw material for making pulp and paper in the 19th century. In a forest-rich country such as Sweden, the forest industry remains one of the most important industries; the country is still one of the world's largest exporters of pulp and paper (Skogsindustrierna, 2013). Greater competition in the pulp and paper market, however, means that the Swedish forest industry is now facing challenges and, consequently, there is a need for new, and more advanced, products and processes. Moreover, general awareness of the gradual decrease in the availability of fossil fuel and ambitions to replace petroleum-based materials and fuels with sustainable alternatives has increased. This has generated enhanced interest in research with respect to developing processes and products that utilize renewable resources as raw materials (Ragauskas et al., 2006).

The concept of "biorefinery" is based on the idea that biomass can be fractionated, analogous to crude oil in a conventional refinery. The various components of the biomass can then be used for producing various new materials, chemicals and fuels. Different types of wood are examples of biomass that may be used in biorefineries, being both an abundant and renewable resource (van Heiningen, 2006). Wood consists mainly of cellulose, lignin and various types of hemicelluloses (a group of heterogeneous polysaccharides). Today, wood-based cellulose is used chiefly in in the production of pulp and paper, but textiles and modified celluloses are other interesting applications. Several

potential products based on lignin and hemicelluloses have also been proposed, some of which are commercialized and others are at the research and development stage. Many studies have, for example, investigated the possibility of using cellulose or hemicelluloses in the production of fuels such as bioethanol (Senthilkumar and Gunasekaran, 2005, Parawira and Tekere, 2011). These processes require, however, that the polysaccharides are converted into sugar monomers which then could be used for fermentation. More intact xylans and glucomannans, with higher molecular weights, are of interest for other applications, such as barrier materials in bioplastic films and as emulsion stabilizers (Gatenholm et al., 2004, Willför et al., 2008).

The most common wood species in Sweden is Norway spruce (*Picea abies*) (SLU, 2013). It contains a high concentration of the hemicellulose (galacto)glucomannan (approx. 15-20%), which is a polysaccharide consisting of glucose and mannose with galactose side-chains (Sjöström, 1993). During ordinary kraft cooking, where wood is converted into pulp by chemical dissolution of the lignin that liberates the cellulose fibres, the hemicelluloses are degraded to varying extents. They are then normally burned together with the lignin in the recovery boiler to recover latent energy (Sixta, 2006). Lignin and xylans are, to a great extent, present as macromolecules in the process while (galacto)glucomannans are disintegrated into monomers. Thus, the kraft pulping process may be considered as being a reasonably good process for separating all the components found in wood, with the exception of (galacto)glucomannan.

The major problem of the fractionation of different wood components is that lignin crosslinks the wood polysaccharides into large networks that act as an obstacle to extraction and separation procedures (Lawoko et al., 2006, Jeffries, 1990). Furthermore, wood has such a compact structure that enzymes, for example, cannot penetrate the cell walls directly (Blanchette et al., 1997). It is therefore important to find selective methods that make the wood structure more accessible. Several techniques have been proposed and are investigated with the aim of increasing the material efficiency of biomass including, for example, fractionation in organic or ionic solvents (Sathitsuksanoh et al., 2010, Leskinen et al., 2011, Varshney and Patel, 1989). Another approach, which is also the focus of this work, is to pre-treat the wood prior to the kraft process with the aim of isolating some of its components in the form of macromolecules. The investigations are centred on a method called "steam explosion", which is an energy-efficient technique when compared to mechanical treatments (Alvira et al., 2010), where biomass is treated with pressurized saturated steam followed by a rapid release to atmospheric pressure. Steam explosion is a hydrothermal treatment aimed at rupturing the structure of the wood and making it more accessible. This pre-separation step must be performed under mild conditions to avoid the degradation of the wood components, which results in a paper pulp of lower quality. Treatments at harsher conditions, on the other hand, make other

potential cellulose products, such as dissolving pulp and microcrystalline cellulose, possible.

1.1 Wallenberg Wood Science Center (WWSC)

This work has been a part of the Wallenberg Wood Science Center, which is a joint Swedish research centre shared between Chalmers University of Technology in Gothenburg and The Royal Institute of Technology in Stockholm, and formed by the Knut and Alice Wallenberg Foundation in 2009. The mission and vision of WWSC is to create new materials from trees, including processes for producing materials. This study has been part of the work undertaken by a research group working under the title "From wood chips to material components", the focus of which is on raw materials and the development of efficient and economically feasible novel processes for the separation of the components in spruce wood.

1.2 Objectives

The main objective of the work in this thesis was to investigate the effects, both mechanical and chemical, of a mild steam explosion treatment of Norway spruce. (Galacto)glucomannan (GGM), which is the main hemicellulose component in spruce, was especially in focus due to its abundance and the fact that it is currently under-utilized. The steam explosion conditions were chosen to be mild in order to avoid extensive degradation of the wood components. Chemical pre-treatments were also studied to protect the GGM further, and the chemical reactions during the mild steam explosion were investigated by characterization of the condensed steam.

1.3 Outline and overview of the papers

This work is based on six papers, all of which can be found at the end of the thesis. A significant difference between the papers is that different equipment was used for the mild steam explosion treatment. An overview of the content of the papers is illustrated in **Fig. 1.1**.

Paper I: The experiments were made in a lab-scale steam explosion apparatus, with wood powder of spruce used as the raw material. The study included investigations of chemical pre-treatments in alkaline and acidic environments, effects of different residence times and temperatures during mild steam explosion as well as a few experiments on extraction in various aqueous liquors after mild steam explosion.

Paper II: The focus of this study was to investigate whether or not there is a threshold for enzymes to access the structure of wood after mild steam explosion. The experiments were performed on wood chips of spruce that were subjected to mild steam explosion in the lab-scale equipment.

Paper III: The effects of a mild steam explosion treatment of wood chips were followed during subsequent kraft cooking and oxygen delignification, and the resulting pulps were analyzed. The experiments were performed in the bench-scale steam explosion apparatus.

Paper IV: The mechanical effects and morphological changes that occur in spruce wood during the different steps of steam explosion treatment were studied. Single pieces of spruce wood were used as samples; both the small-scale equipment and the bench-scale steam explosion apparatus were used.
Paper V: Detailed knowledge of the wood components released into the steam explosion liquor (i.e. the condensed steam) was obtained. Identification of the components and the molecular weight distributions were characterized using several analytical techniques.
Paper VI: The combined effect of impregnation with dithionite prior to mild steam explosion (bench-scale apparatus) and kraft cooking were investigated.

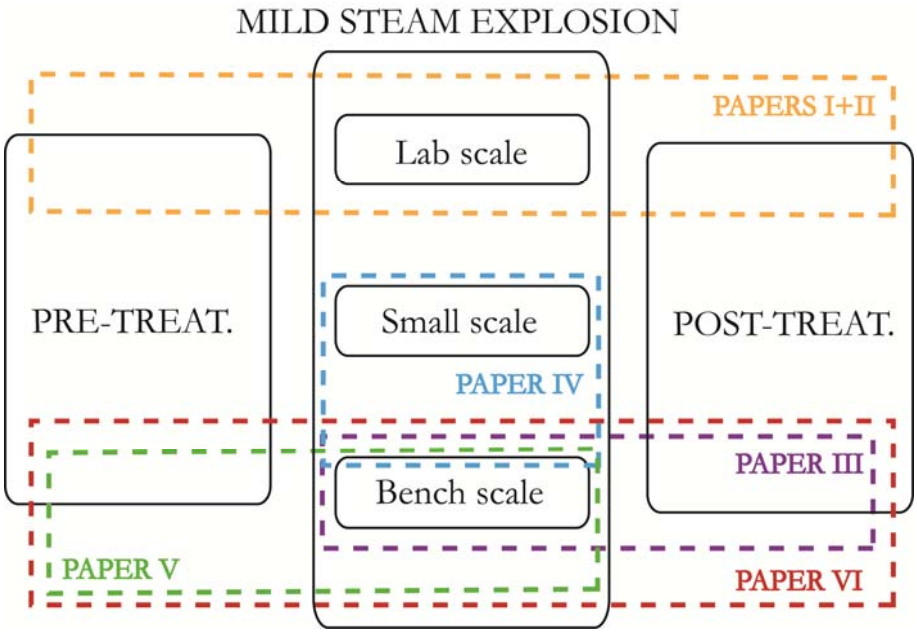

Figure 1.1 Overview of the papers included in this thesis.

The following three chapters introduce the reader to the chemistry of wood and chemical pulping (Chapter 2), the concept of biorefinery (Chapter 3) and also provide some findings in the literature pertaining to steam explosion and methods employed for extracting hemicelluloses (Chapter 3-4). The materials and experimental procedures used in this work are described in some detail in Chapter 5, and Chapter 6 provides the main findings of the various investigations along with some discussion and interpretation of the results. Finally, Chapter 7 summarizes the experimental part with conclusions, reflections and some suggestions for further work.

2. Wood and pulping

Trees are categorized into two kinds of wood: softwood (conifers) and hardwood (deciduous, i.e. broad-leaved) (Sjöström, 1993). There are many more species of hardwood than of softwood in the world. The species that are of industrial importance in Scandinavia include conifers such as Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). The general composition of softwood is: 40-46% cellulose, 23-30% lignin and 19-26% hemicelluloses (e.g. glucomannans and xylans), with extractives and inorganics making up the remaining few percent (Pettersen, 1984, Fengel and Wegener, 1989). The extractives include wood resin as well as some lignans, phenols and sugars. Wood resin is composed of different lipophilic components, such as sterols and fatty acids (Back and Allan, 2000).

2.1 Morphology

The hierarchical structure of softwood is illustrated in **Fig. 2.1**. The tree is protected from physical and biological damage by the bark on the outside of the stem, where the outer part of the bark is composed of dead cells and contains large amounts of wood extractives. The next tissue, on the inside of the bark, is called phloem. It is a living part of the tree and has the function of transporting nutrients and storing products (Rowell, 2005). The next layer is the vascular cambium, which is a thin cellular layer that produces cells for the phloem on the outside and secondary xylem cells on the inside. The main part of the trunk consists of secondary xylem (i.e. the actual wood), which can be divided, in turn, into sapwood and heartwood. Sapwood contains both dead and living cells while heartwood consists exclusively of dead cells. It is the xylem that usually has the characteristic pattern of annual rings: each ring represents one year of growth. Annual

rings are a consequence of differences during the growing season: latewood cells are smaller, and have thicker walls, than early wood cells that are produced earlier in the season. The pith, in the middle of the tree, is the tissue from the initial years of the tree's life (Fengel and Wegener, 1989).

Wood is composed of ordered arrangements of wood cells in both the longitudinal and radial direction. The radial system is made up of ray cells, the main function of which is to redistribute and store nutrients, such as starch. Softwoods have a more homogenous structure than hardwood, and lack the vessel elements for transporting water that hardwoods have (Peters, 2000). Tracheids are the most common type of cell present in softwood. Constituting about 90-95% of the total volume of cells, they have a long and slender shape and are approx. 3 mm in length and 30 μm in width (Fengel and Wegener, 1989). The transport of water occurs through tracheids and via bordered pits. The other kinds of cells present in wood include parenchyma and epithelial cells. The majority of the former are found in the ray canals and are involved in transport of liquids in the horizontal direction; the latter are located around resin canals into which they secrete resins (Rowell, 2005).

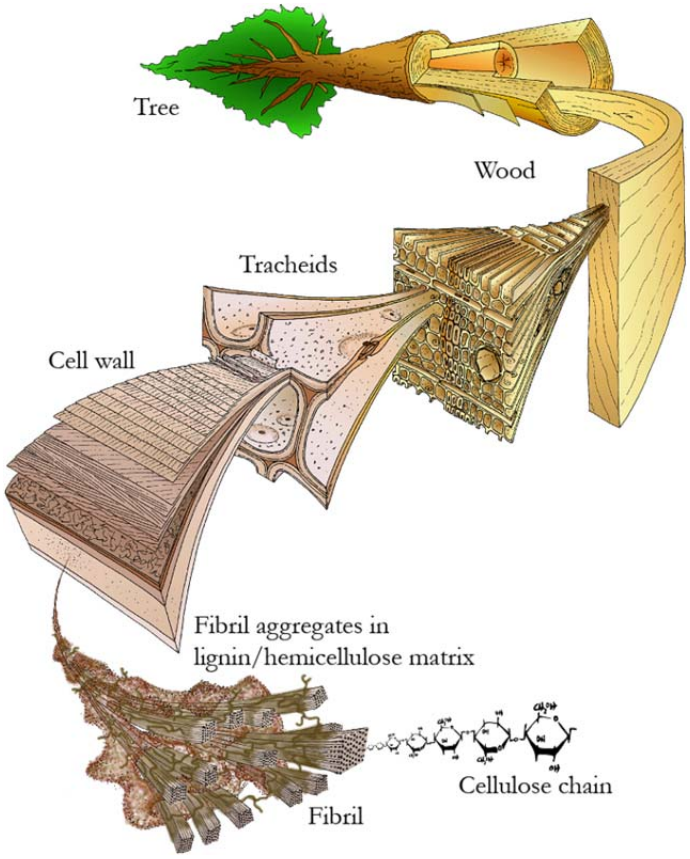

Figure 2.1 Illustration of the hierarchical structure of softwood, from the tree down to the cellulosic chains. Artwork by Mark Harrington, copyright University of Canterbury, 1996 (reprinted with permission).

The cell wall of wood is composed of different layers: a primary layer followed by a few secondary layers. The primary wall consists of randomly distributed cellulose microfibrils:

behind these are located 2-3 secondary layers (S1, S2 and S3) in which the microfibrils are oriented at different angles. The S2-layer is comparatively thick and therefore represents the main wood tissue. It is also characterized by the fibrils having a low angle, and being orientated in a z-helix. The wood cells are tied together by the middle lamella, which has a high lignin and pectin content (Booker and Sell, 1998).

2.2 Cellulose and lignin

A typical composition of Norway spruce is 41.7% cellulose, 27.4% lignin, 16.3% glucomannans, 8.6% xylans, 3.4% other polysaccharides, 1.7% extractives and 0.9% others (Henriksson et al., 2004).

2.2.1 Cellulose

Cellulose is not only the most abundant compound present in wood but also the most common organic compound on Earth (Kamide, 2005). It belongs to the polysaccharides, and its monomers, β-D-glucose units, are linked together by (1→4) glycosidic bonds, see **Fig. 2.2**. The repeating unit consists of two glucose monomers and is known as cellobiose. The degree of polymerization (DP) depends greatly on the origin and treatment to which the cellulose has been subjected. Native wood cellulose has a DP that varies between 800 and 10,000 whereas wood pulp has chain lengths of between 300 and 1700 monomers (Klemm et al., 2005).

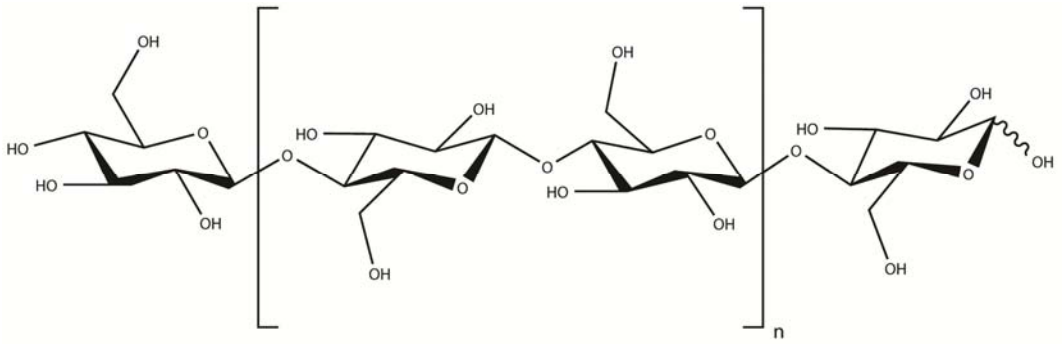

Figure 2.2 The molecular structure of cellulose with the repeating cellobiose unit.

The molecular structure of cellulose presents many possibilities for the formation of strong intra and intermolecular interactions, e.g. hydrogen bonds and hydrophobic interactions. The resulting cellulose chains show the tendency to self-order: they arrange themselves into fibrils that have regions with varying degree of order (O'Sullivan, 1997). The fibrils, in turn, organize themselves into structures of higher order. This complex arrangement into layers of varying texture and density that constitute the major part of the cell wall of wood is important for the accessibility of its components during chemical treatments.

2.2.2 Lignin

Lignin is a complex macromolecule consisting of phenyl propane units. Its main function is to act as an adhesive between the cellulose fibrils and hemicelluloses, thereby providing the fibres and their structure with strength (Sjöström, 1993). The amount of lignin is usually somewhat higher in softwoods than in hardwoods. The three different monomers that constitute the lignin polymer, i.e. *p*-coumaryl alcohol, sinapyl alcohol and coniferyl alcohol, can be connected through several kinds of bonds. The lignin in softwood is called guaicyl lignin and consists almost entirely of coniferyl alcohol and some *p*-coumaryl alcohol (Brunow and Lundquist, 2010).

2.3 Hemicelluloses, pectins and starch

Hemicelluloses form a diverse group of polysaccharides that are substantially smaller in size compared to cellulose, with chain lengths somewhere between 100 and 200 monomers (Sjöström, 1993). This low DP, in combination with a higher degree of branching, means that hemicelluloses have a more amorphous structure than cellulose. Although there are many different sugar monomers that can make up the chains of hemicelluloses, the five that are most common are: D-glucose, D-mannose, D-galactose, D-xylose and L-arabinose. Small amounts of L-rhamnose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid and D-galacturonic acid are also present. Three main types of hemicelluloses occur in softwoods: (galacto)glucomannan, arabinoglucuronoxylan and arabinogalactans, although the latter is found primarily in larch wood.

2.3.1 (Galacto)glucomannan (GGM)

GGM is the principal hemicellulose in spruce. Its backbone is composed of β-(1→4)-linked D-mannopyranose (Man*p*) and β-(1→4)-linked D-glucopyranose (Glc*p*) (Timell, 1967, Fengel and Wegener, 1989) at a ratio of 10:1.9-2.6 (Hannuksela and Herve, 2004) with side units of α-(1→6)-linked D-galactopyranose (Gal*p*). Acetyl (Ac) groups are also common, with some of the mannopyranosyl units being substituted partially by *O*-acetyl groups at the C2 and C3 positions; the degree of acetylation is reported to be about 0.28-0.37 (Hannuksela and Herve, 2004). The principal structure of GGM is illustrated in **Fig 2.3A**. Structural studies have also shown that the hydrogen on the galactosyl sidechains interacts with the mannan backbone intramolecularly, thus providing structural stability (Moreira and Filho, 2008). GGM occurs as two fractions that differ in galactose content, where the one with a low content is often referred to as simply glucomannan (Fengel and Wegener, 1989, Pettersen, 1984). In this thesis, however, GGM is used to represent both of these fractions.

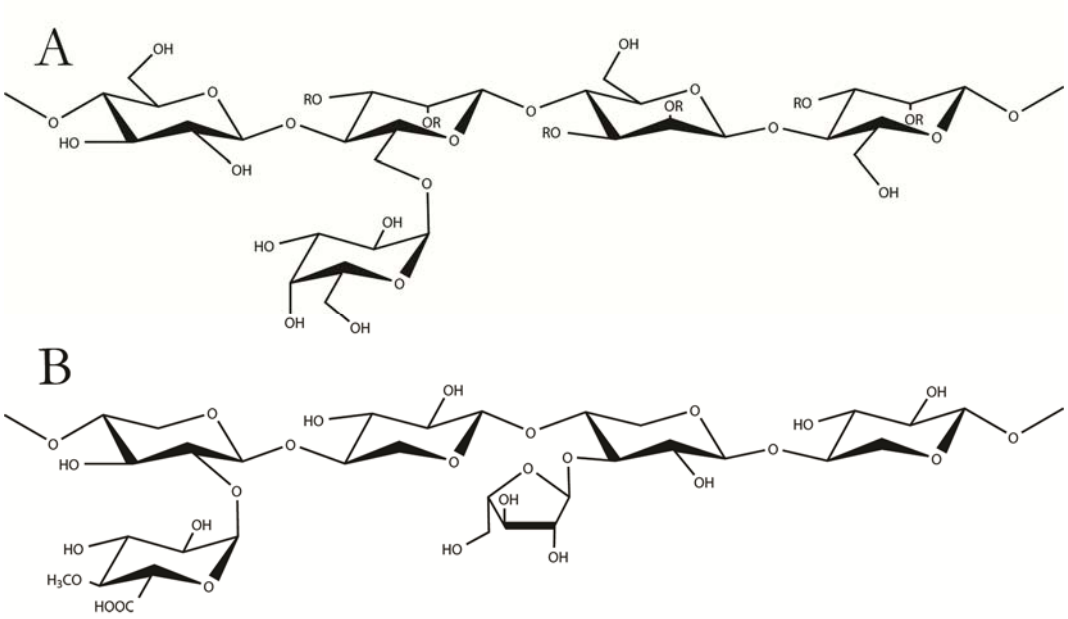

Figure 2.3 A: The principal structure of *O*-acetylated GGM. The sugar units are: β-D-Glc*p*, β-D-Man*p* with an α-D-Gal*p* side chain, β-D-Man*p*, β-D-Man*p*, R = $COCH_3$ (Ac) or H. **B:** The principal structure of AGX with the sugar units: β-D-Xyl*p* with a 4-*O*-Me-α-D-Glc*p*A side chain, β-D-Xylp, β-D-Xyl*p* with α-L-Ara*f* sidechain, β-D-Xyl*p*.

2.3.2 Arabinoglucuronoxylan (AGX)

Arabinoglucuronoxylan is the second most abundant hemicellulose in softwood and consists of β-(1→4)-linked D-xylopyranose (Xyl*p*) units substituted with α-(1→2)-linked 4-O-methyl-α-D-glucuronic acid (Glc*p*A) and α-(1→3)-linked L-arabinofuranose (Ara*f*) units (Ebringerova et al., 2005), see **Fig. 2B**. The arabinose side chains are easily hydrolyzed by acids due to their furanosidic structure (Sjöström, 1993).

2.3.3 Starch and pectins

A minor part of the wood is composed of other polysaccharides, e.g. starch and pectins. Starch, which consists of amyloses and amylopectins, is mainly present in the parenchyma cells of the wood (Sjöström, 1993). Pectins are mostly acidic and neutral sugars (Fogarty and Ward, 1972) and include galactans, galacturonans and arabinans (Fengel and Wegener, 1989).

2.3.4 Reactions

During alkaline treatment, the components in wood are subjected to depolymerization mainly through the hydrolysis of acetyl groups, the dissolution of low weight carbohydrates, peeling reactions and alkaline hydrolysis (Sjöström, 1993). GGM is degraded to a greater extent than both cellulose and xylan. The peeling reactions take place at the reducing end-group of a carbohydrate polymer, where one monomer at a time is rearranged and cleaved off. In the case of GGM, peeling reactions starts at temperatures as low as below 100°C (Wigell et al., 2007). The reaction continues until a competitive stopping reaction occurs, primarily caused by the rearrangement of the

reducing end-group (Franzon and Samuelson, 1957). Xylans in softwood are more stable than GGM since they have alkali-stabilizing arabinose and 4-*O*-methyl glucuronic acid side-groups and are also known to have the ability to resorb on the cellulose fibres (Yllner and Enström, 1956).

Treatments at acidic conditions lead to degradation of the carbohydrates mainly due to acid hydrolysis, which results in a breakage of the glycosidic bond. At elevated temperatures, acid-catalyzed dehydration of the polysaccharides also occurs, resulting in the formation of anhydro sugars with intramolecular glycosidic bonds. These are easily hydrolyzed and further degradation products such as uronic acids and furfurals, may be formed. Other common reactions in acidic media are oxidation reactions, which lead to the hydroxyl groups and reducing end-groups being converted into aldehyde, keto and carboxyl groups (Fengel and Wegener, 1989).

2.4 Chemical pulping

Wood can be converted into pulp through a series of chemical or mechanical processes. The two most common chemical processes for pulp production are the kraft (sulphate) and the sulphite processes. The main purpose for both is to remove the lignin from the cellulose fibres. Then, the fibres are most often transferred to a paper mill for further processing (Biermann, 1993). The sulphite process is operated at acidic or near-neutral conditions, which causes some hydrolysis of the cellulose molecules. As a result, the sulphite fibres are not as strong as the kraft pulp fibres. That, in combination with the more efficient chemical recovery process of the kraft process, has made it the dominant process for manufacturing pulp (Kleppe, 1970, Sjöström, 1993, Sixta, 2006). A schematic diagram of the kraft process is shown in **Fig. 2.4**.

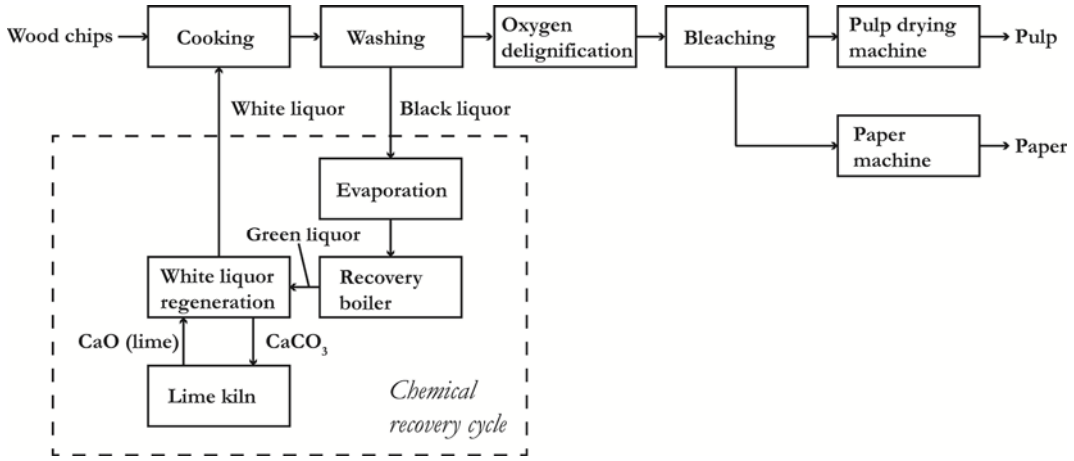

Figure 2.4 Schematic diagram of the kraft pulp process, including the chemical recovery process.

Wood chips are usually subjected to steaming prior to impregnation with the cooking liquor (white liquor). The air inside the wood chips is thus removed and the degree of impregnation is improved, which is important for achieving uniformity in the pulping

process (Malkov et al., 2002). Impregnation is a complex process that can be divided into two main mass transport mechanisms, namely penetration and diffusion. Penetration is a fast phenomenon driven by a pressure gradient. Diffusion, on the other hand, is a slow process that strives to equalize concentration gradients (Hultholm, 2004).

The white liquor in the kraft process has a high pH and contains the main active cooking chemicals, i.e. hydroxide and hydrosulphide ions (Sixta, 2006). The components in the wood are then subjected to degradation by several chemical reactions, including favorable fragmentation reactions of lignin as well as unfavorable degradation reactions of carbohydrates (Sjöström, 1993). Lignin is broken down into smaller fragments, which become ionized in the alkaline conditions. This increases their hydrophilicity and, finally, makes them water soluble (Gierer, 1980). The β-*O*-4 linkages are cleaved rapidly in the phenolic structures and more slowly in the non-phenolic (Ljunggren, 1980). Carbohydrates are degraded mainly by alkaline hydrolysis and peeling reactions (Matthews, 1974).

Oxygen delignification is often used as a first step prior to a bleaching sequence, with the purpose of removing more of the lignin from the unbleached pulp through the use of oxygen and alkali. Complex oxidation reactions occur, including radical chain reactions that act on both lignin and, unfortunately, also carbohydrates (Dence and Reeve, 1996).

2.4.1 Preservation of carbohydrates

Stabilizing the polysaccharides during alkaline degradation can be achieved by eliminating the aldehyde functions of the end-groups. Several reducing methods have been suggested with the aim of improving the pulp yield and the properties of the cellulose in the kraft process. Pre-treatment of the wood chips with hydrogen sulphide has been considered to be the most technically feasible method (Sjöström, 1993). In a study on pine wood chips, this treatment increased the pulp yield by 4-5% (Pekkala and Palenius, 1973). The increase in yield may reach as much as 8% on a dry wood basis, but the reaction requires high pressures and a large excess of H_2S (Sjöström, 1993). Other reducing agents, e.g. sodium borohydride ($NaBH_4$), have also been reported to make the pulp more resistant to alkali (Meller, 1953, Richtzenhain et al., 1954). Studies have shown that adding 1% $NaBH_4$ (on wood) can increase the pulp yield by 10% (Hartler, 1959), and increase the brightness of the pulp (Giertz and McPherson, 1956). In one study in which the effects of $NaBH_4$ and antraquinone (AQ) on microbiological pre-treated pine chips (bio-kraft pulp) were studied, it was found that the quantity of pulp rejects decreased with the addition of $NaBH_4$ (Cöpür and Tozluoglu, 2007).

Another approach to stabilize the carbohydrates is to oxidize the end-groups into carboxyl groups or other stable derivatives (Sjöström, 1993). Oxidative treatment in kraft mills has also been investigated in order to improve both delignification and bleaching,

thus giving several benefits (Allison, 1985). The use of polysulphides is one potential method of stabilization, and is based on a specific oxidation of the end groups to carboxyl groups (Sjöström, 1993, Venemark, 1964). It has been shown to give an increase in the pulp yield as well as a somewhat faster delignification rate (Lindström and Teder, 1995). Other possible oxidizing agents are superoxide radicals and hydroxyl radicals, which, for example, are used in chlorine-free bleaching processes (Gierer, 1997).

For acidic treatments, measurements on pulps after pre-hydrolysis and kraft cooking have shown that a pre-treatment at pH 2.5 and a temperature of 100°C do not affect the pulp yield and viscosity negatively, while an increase to a temperature of 120°C leads to significant lowering of the pulp yield (Brelid, 2002). Studies on the stabilization of GGM before sulphite pulping have shown that deacetylation of the GGM leads to molecules that are more resistant towards acidic hydrolysis. This can be achieved either by a prolonged impregnation at a low temperature or by a slightly alkaline precook. Stabilization cannot, however, be accomplished by weak acidic conditions in the precook (Annergren and Rydholm, 1960, Annergren et al., 1961). The reason for the stabilization seems to be that the fragments of deacetylated GGM become more linear, which provides them with the ability to sorb onto the cellulose fibres.

3. Biorefinery

The concept of biorefinery involves the conversion of biomass into new materials, chemicals and fuels in a manner analogous to that of the fractionation of crude oil in a conventional refinery (Fatih, 2009). Increasing environmental concerns and the limited availability of fossil fuels are also factors leading toward the development of more sustainable technologies, such as biorefineries (Fernando et al., 2006). Different types of biomass, e.g. agricultural products and lignocellulose feedstock (LCF), are considered suitable raw materials for biorefineries. The focus of this work is on forest material, where the wood components (cellulose, hemicelluloses and lignin) may be used for various new applications. Wood has several advantages, which include not only being a renewable resource that is not competitive with food but also available in large quantities (van Heiningen, 2006, Ragauskas et al., 2006). Pulp mills could actually be considered as being the first generation of biorefineries, in which cellulose is isolated and transformed into value-added products and lignin is used to produce steam and power (Chirat et al., 2010). However, most of the hemicelluloses are degraded during the pulping process and this emphasizes the main issue of this type of biorefinery: that all the main constituents of wood cannot be separated simultaneously without some extent of degradation.

3.1 General aspects

Several types of biorefineries exist, with different raw materials, pathways and final products. The main biorefinery technologies are based on three very different groups of products: fuels, energy and bio-based products (Kamm et al., 2006). A distinction can also be made between the processes upon which these biorefineries are based: biochemical or thermomechanical. The former process is often carried out at relatively

low temperatures and may involve, for example, enzymatic conversion of the biomass. The products of this type of biorefinery include material compounds, chemicals and fuels. The latter process, on the other hand, is performed at higher temperatures, and include gasification and pyrolysis of the biomass (Demirbas, 2009). The products of these processes, such as syngas and bio-oil, can then be used as intermediates for conversion into energy, fuels or chemicals via, for example Fischer-Tropsch synthesis.

Biorefineries based on LCF often employ an integrated concept with both high value-low volume and low value-high volume outputs (Kamm and Kamm, 2004). The simultaneous production of several products has been shown to lead to both environmental and economic advantages (Luo et al., 2010).

3.2 Lignocellulose biorefineries in Scandinavia

The idea of a wood-based biorefinery is not new. It has been brought up several times in the past, and especially during times of crisis. An example of an LCF biorefinery process is the fermentation of sugars from wood into alcohols, which was introduced early on in sulphite mills. The Domsjö mill in Sweden started to produce ethanol from biomass as far back as in the 1940s. The same mill was also a forerunner in the production of dissolving pulp for making viscose. This process was initiated in the 1930s, but was later taken out of use (Domsjö, 2014). Production was, however, resumed in the 1990s and comparatively large volumes are currently being produced. Today, a lot of research is invested in producing textile fibres from cellulose and finding alternatives to the viscose process: an example is the Södra Cell mill in Mörrum (Sweden), which was recently partially converted to produce dissolving pulp for textile applications (Södra, 2014). The Borregaard mill in Norway is another example of a sulphite mill that was converted into a biorefinery at an early stage. After the Second World War, Borregaard expanded their activities from producing only cellulose products to being a manufacturer of a wider range of chemical products. They are now one of the largest biorefineries in the world, with products that include lignosulphonates and vanillin made from lignin, and specialty cellulose products (Borregaard, 2014). Pulp and paper have remained the key products of kraft pulp mills, although companies such as LignoTech and Westvaco (now MeadWestwaco) developed products from lignin early on, e.g. lignosulphonates. Lignosulphonates can be used as surfactants, for example, as dispersing agents in concrete (Areskogh et al., 2010). It is also possible to obtain a relatively pure lignin from the kraft process via the so-called "LignoBoost process" (Öhman et al., 2007, Theliander, 2008). This lignin could be used as a source of energy as well as a raw material for value-added products. Another product from kraft pulp mills is tall oil, which originates from the wood extractives, and is used as a raw material for soap and biodiesel (Nogueira, 1996).

Great interest is also being shown for research to be carried out in the area of LCF biorefinery. One interesting product currently in the research phase is carbon fibres made

from lignin (Gellerstedt et al., 2010). There are also some products and applications based on different types of hemicelluloses. Some hemicelluloses were previously considered as a secondary production stream, mainly due to the difficulties of fermenting pentoses into ethanol. The integration of hemicellulose fractions and the production of other value-added products have since become attractive options (Carvalheiro et al., 2008). The xylose fraction can, for example, be converted into furfural, which is one of the basic components of several materials and chemicals, e.g. nylon 6 (Fernando et al., 2006). Several potential products made from GGM have also been suggested that could be used, for instance, in abrasion-resistant clothing, specialty papers or antibacterial bandages by modifying cellulose surfaces (Willför et al., 2008). Another example is a product that can be used as an emulsion stabilizer in the food and health industries. It has been shown that hemicelluloses the size of oligomers, prepared by steam explosion and fractionated with size exclusion chromatography (SEC), are easy to purify and modify before being used in the production of hydrogels (Söderqvist-Lindblad et al., 2001, Edlund and Albertsson, 2008). Furthermore, both GGM and xylan have been considered as potential barriers in bioplastic films (Gatenholm et al., 2004, Zhu et al., 2011, Mikkonen et al., 2010).

3.3 Extraction of hemicelluloses

The earliest investigations into methods for extracting various hemicelluloses from wood were performed on a lab scale and had the aim of gaining a better understanding of the chemistry of different wood species. The experiments were often conducted on delignified wood, also known as holocellulose. Hemicelluloses were isolated in successive steps, usually with different types and concentrations of alkali, depending on the source of the wood (Fengel and Wegener, 1989, Sjöström, 1993). The methods and results from these experiments have been summarized in various review articles, e.g. Timell (1967). It was shown that GGM could be isolated using barium hydroxide, which forms an insoluble complex with mannans and glucomannans (Meier, 1958). Many of the methods presented in these early works still dominate current research in the area: the use of barium hydroxide, for instance, was used recently to fractionate lignin-carbohydrate complexes (LCCs) from spruce wood (Du et al., 2013). Different types of organic solvents have also been used for extracting hemicelluloses on a lab scale (McPherson, 1958). A large-scale organosolv process (Organocell) was commercialized at the Kelheim mill in Germany (Young, 1992), but was later shut down.

The extraction and isolation of hemicelluloses on a larger scale have been investigated for various resources and with several different processes dependent on factors such application, cost and performance. Research has, however, been dominated by investigations aimed at the production of fuels such as ethanol (Bozell, 2010). Technologies for converting biomass into fermentable sugars have been covered in several review articles (e.g. Hamelinck et al. (2005) Gnansounou and Dauriat (2005) Fatehi (2013)) and many pre-treatment methods have been investigated in order to

improve the hydrolysis of lignocellulosic materials. These methods include mechanical (grinding, extrusion etc.), physico-chemical (e.g. steam explosion, ammonia fibre explosion, wet oxidation, CO_2 explosion, microwave treatment and ultrasound treatment) and chemical (ozonolysis, acid/alkaline hydrolysis, organosolv processes and treatment with ionic liquids) treatments (Sun and Cheng, 2002, Alvira et al., 2010).

The extraction of hemicelluloses has also been conducted prior to the production of dissolving pulp. The resulting hemicelluloses were used as a source of fermentable sugars while the final pulp benefitted from being more cellulose-rich (Li et al., 2010, Liu et al., 2011, Huang et al., 2010). Process waters from thermomechanical pulping have also been shown to be a potential source of hemicelluloses (Willför et al., 2003, Andersson et al., 2007). In the former study, GGM was isolated from process water removed from a softwood process by precipitation in ethanol. In the latter, the process waters were ultrafiltered and purified by diafiltration or SEC. The extraction of GGM from spruce wood has also been accomplished by using pressurized hot water (Song et al., 2008). That particular study also found that the pH profile is an important factor which needs to be controlled in order to reach reasonably high yields of hemicelluloses of high molecular weight. Succeeding investigations, where the pH was controlled with phthalate buffers, showed that the optimal pH for extracting GGM was a pH value of 4. Lower values resulted in GGM with lower molecular weights, and higher values led to lower yields (Song et al., 2011b). Analogous results were found for hot-water extractions with the addition of $NaHCO_3$ (Song et al., 2011a).

Methods based on treatment in a microwave oven and treatment with steam for extracting oligosaccharides from hemicelluloses from spruce have also been published (Palm and Zacchi, 2003). The treatments were carried out for a few minutes at temperatures around 200°C and resulted in hemicelluloses with molecular weights of a few thousands Daltons. The effect of impregnation with alkali (NaOH) was also investigated in a similar study, where GGM was characterized after extraction by microwave heat-fractionation. It was, for example, shown that deacetylation of the GGM occurred when the NaOH concentration was above 0.05% (Lundqvist et al., 2002). Extraction with aqueous liquors at acidic, near-neutral and alkaline conditions was investigated in a study where the impact of the extraction conditions and wood species on the chemical composition was compared (Perez et al., 2011). In the case of spruce wood, the conclusion was that extraction at near-neutral conditions resulted in GGM-rich fractions with low contents of residual lignin.

4. Steam explosion

Steam explosion (STEX) of wood was introduced in two patents in 1928 and 1932, respectively. In the former patent, descriptions of how STEX can be used on an industrial scale were explained (Mason, 1928); it also formed the basis for the production of Masonite boards (Boehm, 1930). The latter describes the treatment of pine wood chips with saturated steam at elevated pressure. The pressure was released quickly and the wood chips were discharged; they were subsequently treated with extraction liquors to obtain sugars which were fermented into alcohols (Babcock, 1932).

The STEX technique can be described as a hydrothermal treatment whereby wood, or other types of biomass, are subjected to saturated steam at elevated pressure. This pressure is then rapidly released to a considerably lower level, usually atmospheric pressure. For wood, the process could be described as a three-step process: (i) treatment with pressurized steam for a certain amount of time, (ii) explosion through quick pressure release and, most frequently, (iii) highly softened wood chips impact with each other and the walls of the equipment. During the treatment, the wood components are exposed to several chemical reactions. One of the main type of reactions is acid hydrolysis, which occurs due to the release of acetic acid from the wood itself: it is often referred to as "autohydrolysis" (Marchessault, 1988). The two latter steps of the steam explosion process lead to physical rupture of the wood structure. Thus, STEX treatment results in the wood being subjected to a combination of mechanical and chemical effects.

Steam explosion has been suggested as a pre-treatment step in biorefinery contexts as well as being an environmentally friendly pulping process (Kokta and Ahmed, 1998). The

most common application for STEX as a pre-treatment is to produce fermentable sugars, e.g. for the production of ethanol. The treatment conditions in this case are usually harsh, as acidification is often enhanced (e.g. by the addition of sulphur dioxide (SO_2)) to aid the decomposition of the biomass (Shimizu et al., 1998, Mabee et al., 2006, Grethlein and Converse, 1991).

4.1 Severity factor

Subjecting wood to steam explosion could result in anything from introducing small cracks in its structure to full defibrillation (Tanahashi, 1990). The temperature and treatment time have been identified as the most important parameters that govern the effects of the treatment; Overend and Chornet (1987) observed that it was possible to trade these factors and attain nearly equivalent results. They discussed a severity factor for steam-aqueous pre-treatments, where the temperature and the residence time were combined into a single parameter, analogous to the H-factor often used for pulping processes (Sjöström, 1993). The P-factor (Brasch and Free, 1965), which describes the severity of a treatment based on the reaction temperature was rewritten for steam explosion treatments by Heitz et al. (1991), and the severity parameter, R, was thus introduced:

$$R = t \times exp[(T_r - T_b)/14.75]$$

where T_r is the reaction temperature, T_b the base temperature (i.e. 100°C) and t the residence time. This concept indirectly includes the assumptions that the overall kinetics follow first-law concentration dependence, and that the rate constant has an Arrhenius-type dependence on temperature, even though the apparent activation energy may itself be a function of temperature. There are also other parameters that influence the STEX treatment of wood, such as the wood species, size of the wood chips, moisture content and equipment design. Some of these factors were investigated by Cullis et al. (2004), who found, for example, that the "relative severity" decreased when the size of the wood chips increased.

4.2 Steam explosion and pulping

The process described in Mason's patent, whereby wood chips were subjected to steam at temperatures around 285°C and then discharged through slotted ports and exploded to atmospheric pressure, resulted in completely defibrillated fibres (Mason, 1928). This treatment was an energy-efficient way of producing mechanical pulp, and provided a high yield. However, the pulp had a dark colour and rough texture, which made it unsuitable for making paper. Modifications to the process were therefore suggested, such as chemical impregnation of the wood chips prior to the STEX treatment, along with refining and bleaching steps afterward (Kokta and Ahmed, 1998, Vit and Kokta, 1986). Some examples of this include treatment of the wood chips with antioxidants in order to

protect the wood components from degradation during the STEX, as well as using sodium hydroxide to increase fibre swelling (Kokta and Ahmed, 1998). The pulps resulting from the STEX process were somewhat different from other mechanical pulps, showing higher porosity, greater specific surface area and a more hydrophilic character.

Steam explosion has also been investigated as a pre-treatment step prior to conventional pulping. A STEX treatment was suggested as a method of producing ethanol and kraft pulp simultaneously (Martin et al., 1995). The resulting kraft pulp required less pulping chemicals, but it had a lower quality than pulp produced by conventional pulping methods. In a more recent study, however, in which eucalyptus wood chips were steam-exploded prior to kraft cooking, the resulting pulps showed lower kappa numbers with no noticeable change in viscosity (Martin-Sampedro et al., 2011). STEX has also been used as a pre-treatment prior to soda/anthraquinone pulping of softwood in a study of the characteristics of sulphur-free lignins (Anglès et al., 2003).

4.3 Effects of steam explosion on biomass

The effects of STEX treatments on the appearance of wood have been studied for different wood species, e.g. sub-alpine fir (Zhang and Cai, 2006) and aspen (Kallavus and Gravitis, 2009). In the former study, microscopy images showed fractures in the structure of the cells (especially in the earlywood tracheids), at bordered pit pairs and in pits between the parenchyma and ray cells. In the latter, it was revealed that vessels and ray cells of aspen functioned in opposite ways: vessels accelerated separation while the ray cells retarded it. In another study on aspen wood chips (Josefsson et al., 2002), it was shown that the amount of hemicellulose and molecular weights in the exploded material could be changed considerably by varying the STEX conditions.

The components of wood are affected by a STEX treatment in several ways: accessible glycosidic bonds and β-ether linkages in lignin are cleaved, as are potential lignin-carbohydrate complex (LCC) bonds (Marchessault, 1988). Studies on lignin in steam-exploded wood have also shown that the lignin becomes softened and depolymerized at relatively high temperatures (200-250°C), which lead to the formation of droplets (Excoffier et al., 1988). The lignin can become redistributed on the inner and outer surfaces of the cell wall, as well as within the actual cell wall (Capretti and Focher, 1988). Two processes responsible for morphological changes in biomass during STEX were published by Langan et al. (2014), who identified cellulose dehydration (expulsion of water molecules from between the bundles of fibrils) and the aggregation of lignin into globules, i.e. phase separation between lignin and hemicelluloses. The study was conducted on aspen wood chips treated at 180°C for 28 minutes, and characterization data was compared with molecular dynamics simulations. Studies on lignin from softwood after STEX, and with the addition of SO_2, have also been performed (Shevchenko et al., 1999, Li et al., 2009). The resulting changes in the chemical

composition were investigated and it was shown that the reactivity of lignin was lowered after STEX, probably due to oxidation or condensation reactions.

Steam explosion has also been used on other types of biomass. The cellulose and lignin in corn cobs, banana plants, cotton stalks and cotton gin waste were characterized after different residence times of a STEX treatment at 220°C by Ibrahim et al. (2010). Their findings showed that the STEX led to the extensive cleavage of ether bonds, condensation reactions and some demethylation of aromatic methoxyl groups in the lignin. It was also shown that the degree of crystallinity of the cellulose increased with the length of the treatment times. A similar study was conducted by Schultz et al. (1984), in which steam-exploded hardwood chips, rice hulls, corn stalks and sugar cane bagasse were characterized. It was shown that STEX caused partial degradation of the hemicelluloses, that the residual fibres consisted mainly of cellulose and that a proportion of the lignin became soluble in hot alkali. Equivalent results were found by Glasser and Wright (1998), who studied steam-exploded yellow poplar, peanut hulls and sugar cane. Continuous pilot-scale STEX equipment was used in a study aimed at improving the digestibility of wheat, barley and oat straw for animal feed (Viola et al., 2008), with the STEX treatment being followed by washing with sodium hydroxide. It was found that both the STEX and the alkaline wash increased the digestibility of these materials. A similar treatment was described by Montané et al. (1998), who fractionated wheat straw.

4.4 Steam explosion as a pre-treatment

A very common application of STEX, as mentioned previously, is as a pre-treatment to prepare biomass for enzymatic treatment and the fermentation of sugars into alcohols (Schell et al., 1991, Söderström et al., 2003, Parawira and Tekere, 2011). Sulphur dioxide (SO_2), which is transformed into sulphuric acid, is often added in order to depolymerize the cellulose and hemicelluloses further: Shevchenko et al. (2000), for example, studied solubilized hemicelluloses with the aim of achieving a high degree of monosaccharides. Also, in studies that have focused on the utilization of all of the components of wood, the application considered for the hemicellulose fractions has been the production of fermentable sugars (Jain et al., 1999, Li et al., 2005, Shimizu et al., 1998).

Steam explosion has also been evaluated and compared with other pre-treatments prior to enzymatic hydrolysis and fermentation processes. In a study using aspen, STEX was compared to treatment with dilute nitric acid and was shown to be somewhat more effective (Saddler et al., 1982). The cellulosic structures and kinetics after STEX and dilute acid treatment were also studied by Carrasco et al. (1994), who found that the STEX treatment was very effective and resulted in intense degradation of the hemicelluloses into fermentable monomeric sugars. However, an important factor in STEX treatments at harsh conditions is the formation of degradation products, such as furfurals and hydroxymethyl furfurals, which occur at severe (acidic) conditions (Li et al., 2005). These

compounds can act as inhibitors during enzymatic hydrolysis (Tofighi et al., 2010) and thereby result in lower yields of ethanol. STEX has also been compared with other steam treatments that lack the actual explosion step. In a study based on two hardwood species, the rate of enzymatic hydrolysis and depolymerization of the lignin increased for both steam treatments, although STEX resulted in somewhat more degraded hemicelluloses (Schultz et al., 1989). No significant changes in enzymatic hydrolysis were seen in poplar wood chips, however, when steam explosion and steam refining were compared (Schuett et al., 2012). Comparative data from different pre-treatment technologies has also been prepared by the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI), where corn stover was converted into sugars. The findings showed that the more acidic pre-treatments lead to a greater amount of xylose in the pre-treatment step than alkaline treatments. The overall yields after pre-treatment and enzymatic hydrolysis resulted in similar values for all the pre-treatments investigated, including STEX with the addition of SO_2 (Elander et al., 2009).

5. Materials and Methods

This section introduces the various materials and experimental methods used in this work. Mild steam explosion treatments were investigated in three different pieces of equipment, all described in detail below. The effects of mild steam explosion were studied with both physical and chemical characterization methods, after steam explosion only, as well as after various post-treatments. Some investigations of chemical pre-treatments of the wood material and an investigation of the steam explosion liquor were also conducted. More detailed descriptions of the methods used are found in the appended papers.

5.1 Materials

5.1.1 Raw materials

The samples in most of the studies originate from industrially-cut wood chips of Norway spruce (*Picea abies*) obtained from a Scandinavian pulp mill. The wood chips were air-dried and selected manually prior to the experiments in order to avoid dirt, knots and bark. In Paper I, the wood chips were also ground into wood powder in a Wiley-type mill (< 1 mm).

Sawn pieces of Norway spruce, with dimensions of 90 mm x 20 mm x 4 mm and taken from the same annual rings in the wood trunk, were used in Paper IV. Each of these pieces was divided into three equally-sized samples which were used for comparison after the different experiments.

5.1.2 Chemicals

The chemicals used for the pre-treatments, analyses and as standards were all of analytical grade. The cooking chemicals used for kraft pulping were of reagent and analytical grade. The enzymes used in Paper II was a cellulolytic culture filtrate, containing mainly endoglucanase, exoglucanase and β-glucosidase (Liu et al., 2009) with an original activity of 90 ECU/mL. A more detailed description of the chemicals is given in Appendix A.

5.2 Pre-treatments

Some investigations into chemical pre-treatments of spruce wood were performed, primarily as a way of stabilizing the easily degradable GGM. The focus was on treatment employing a reducing agent, so $NaBH_4$ (described in **5.2.1**) was used in several of the studies. An alternative reducing agent was used in Paper VI, i.e. sodium dithionite ($Na_2S_2O_4$).

5.2.1 Reference study (Paper I)

The first part of the study in Paper I was a reference study made to obtain data on the chemical effects of various aqueous solutions on spruce wood powder. Milled wood was chosen as the sample material in order to minimize mass transport resistances, thereby making it easier to study the chemical effects alone. The samples were subjected to either an alkaline (NaOH, 0.75 M, pH 13.9 at RT) or acidic solution (H_2SO_4, 0.005 M, pH 2.0 at RT). All of the experiments were performed in steel autoclaves with a volume of approx. 1.2 L.

Some samples were pre-treated with $NaBH_4$ before being subjected to the alkaline treatment. The pre-treatment was performed by adding the wood powder to beakers containing an aqueous $NaBH_4$ solution (7 w/w% based on wood), with a liquor-to-wood ratio of 7:1. The treatment time was 100-120 hours.

The wood powder samples (10 g on dry basis) were mixed with alkaline or acidic liquor to a liquor-to-wood ratio of 100:1. The ratio was kept high in order to maintain a virtually constant concentration of the ions in the solution. The autoclaves were subjected to vacuum followed by overpressure, before being placed in a pre-heated polyethylene glycol (PEG) bath. The temperatures applied were 90, 110 and 130°C, which corresponded to 87, 108, and 127°C inside the autoclaves at equilibrium temperature, see **Fig. 5.1**, (Ribe et al., 2010); the first fifteen minutes could be considered as a heat-up time and the final equilibrium temperatures end up a few degrees below the applied temperatures. The treatment times were 10, 30, 60 and 120 minutes. Afterwards, the wood powders were analyzed with respect to the content of Klason lignin, acid-soluble lignin (ASL) and carbohydrates. In addition, NIR spectroscopy measurements were performed and correlated with the results obtained from the chemical analyses using multivariate data analysis.

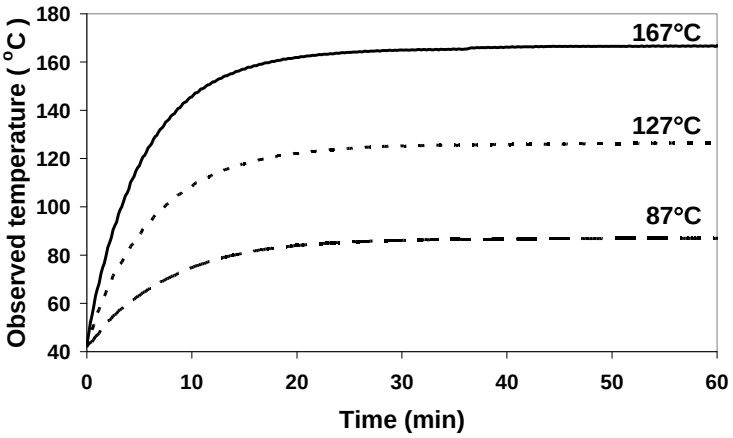

Figure 5.1 Temperature profiles inside the autoclaves at PEG-bath temperatures of 90, 130 and 170°C, monitored using a 175-T3 logger with a Teflon thermocouple (Ribe et al., 2010).

5.2.2 $NaBH_4$ pre-treatment study (Paper V)

A minor study was conducted on the actual $NaBH_4$ pre-treatment of wood chips. The concentrations of $NaBH_4$ in the aqueous solutions and the treatment times were varied as illustrated in **Table 5.1**. In each experiment, 30 g (dry basis) of wood chips were submerged in aqueous $NaBH_4$ solutions with concentrations based on wood (w/w%) and with a liquor-to-wood ratio of 10:1. After treatment, the wood chips were washed and dried at RT prior to being subjected to the mild steam explosions and following chemical analysis.

Table 5.1 $NaBH_4$ pre-treatment conditions. Concentrations of $NaBH_4$ are presented as weight percentages (w/w%) based on wood.

Experiment	Conc. $NaBH_4$	Treatment time	pH level of liquor
1	2%	24 h	10.2
2	7%	24 h	10.8
3	2%	1 week	9.6
4	7%	1 week	10.3
5	0.5%	3 days	9.2

A 20 g (dry basis) sample of wood chips from the pre-treatment study was subsequently exploded in the lab-scale steam explosion equipment (see **5.3.1**) at 160°C for ten minutes. The chips were washed and analyzed with respect to content of lignin, carbohydrates and acetate.

5.2.3 Dithionite impregnation (Paper VI)

The impregnations made using dithionite in Paper VI were performed in steel autoclaves. Wood chips (500 g) were put in aqueous solutions containing 21 g of $Na_2S_2O_4$ and 200 g of $NaHCO_3$ at a liquor-to-wood ratio of 4:1. The autoclaves were evacuated for five minutes and then pressurized with 5 bar of nitrogen gas for five additional minutes. They were placed in a pre-heated PEG bath with a temperature of 85°C for four hours under constant rotation. Subsequently, the autoclaves were put in a cooling bath and the wood

chips and impregnation liquor were transferred into a larger steel vessel. The vessel was evacuated, followed by pressurization with nitrogen gas, and then left overnight.

5.3 Mild steam explosion

Three different steam explosion apparatuses of various sizes were used in this work. Each has its own distinguishable characteristics that influence the effects of the treatment, even when the STEX conditions are the same. In both the lab-scale and small-scale equipment, only the steam is released: the wood samples are exploded inside the apparatus. In the bench-scale apparatus, both the wood chips and the steam are released into another vessel. The small-scale equipment is designed to explode only one or a few single wood pieces at the time. The different pieces of equipment, and the experiments performed in each of them, are described below.

5.3.1 Lab scale (Papers I-II and V)

The lab-scale equipment consists of a modified steel autoclave approx. 1.2 L in volume with a lid equipped with an inlet for steam and a temperature sensor. The valve in the middle of the lid is used to release the pressure, see **Fig. 5.2**. An insulated outer beaker is used to maintain the temperature inside the autoclave during the treatment.

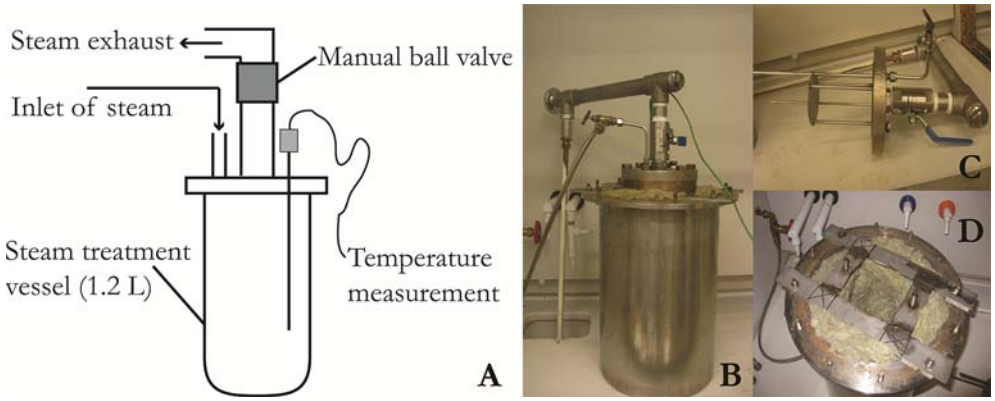

Figure 5.2 The lab-scale steam explosion equipment. **A)** schematic diagram of the autoclave and lid. **B)** the autoclave connected to the steam inlet. **C)** the modified lid showing the temperature sensor and steam inlet and outlet. **D)** the insulated outer beaker.

In Paper I, all of the samples consisted of wood powder. Mild explosion experiments were performed on either water-impregnated or $NaBH_4$ pre-treated samples and the effects of different residence times during the steam explosion treatment were investigated. The water impregnation procedure was performed to correspond to that of fully impregnated wood chips, i.e. with a liquor-to-wood ratio of 2.3:1. All the experiments were conducted at a steam explosion temperature of 160°C, which was kept constant during the residence time, and then the pressure was rapidly released. Seven different residence times were applied: 5, 10, 15, 20, 25, 30 and 35 min. The samples were subsequently washed and their chemical composition analyzed.

The samples pre-treated with $NaBH_4$ were first prepared in the same way as described in **5.2.1** before being subjected to mild steam explosion at 160°C for four different residence times: 5, 10, 20 and 30 min.

The experiments in Paper II were conducted on samples of wood chips, with the aim of investigating the effects of a mild steam explosion treatment during different types of post-treatments, such as enzymatic reactions, alkaline leaching and kraft pulping. 50 g of water-impregnated wood chips (dry basis) were used in each experiment. The steam explosion temperatures were varied: the chips for the enzyme experiments were treated at 115, 130, 145 or 160°C, those for alkaline leaching at 140 or 160°C and those for kraft cooking at 160°C. However, the residence time was set to 10 min in all cases to ensure that the temperature was also reached inside the chips. This time was estimated by using an approximation of Fourier's law of heat conduction.

5.3.2 Small scale (Paper IV)

The small-scale STEX equipment is depicted in **Fig. 5.3**. The steam explosion chamber has a vessel volume of 3.8 L; steam is introduced through a steam inlet pipe at the top of the chamber and the pressure is released by opening the ball valve to the blow tank.

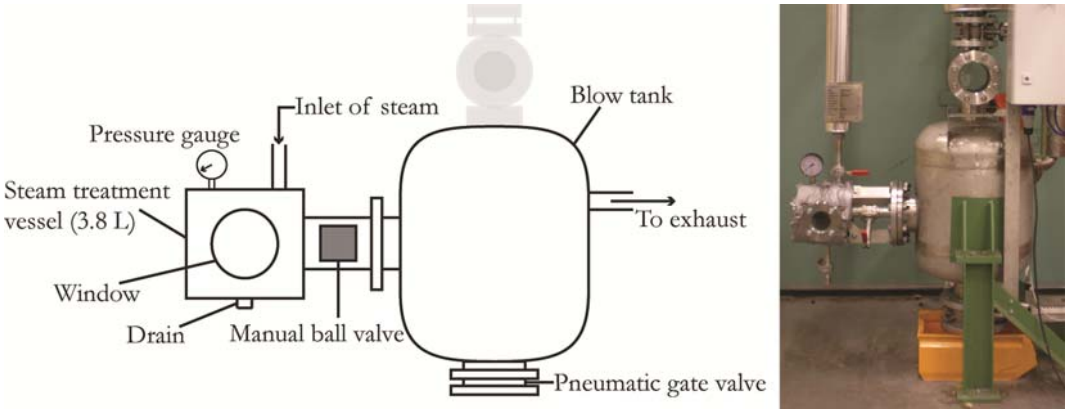

Figure 5.3 Small scale steam explosion equipment. Left: schematic diagram of the steam explosion unit (with the blow tank on the right hand side). Right: photograph of the equipment.

In Paper IV, the samples used were single, sawn pieces of wood and the different effects of heating with steam, pressure release and impact with other wood pieces and the walls of the equipment were investigated. The effect of impact was studied in the bench-scale equipment; the explosion step of the treatment was isolated from the impact step by containing the wood pieces in small steel-wire baskets. The steam explosion valve in the small-scale equipment could be opened rapidly or slowly, depending on whether or not an actual explosion step (i.e. rapid release of pressure) was desired. The experimental conditions used in the different experiments in Paper IV are shown in **Table 5.2**.

Table 5.2 Experimental conditions of the steam explosion experiments in Paper IV.

Experiment	Pressure	Time	Explosion	Impact
STEX 7_10	7 bar (165°C)	10 min	Yes	No
STEX 14_10	14 bar (195°C)	10 min	Yes	No
STEX 7_5	7 bar	5 min	Yes	No
STEX 14_5	14 bar	5 min	Yes	No
Heat 14_10	14 bar	10 min	No	No
Expl. 14_10	14 bar	10 min	Yes	No
No imp. 7_10	7 bar	10 min	Yes	No
Imp. 7_10	7 bar	10 min	Yes	Yes

5.3.3 Bench scale (Papers III and VI)

Most mild steam explosion treatments were performed in the bench-scale steam explosion equipment shown in **Fig. 5.4**. The wood chips were filled into the top chamber and heated with saturated steam from the boiler until the desired pressure was reached. At the moment of pressure release, the wood chips were discharged into the lower vessel together with the steam and the condensate.

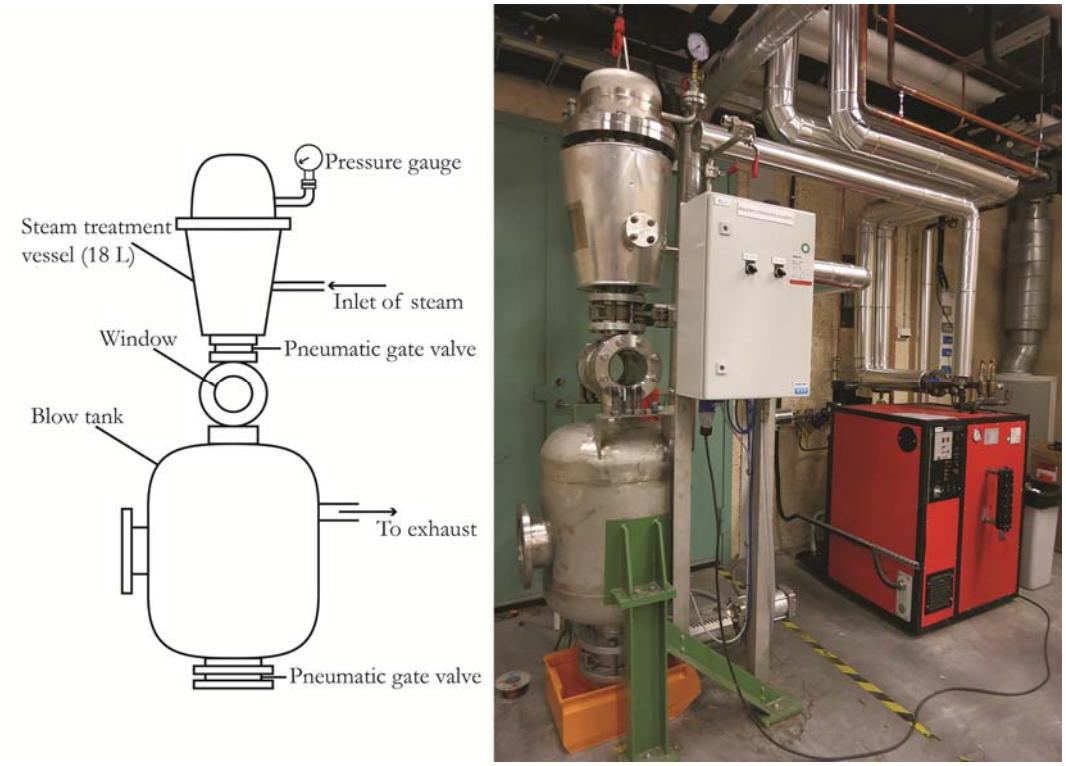

Figure 5.4 The bench-scale steam explosion equipment. Left: schematic diagram of the steam explosion unit with its upper and lower vessels and valve controls. Right: photograph of the STEX unit together with the boiler (on the right hand side).

For each steam explosion experiment, 500 g of o. d. wood chips were used. The treatment conditions varied somewhat in the different studies. In Paper III, water-impregnated wood chips were subjected to steam explosion at 4 bar (i.e. 144°C) or 7 bar (i.e. 165°C) after ten minutes, before being subjected to kraft cooking experiments. The same STEX conditions were used in Paper VI, where water-impregnated wood chips were compared with wood chips pre-impregnated with $Na_2S_2O_4$, which were also

subsequently subjected to kraft cooking. The focus of Paper V was on the components in the steam explosion liquors, i.e. the condensed steam. Two different batches of wood chips were steam exploded at 4 and 7 bar after ten minutes. A batch of never-dried wood chips and a batch of $NaBH_4$ pre-treated wood were also exploded at 7 bar after ten minutes. The liquors were then collected, evaporated and freeze-dried (FreeZone, Triad™, Labconco Corp.) prior to chemical analysis. The wood chips in Paper IV were only used as a bulk material, as the aim was to investigate the mechanical effects of impact on sawn pieces of wood. The STEX condition in this case was set to 7 bar and the treatment time was ten minutes.

5.4 Post-treatments

The effects of mild steam explosion during a few different post-treatments were also investigated. Extractions, or leaching, in aqueous solutions of different levels of pH were studied in Paper I and, to some extent, also in Paper II in which the wood chips were also subjected to enzymes after mild STEX. Kraft cooking experiments of steam-exploded wood chips were conducted in Papers II, III and VI.

5.4.1 Extraction/Leaching (Papers I - II)

Experiments using steam-exploded wood samples subjected to alkaline or acidic aqueous solutions were performed in order to investigate the possibility of extracting wood components after a mild steam explosion treatment. The extractions were performed in steel autoclaves that were placed in a pre-heated PEG bath. In Paper I, two experimental series were carried out on mild steam-exploded wood powder: one with a high liquor-to-wood ratio and the other with a low liquor-to-wood ratio. The former was done to preserve an almost constant concentration of the ions in the liquor whereas the intention of the latter, which also used larger quantities of material and harsher extraction conditions, was to extract higher amounts of wood components into the liquid. In both cases, the samples of wood powder were either water-impregnated or pre-treated with $NaBH_4$ and then steam exploded at 160°C after 25 minutes.

In the series with a high liquor-to-wood ratio (100:1), 15 g of wood powder (dry basis) was used for the mild STEX of which 10 g were used in the subsequent extraction experiment; the extraction conditions are presented in **Table 5.3**. The sample amounts were somewhat larger in the series with a low liquor-to-wood ratio (10:1): 50 g of wood powder was used for the mild STEX of which 40 g were used in the following extractions. After undergoing treatment, the wood samples were washed and analyzed with respect to chemical composition.

Table 5.3 The experimental conditions prevalent during the extractions of wood powder

Experimental condition	Liquor-to-wood ratio	Level of pH	Temperature (°C)	Time (h)
1_high L:W	100:1	8	130	1
2_high L:W	100:1	4.5	130	1
3_high L:W	100:1	4.5	130	2
4_high L:W	100:1	2.5	130	1
1_low L:W	10:1	2.5	160	1
2_low L:W	10:1	11	110	1
3_low L:W	10:1	11	130	1

The extraction liquors from the extractions performed at low liquor-to-wood ratios were collected and neutralized to pH 7. Then they were put in a rotary evaporator and, finally, freeze-dried. These samples were subjected to complete hydrolysis and analyzed for chemical composition; some of them were also analyzed for molecular weights and molecular weight distributions.

The experiments in Paper II were performed on wood chips and the extraction liquor was a 0.75 M NaOH solution. The liquor-to-wood ratio was 10:1 and the temperature was 130°C, which was kept for two hours. Samples that were steam-exploded at 160°C (i.e. 6.2 bar) after ten minutes were compared to untreated wood chips. After the extractions, the wood chips were washed with ten liters of de-ionized water and then placed in de-ionized water for a week, during which time the water was replaced several times. This was done in order to leach out the extraction liquor before the chemical analyses were made.

5.4.2 Enzymatic treatment (Paper II)

The enzymatic treatments were performed on wood samples of 20 mg (dry basis). Experiments were performed on samples cut into small pieces (cuboids) from different parts of the wood chips as well as on disintegrated samples. The samples were put in Eppendorf reaction tubes together with 20 mM of phosphate buffer of pH 7 and 20 µL of enzymes. The reaction took place at 40°C and 600 rpm for 24 h and was terminated by increasing the temperature to 99°C.

5.4.3 Kraft cooking (Papers II, III and VI)

Kraft cooking experiments were performed in several of the studies. An initial study was made in Paper II in which wood chips were steam-exploded in the lab-scale equipment at 160°C for 10 min. The cooking chemicals (NaOH, Na_2S and Na_2CO_3) were charged at a liquor-to-wood ratio of 4.5:1 (kg/kg); the sulphidity was 35%, the effective alkali (EA) charge was 22% and the carbonate concentration in the white liquor was 0.1 M. The cooks were performed in steel autoclaves that were placed in a pre-heated PEG bath at 80°C for 20 min. The temperature was then raised, at a rate of 0.8°C/min, to the cooking temperature of 170°C, which was then maintained for 40, 60, 80 or 100 min. After the cooks, the pulp was separated from the black liquor, which was re-filtered once. The pulp was washed, disintegrated in a laboratory defibrator and, finally, subjected to a second wash.

In Paper III, two batches of chips were steam exploded in the bench-scale equipment at 4 and 7 bar after 10 min prior to cooking. The kraft cooking conditions were analogous to those in Paper II (cooking temperature of 170°C) but with two differences: the times at the cooking temperature were 20, 40, 60 and 80 min, and the washed pulps were also subjected to subsequent oxygen delignification. Pulp (35 g on dry basis), $MgSO_4$ (corresponding to 1 kg magnesium per ton dry pulp), NaOH (25 kg/ton pulp) and de-ionized water were used to prepare suspensions with a pulp concentration of 12 w/w%. The samples were placed in autoclaves and pressurized with 6 bar oxygen gas for two minutes. The autoclaves were placed in a pre-heated PEG bath at 105°C for one hour under constant rotation. The autoclaves were then cooled, the overpressure was released and the pulps were subjected to a series of washing processes.

The kraft cooking experiments in Paper VI were performed on wood chips impregnated with either water or $Na_2S_2O_4$ and steam-exploded in the bench-scale equipment at 4 or 7 bar after ten minutes. Each cook was conducted with 500 g (o. d.) wood chips in a pilot-scale circulation digester. The chips were steamed at 15 bars for five minutes prior to being subjected to the white liquor circulation. The starting temperature was 100°C and the temperature was increased gradually at 1°C/min until a cooking temperature of 160°C was reached. The sulphidity was 35%, the concentration of hydroxide ions was 35 g/L and the liquor-to-wood ratio was 4:1. A cook where 4 (w/w)% $Na_2S_2O_4$ was added into the white liquor was also conducted. The cooking temperature was maintained for four hours and the chips were then washed overnight with de-ionized water at a flow rate of 1.8 L/min. The chips were disintegrated after undergoing 50,000 revolutions in a disintegrator and then defibrated with a water jet defibrator. Finally, the pulps were centrifuged to a dry content of about 30%.

5.5 Characterization

Physical characterization of steam-exploded wood was conducted mainly in the work in Paper IV; chemical analyses were conducted in most of the papers.

5.5.1 Electron microscopy (Paper IV)

An environmental scanning electron microscope (ESEM) was used for characterization of changes in morphology in steam-exploded wood. One advantage ESEM has over SEM is that its chamber can be operated at humid conditions, so the material being examined does not need to be dried prior to analysis. Samples were cut from the surfaces and insides of the steam-exploded wood chips. Images were taken in a Carl Zeiss EVO-HD15 ESEM instrument; the backscatter detector was the preferred detector and the software used was SmartSEM, Version 5.05.

5.5.2 Mercury porosimetry (Paper IV)

Mercury porosimetry is a technique that can provide important structural information of a wood sample such as, for example, the total pore volume and the pore size distribution

distribution (Moura et al., 2005). Mercury has a very high surface tension when it comes into contact with most materials: it therefore does not penetrate through pores unless a force is applied. The theory behind mercury porosimetry is based on the assumption that pores are cylindrical and that an increase in applied pressure will lead to penetration into pores of diminishing size. Pore diameters are related to the pressure using the Washburn equation (Washburn, 1921):

$$D = -\frac{4\gamma cos\theta}{P}$$

where D is the pore diameter, γ is the surface tension of mercury (0.485 N/m), θ is the contact angle (130°) and P is the applied pressure (Pfriem et al., 2009). Samples of freeze-dried, steam-exploded wood chips were studied in a Micromeritics AutoPore IV Mercury Porosimeter and analyzed with the software AutoPore IV 9500, Version 1.09. The average pore diameter was calculated as being four times the total intrusion volume divided by the total pore area.

5.5.3 Lignin analyses (Papers I-III and Papers V-VI)

Klason lignin is defined as the residual material after acid hydrolysis and was determined gravimetrically after complete acid hydrolysis with 72% H_2SO_4. The method used was based on the procedure presented by Theander and Westerlund (1986). The filtrate from the hydrolysis was later used for measurement of the content of acid soluble lignin (ASL) and carbohydrate analysis.

The content of ASL was calculated in relation to the absorbance value measured with UV at a wavelength of 205 nm in a Specord 205, Analytik Jena. It was calculated assuming an absorptivity constant of 110 $dm^3g^{-1}cm^{-1}$ (Lin and Dence, 1992).

5.5.4 Carbohydrate analyses (HPAEC) (Papers I-III and Papers V-VI)

Analyses of the composition of monomeric sugar after hydrolysis with sulphuric acid were performed on two different high performance anion exchange chromatography (HPAEC) instruments. The experiments in Paper I were performed on set-up 1: a Varian Pro-Star with an electrochemical detector. The system consisted of CarboPac™ PA1 columns and the software was Star Chromatography Workstation, System Control Version 5.50 by Varian. The results of the carbohydrate contents in Papers II-III and Papers V-VI were analyzed on the second set-up: a Dionex ICS-5000 system, which was also equipped with CarboPac™ PA1 columns and an electrochemical detector. The software used was Chromeleon 7, Chromatography Data System, Version 7.1.0.898. Both systems used NaOH and NaOH+NaAc (0.2 M) as eluents.

The amounts of sugars analyzed were corrected for the acid hydrolysis yield (Janson, 1974); the values of the hydrolysis yield were taken from experimental results presented

by Wigell (2007). The amounts of cellulose, GGM and AGX were then calculated from the carbohydrate analysis using the assumptions and corrections described in Appendix B.

5.5.5 Methanolysis, silylation and GC-MS (Paper V)

The compounds in the freeze-dried samples of the STEX liquors in Paper V were investigated using gas chromatography (GC) coupled with mass spectroscopy (MS) after methanolysis and per-silylation. The method used was adapted from Sundberg et al. (1996). A reagent for the methanolysis was prepared by mixing 2.8 mL acetyl chloride (Ac-Cl) in 17.2 mL anhydrous methanol (on ice). Four milligrams of the freeze-dried samples were put in small, precision glass vials and 2 mL of the methanolysis reagent was added. The samples were put in a pre-heated oil bath at 100°C and were left to react for three hours. Afterwards, 100 µL of pyridine was added together with 4 mL of a sorbitol standard (conc. 0.1 mg/mL), which was used later for semi-quantification. One milliliter of this solution was removed and the solvent was evaporated under a flow of nitrogen gas. When the samples were dry, 100 µL of pyridine was added to each sample together with the silylation reagents: 150 µL hexamethyldisilazane (HMDS) and 80 µL trimethylchlorosilane (TMCS). The vials were put onto a shaking machine and left overnight. The samples of the standards were prepared by weighing 2 mg of the standards to 20 mL of anhydrous methanol and 1.5 mL of the solution was pipetted into small vials. The solvent was evaporated under a flow of nitrogen gas and pyridine before the silylation agents were added in the same amounts as for the samples.

After silylation, the solvents were evaporated from the vials and 2 mL of diethyl ether was added to the dry samples. These samples were filtered through 0.2 µm syringe filters (GHP, Acrodisc) and then injected into the GC-MS (Agilent technologies 7890A GC system, 5975C inert XL EI/CI MSD with Triple-Axis Detector and 7693 Autosampler). The spectra were analyzed with the software Enhanced ChemStation (MSD ChemStation E.02.02.1431 by Agilent) equipped with the NIST Mass Spectral Search Program (V. 2.0).

5.5.6 Acetate content (Paper V)

The content of acetyl groups in Paper V was analyzed with an 850 Professional IC from Metrohm equipped with a Metrosup A Supp7 column. The eluent consisted of 3.6 mM Na_2CO_3-solution. The calibration curve was obtained from five different standards prepared from "Acetate std for IC", 1000 mg/L, from Sigma-Aldrich (FLUKA) and, diluted to the following concentrations: 10, 50, 100, 150 and 200 mg/L.

5.5.7 Determination of molecular weight (Papers I and V)

Distributions of molecular weights were determined in a PL-GPC 50 Plus Integrated GPC System from Polymer Laboratories (A Varian Inc. Company) using both RI and UV detection. The system was equipped with PolarGel-M columns and run with DMSO, with the addition of 10 mM LiBr as eluent. The results were evaluated using the software Cirrus GPC Version 3.2. Pullulan of ten different molecular weights (708, 375, 200, 107,

47.1, 21.1, 11.1, 5.9, 0.667 and 0.180 kDa) was used for calibration. An example of a calibration curve with molecular weights and corresponding retention times can be found in Appendix C.

5.5.8 Analysis of the reducing sugars released (Paper II)

The amount of reducing sugars produced was measured in order to investigate the extent of the enzymatic reactions after mild STEX. The method used was the dinitrosalicylic acid (DNS) method described by Miller (1959), where the colour reaction of the DNS reagent to the reducing sugar is used to determine its concentration. Absorbance values from a UV-2550 UV/VIS spectrometer (Shimadzu, Japan) at 575 nm were correlated with a glucose standard calibration curve. After the enzymatic reaction, samples of 1 mL were mixed with 1 mL of DNS reagent solution and centrifuged for three minutes at 14,500 rpm. Then 1 mL supernatant was transferred to a new Eppendorf tube and boiled for five min together with a glucose standard of known concentration, and a reference sample that contained only water and DNS reagent. The samples were cooled, the absorbance values were measured and the contents of the reducing sugars were calculated. The glucose calibration curves are shown in Appendix D.

5.5.9 Near-infrared (NIR) spectroscopy (Paper I)

The NIR experiments were performed on Foss NIR Systems 6500 with a Rapid-Content™ Analyzer, using silica and PbS-detectors. The software was Vision 2.51 and the wavelengths of the electromagnetic spectrum applied were between 400 to 2500 nm, i.e. both in the visible and near-infrared range. Since the instrument switches detector in the shift between these regions, i.e. wavelengths between 1090 and 1110 nm, these signals were removed. There were 32 scans per sample and the results were analyzed using multivariate analysis: PCA (principal component analysis) and PLS (partial least squares) models (Naes et al., 2002). The software employed was Simca-P+ Version 12.0.1.0 by Umetrics. The second derivative was used as a spectral filter with a quadratic polynomial order, with 13 points in each sub-model (Savitzky and Golay, 1964).

5.5.10 Nuclear magnetic resonance (NMR) (Paper V)

HSQC-NMR measurements were performed on a Varian 400-MR 400 MHz NMR spectrometer at RT. Samples were prepared by dissolving 100 mg of freeze-dried STEX liquors in 0.75 mL DMSO-d_6. The acquisition parameters of the HSQC were: 10 ppm spectra width in the F2 (^{1}H) dimension with 962 data points (150.0 ms acquisition time), 180 ppm spectra width in the F1 (^{13}C) dimension with 96 data points (4.7 ms acquisition time), 1 s pulse delay, $^1J_{C\text{-}H}$ of 145 Hz, and 16 scans. The solvent peak at δ_C 39.5 ppm and δ_H 2.5 ppm was used to calibrate the chemical shift. The software MestReNova 9.0.0.0 suit was used to process the NMR data.

5.5.11 Properties of pulp (Papers II, III and VI)

The properties of the pulp samples used in Papers II, III and VI were measured using standard methods. The kappa numbers were analyzed according to SCAN-C 1:77 and the

intrinsic viscosity was analyzed according to SCAN-C 15:62. In Papers II and III, laboratory sheets for measuring ISO brightness were made from each pulp and were analyzed according to SCAN-C 11:75. A Kajaani FS300 was used to measure fibre lengths and fines, according to the Tappi standard. Residual alkali was measured using titration according to Wilson (1968). In Paper VI, brightness values were instead determined according to ISO 2470, where absorbance values at a wavelength of 457 nm were determined. The strengths of the pulps in Paper VI were also investigated. Paper sheets were prepared by disintegrating pulp samples and then allowing the pulp cake to be beaten according to ISO 5264-2:2002. Finally, sheets were formed according to ISO 5269-2:2004. The paper sheets were conditioned prior to measurements of tensile index and stiffness, which were measured with a Frank Tensile Tester according to ISO 1924-2:2008. The thickness and structural density of the sheets were determined according to SCAN-P 88:01.

6. Results and Discussion

The main findings of the different studies are presented in this section. All the results are presented as a coherent report, although the different papers are indicated in parenthesis. There is also some discussion in order to interpret the results and put them into context vis-à-vis other published works.

6.1 Pre-treatments

6.1.1 Reference study (Paper I)

The initial studies, where water-impregnated and $NaBH_4$ pre-treated wood powder samples were treated with aqueous solutions with different levels of pH, showed how the chemical composition varied according to treatment time and temperature. The results of the analyses of chemical composition are tabulated in Appendix E. A reference sample of untreated spruce wood powder was analyzed four times, from which the standard deviations of the analyses (set-up 1) were as being 0.88% for Klason lignin, 0.51% for cellulose, 0.25% for xylan and 0.22% for GGM. The overall degradation of the wood components was generally higher for the alkaline treatment (**Fig. 6.1**) than for the corresponding conditions during the acidic treatment (**Fig. 6.2**); the total loss of mass for the most severe conditions (130°C, 2 h) was 17.3% for the acidic treatment and 25.6% for the water-impregnated sample treated with alkali. For both the alkaline and the acidic conditions, considerable chemical differences were found mainly in the amounts of GGM.

During the alkaline treatment, the content of GGM was clearly retained for the samples pre-treated with $NaBH_4$, which is shown in **Fig. 6.1**. For example, for samples treated at 110°C for 60 min, the remaining amount of GGM was 9.4% for the water-impregnated sample whereas it was 17.9% for the sample pre-treated with $NaBH_4$.

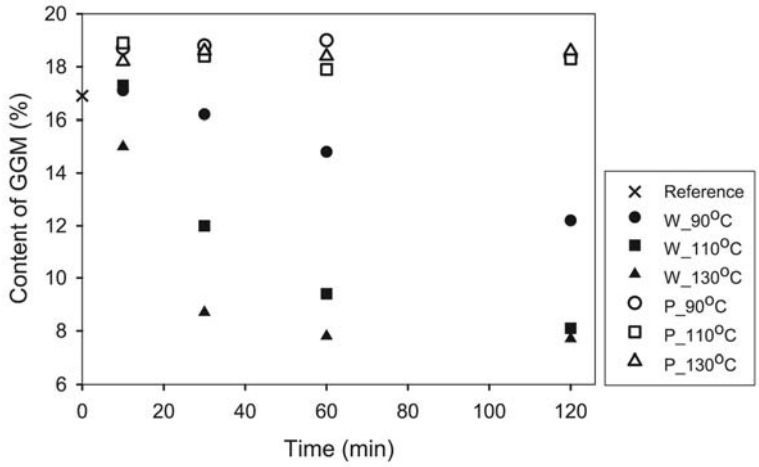

Figure 6.1 Content of GGM for the water-impregnated (W) and $NaBH_4$ pre-treated (P) samples after alkaline treatment at various temperatures and treatment times. The values are presented as (w/w)% of the sample weights.

Losses of polysaccharides and decreases in chain lengths are known to occur at alkaline conditions; the most important degradation reactions in alkaline pulping are peeling and hydrolytic reactions (Fengel and Wegener, 1989). At low temperatures, a swollen state is created and peeling reactions start at temperatures below 100°C. Alkaline hydrolysis of glycosidic bonds, which causes chain splitting, becomes more dominant at higher temperatures (above 150°C). Treatment with a reducing agent, e.g. $NaBH_4$, has been suggested as a method of increasing pulp yields (Meller, 1953, Richtzenhain et al., 1954, Hartler, 1959). The reducing end-group of the polysaccharide is converted into an alcohol during such a treatment, and an alditol is formed, which is more resistant to peeling reactions. This re-arrangement of the end-group is most likely the reason for the stabilized GGM in **Fig. 6.1**: these results formed the basis for the continuing use of a pre-treatment with $NaBH_4$ in following experiments. Treatment with $NaBH_4$ also resulted in increased brightness of the wood, which could be explained by a reduction of the chromophoric groups in the lignin, e.g. coniferyl aldehydes (Pew and Connors, 1971). An increase in brightness when $NaBH_4$ is used has also been observed during pulp bleaching, being explained by reactions on carbonyl groups in oxidized hemicelluloses (Giertz and McPherson, 1956). The contents of the other chemical components, such as Klason lignin and cellulose, were relatively constant after the alkaline experiments at these conditions (see Appendix E).

For the treatment at acidic pH, both the xylan and the GGM were degraded to a great extent, with the decrease being more pronounced with increasing temperature and time.

The results of the decrease in content of GGM are shown in **Fig. 6.2**. The degradation was more severe for the samples treated at 130°C: the content of GGM was almost half of the reference sample after the 2 hour treatment. The degradation was even more severe examining the values of arabinose alone: after acidic treatment at harsh conditions the concentration of arabinose was virtually zero. The loss of arabinose side-chains also explains part of the reduction in the content of arabinoglucuronoxylan. The acidic treatment did not result in any large differences for the content of either cellulose or Klason lignin (see Appendix E).

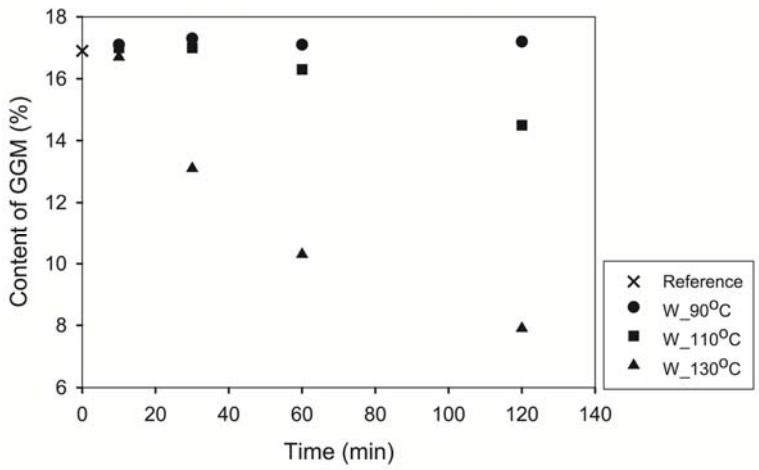

Figure 6.2 The content of GGM after acidic treatment for the different temperatures and residence times (W = water-impregnated samples). The values are presented as (w/w)% of the sample weights.

The most common reaction on polysaccharides at acidic pH is acid hydrolysis; the hydrolytic behavior of glycosidic bonds is mainly influenced by two factors, namely the conformation of the sugar units and the so-called "inductive effect" caused by some substituents (Fengel and Wegener, 1989). Generally, hydrolysis is supported if the axial substituents change to an equatorial position. The hydroxyl at C2 in β-D-mannose is in an equatorial position (while it is in axial position for β-D-glucose), so mannose is hydrolyzed more quickly than glucose, which explains some of the degradation of GGM in **Fig. 6.2**. There is also a difference in the rate of hydrolysis of furanoses and pyranoses, where the former are hydrolyzed much faster than the latter due to a higher structural angle strain in the conformation of furanosidic sugar units. This makes it reasonable to conclude that the arabinose side-chains are easily hydrolyzed from the xylan backbone.

The wood powder samples from the reference study were also investigated with NIR spectroscopy; the spectra of the different samples can be found in Appendix F. Principal component analysis (PCA) and partial least squares (PLS) models were both applied: in the latter, NIR spectra were compared with data of the chemical composition.

The PCA models showed that the treatment applied to the wood samples (alkaline or acidic liquor or $NaBH_4$ pre-treatment followed by alkaline treatment) resulted in spectra

that could be divided into three distinct groups, i.e. the various treatments led to different chemical reactions that could be distinguished in the plot (see **Fig. 6.3A**). However, the various temperatures and residence times of the treatments did not result in additional grouping of the spectra. This is most likely due to the fact that although the samples contained the same type of chemical components after a specific treatment, the concentrations differed.

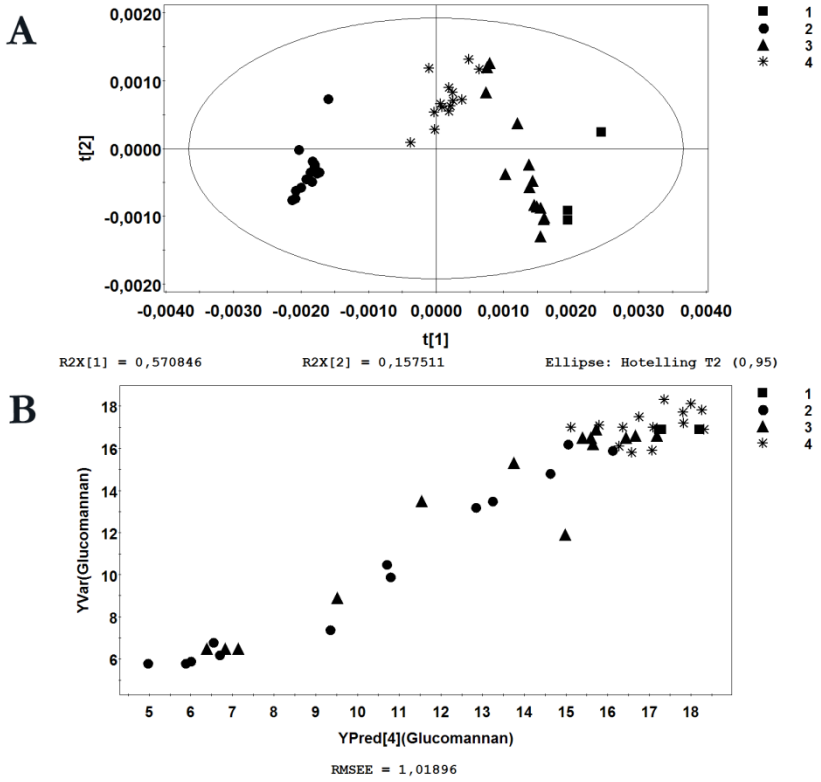

Figure 6.3 A) The reference (1.) and the three groups in the PCA model (2. Alkaline treatment, 3. Acidic treatment, and 4. $NaBH_4$ pre-treated samples). **B)** Observed vs. predicted values for the content of GGM in a PLS model based on four significant components: 1. Reference, 2. Alkaline treatment, 3. Acidic treatment, and 4. $NaBH_4$ pre-treated samples.

The PLS model worked well for GGM, with a good agreement between observed and predicted values, see **Fig. 6.3B**. This is most likely due to the samples having a large variation in the concentration of GGM, which was not the case for the other components of the wood.

NIR spectroscopy is a rapid method that requires minimal sample preparation and can, together with other optical methods, be used to obtain much information of wood in a non-destructive way (Bargigia et al., 2013). Databases of NIR spectroscopy data can form the basis of reliable PLS models for larger sets of samples of varying composition. These may then be used to analyze new samples and predict the value of some property of the sample by measuring only one NIR spectrum.

6.1.2 Sodium borohydride pre-treatment (Paper V)

An additional study was conducted in Paper V to further investigate the effects of the $NaBH_4$ pre-treatment on wood samples. A few different experimental conditions were tested (see Table 5.1) and wood samples were collected exclusively after pre-treatment, as well as after both pre-treatment and subsequent mild STEX (160°C, 10 min) in the lab-scale equipment. The chemical compositions after acid hydrolysis were then measured for all of the samples; the results of the content of lignin and monomeric sugars are presented in **Fig. 6.4**. The content of acetate is showed in **Fig. 6.5**.

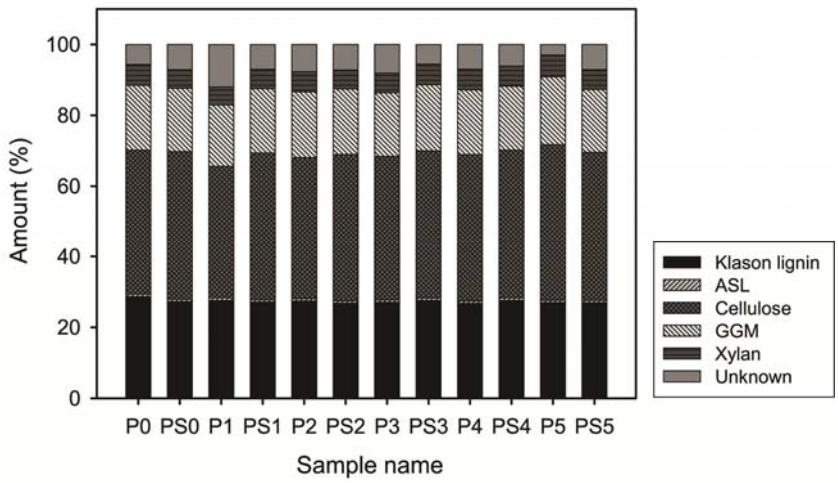

Figure 6.4 Chemical composition in the samples after $NaBH_4$ pre-treatment and mild steam explosion. P = pre-treatment, S = steam explosion, **0** = raw material, **1** = 2% $NaBH_4$, 24 h, **2** = 7% $NaBH_4$, 24 h, **3** = 2% $NaBH_4$, 1 week, **4** = 7% $NaBH_4$, 1 week, **5** = 0.5% $NaBH_4$, 3 days.

Only small differences were found between the chemical compositions of the samples after the pre-treatment and mild steam explosions: this can be explained by the fact that the conditions of both the pre-treatment (0.5-7% $NaBH_4$, RT) and the STEX were mild (160°C), and would therefore not lead to extensive degradation of any of the components. Since the experiments were performed in the lab-scale equipment, where almost no condensed steam was present, it could also be that some of the components were degraded but remained within the wood structure.

The amount of acetyl groups decreased after $NaBH_4$ pre-treatment, especially at a higher concentration (7% $NaBH_4$) and after a long treatment time (1 week). The mild STEX also decreased the acetate concentration in the wood, especially for the samples pre-treated at the high concentration of $NaBH_4$ or for long time. One probable explanation for this could be that cleaved acetyl groups from the pre-treatment were transported out of the wood during the mild STEX; another explanation is that base-catalyzed deacetylation reactions also occurred during the STEX due to the increase in pH of the $NaBH_4$ solution.

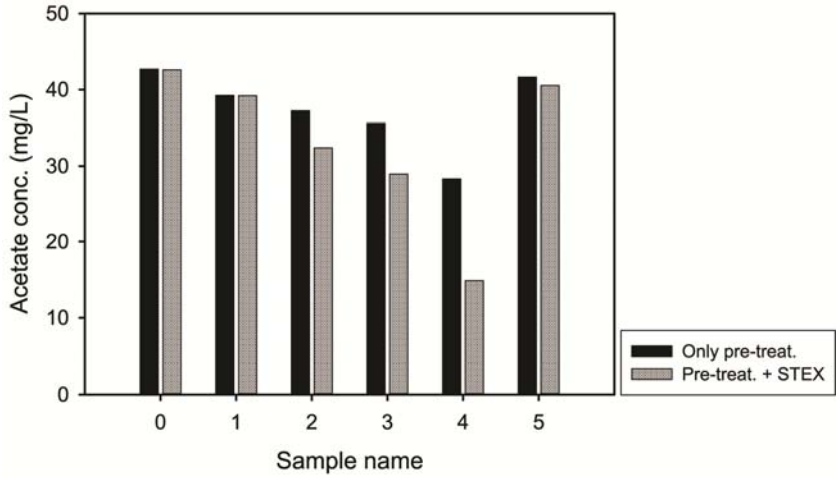

Figure 6.5 Acetate concentrations in the samples from the $NaBH_4$ pre-treatment study, both after pre-treatment and mild steam explosion, **0** = raw material, **1** = 2% $NaBH_4$, 24 h, **2** = 7% $NaBH_4$, 24 h, **3** = 2% $NaBH_4$, 1 week, **4** = 7% $NaBH_4$, 1 week, **5** = 0.5% $NaBH_4$, 3 days.

6.2 Mild steam explosion

The acetyl groups in the wood are cleaved during mild steam explosion and acetic acid is formed which, in turn, leads to further degradation of the components of the wood. The fast release in pressure leads to expansion of the steam: this, in combination with the discharge of the softened wood chips, leads to the physical rupturing of the wood structure. Thus, it should be kept in mind that STEX treatment affects wood both mechanically and chemically. As shown in **Fig. 6.6**, a treatment applied at 7 bar after ten minutes in the bench-scale equipment led to noticeable breakages and cracks in the wood chips, as well as to a change in colour, which moved towards a darker brown. This could be derived from thermally-induced changes in the chemical structure of the lignin, e.g. condensation reactions (Zhang and Cai, 2006). Treatment at 4 bar did not result in any visible changes when compared to the original untreated wood.

Figure 6.6 Steam-exploded wood chips from the bench-scale equipment. Left: chips exploded at four bar. Right: chips exploded at seven bar.

6.2.1 Morphological changes (Paper IV)

Paper IV isolated and investigated the effects of the different steps of the steam explosion process, namely (i) heating with steam (ii) pressure release (explosion) and (iii) impact with equipment and other material. The samples were characterized using mercury porosimetry

and electron microscopy (ESEM); the experimental details can be found in **Table 5.2**. As mentioned above, the result of an increase in the steam temperature (165°C/195°C) was pieces of wood that were darker in colour. However, there were no clear effects of physical rupture: each wood piece remained intact after the explosion when there was no impact, see **Fig. 6.7A**. This indicates that the conditions during the STEX in these experiments were not harsh enough to cause the wood to disintegrate into small pieces due to the release in pressure.

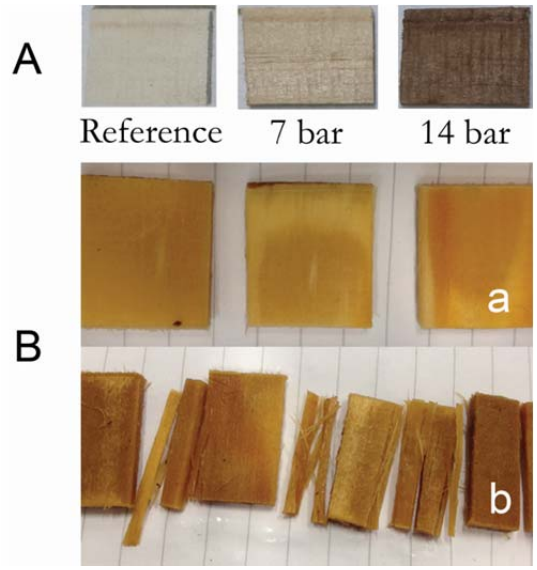

Figure 6.7 A) Change in appearance of dry wood samples caused by steam treatment in the small-scale equipment. From left: reference, STEX 7 bar (165°C), 10 min, STEX 14 bar (195°C), 10 min. **B)** Effect of impact: a) wood pieces after STEX at 7 bar (165°C) without impact and b) after STEX at 7 bar with impact.

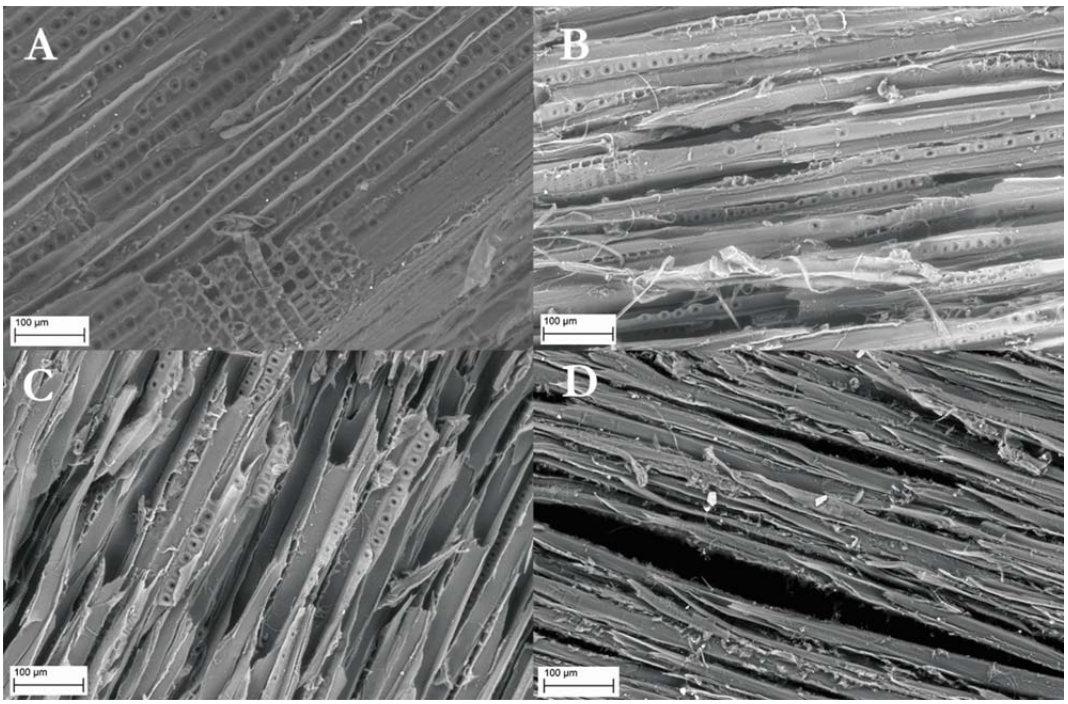

Figure 6.8 ESEM images of **A)** untreated wood, 500x magn., **B)** steam-exploded wood at 14 bar, 10 min (no impact), 500x magn., **C)** steam-exploded wood at 7 bar, 10 min (no impact), 500x magn. **D)** steam-exploded wood at 7 bar, 10 min with impact, 500x magn. The scale bars represent 100 µm.

The effect of impact, i.e. collisions with the equipment and other wood material during the discharge stage, was evident through simple visual inspection, as shown in **Fig. 6.7B**. The samples that were subjected to both STEX (165°C) and impact showed distinct cracks and were even broken into smaller pieces. This is in agreement with the results obtained by Law and Valde (1990), who investigated the effect of impact on both softwood and hardwood at 170-200°C for 3-13 minutes. They found that STEX itself cannot cause defibrillation, and that disintegration is instead due to the impact of highly softened chips in a blow tank.

The difference between the samples that had been subjected to impact and those that weren't was also observed in the ESEM-images, see **Fig. 6.8**. The samples that were subjected to impact show clear cracks, particularly between the wood fibres. Although the sizes of the cracks differed, they were in the range of 1-70 μm. It was more difficult to observe differences between the wood pieces that were steam-exploded without impact and the untreated wood material when these were compared. There were some differences between different samples, and some cracks around pits were observed in the steam-exploded samples. It was nevertheless difficult to distinguish whether the features observed derived from the steam treatment or the preparation of the sample, since the latter (cutting or sawing) was performed prior to the ESEM characterization and affected the surfaces of the samples.

The results of the mercury porosimetry measurements showed that, although the apparent structure of the wood pieces had not changed significantly after the STEX treatment (**Fig. 6.7**), there were differences in the internal structure. The differences in intrusion volume of mercury for different pore sizes are summarized in **Table 6.1**, where each value is an average of four individual measurements.

Table 6.1 Average pore diameter and total intrusion volume of the different STEX experiments [a]

Experiment	Average pore diameter (nm)	Total intrusion volume (mL/g)	Impact
Temp. & residence time			
Reference	595	1.44	No
STEX 7_10	680	1.44	No
STEX 14_10	883	1.57	No
Reference	638	1.46	No
STEX 7_5	970	1.41	No
STEX 14_5	789	1.40	No
Heating vs. explosion			
Reference	553	1.50	No
Heat 14_10	711	1.51	No
Expl. 14_10	933	1.61	No
With & without impact			
Reference	637	1.46	No
No imp. 7_10	945	1.43	No
Impact 7_10	1130	1.52	Yes

a. Experimental conditions are explained in **Table 5.2**.

Fig. 6.9 shows the incremental intrusion volumes for different pore diameters. The STEX experiment with a treatment time of five minutes (**Fig. 6.9A**) did not show much difference in structure when compared to the untreated wood. Although there were some structural changes at 14 bar pressure, they did not increase the total intrusion volume or the average pore diameter. Therefore, a treatment time of five minutes is probably not long enough to heat the whole piece of wood completely, and soften it sufficiently, to lead to deformation of the cell wall.

The two major peaks in **Fig. 6.9B** are in the range of 1-2 and 3-4 μm, which corresponds to the size of cross-field and bordered pits in the cell wall of wood. It indicates that most of the penetration into the cells occurred through pits. In the case of the steam-exploded samples, the increase in intrusion volume could mean that some of the pits in the untreated wood were first aspirated (closed) and then either opened or ruptured after the STEX treatment, thus permitting penetration. This is in agreement with the study by Zhang and Cai (2006), where it was shown that a steam explosion process led to cracks in cell walls and ruptures in pits, thereby increasing the permeability of the wood. There was also some penetration in the very small diameter range (0.2-1 μm) which, after treatment, had relocated to a larger diameter. An increase in pore size increases the accessibility of chemical reagents and enzymes, consequently improving the effectiveness of a STEX pre-treatment, as discussed by Grous et al. (1986).

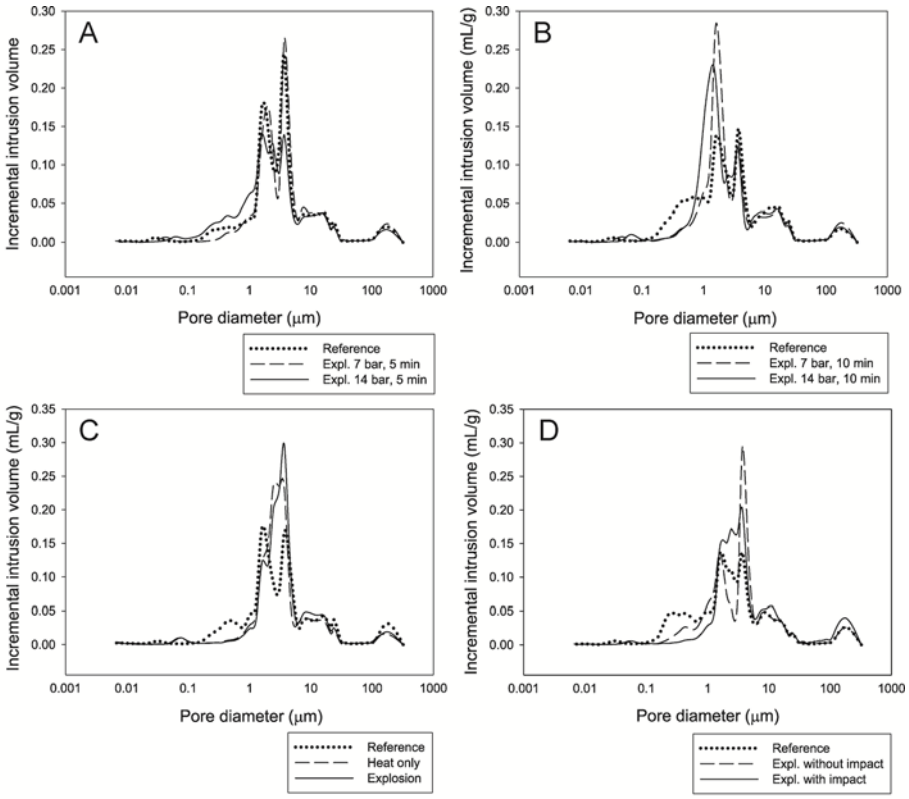

Figure 6.9 Incremental intrusion volume for samples **A:** steam-exploded at 7 or 14 bar, after 5 min, **B:** steam-exploded at 7 or 14 bar, after 10 min. **C:** heated with steam at 14 bar for 10 min, compared to the sample steam-exploded at 14 bar after 10 min. **D:** steam-exploded at 7 bar after 10 min restricted from impact compared to the sample steam-exploded at 7 bar after 10 min and with impact.

The effect of a rapid pressure release, i.e. the actual explosion, was compared with a slow release, see **Fig. 6.9C**. Brownell and Saddler (1987), who studied steam pre-treatments of lignocellulosic material for enhanced enzymatic hydrolysis, discuss the fact that the explosion step of the STEX process is not very effective. In the present study, the average pore diameter increased from 553 nm in untreated wood to 711 nm in steam-heated (unexploded wood) and 933 nm in exploded wood for treatment at 14 bar (195°C) for ten minutes. The structural changes in the unexploded wood might be due to the degradation and removal of hemicelluloses, extractives and lignin fragments during the steam treatment (Donaldson et al., 1988). For the exploded wood, the quick release of pressure could have ruptured the internal structure of the wood material and resulted in additional intrusion into the exploded wood material. Another interesting observation from both cases is that pores of 25-45 nm in size expanded to 65-95 nm after heating and explosion. This may also be due to a re-arrangement of the chemical constituents in the wood during the actual steam treatment.

The total intrusion volume was higher for the samples that were subjected to impact, while it was more or less similar for the reference and the samples subjected to explosion only, as can be seen in **Table 6.1**. However, the average pore diameter in the exploded samples was larger than in the reference, and was larger still in the samples that were subjected to both explosion and impact. The two peaks in the region between 1-5 μm of pore diameter in **Fig. 6.9D** seem to combine into one, broader, peak for the samples exploded with impact. This may be due to breakages or cracks inside and/or between different cell walls in the wood.

6.2.2 Chemical effects of the steam-exploded wood (Papers I-III + Papers V-VI)

The chemical compositions of wood before and after STEX treatments were measured in most of the studies. Some samples were also pre-treated chemically prior to the mild STEX.

Measurements of wood powder samples

In Paper I, the samples of wood powder were subjected to mild STEX in the lab-scale equipment. Steam explosion treatment at 160°C left the major wood components, e.g. cellulose and lignin, almost unaffected, which can be seen in **Fig. 6.10**. The GGM was the component that was most degraded, with a decrease from 17.4% to 11.0% for the longest residence times. The amount of cellulose and lignin increased somewhat as the hemicelluloses decreased; the content of xylan decreased from 5.8% to 4.2%.

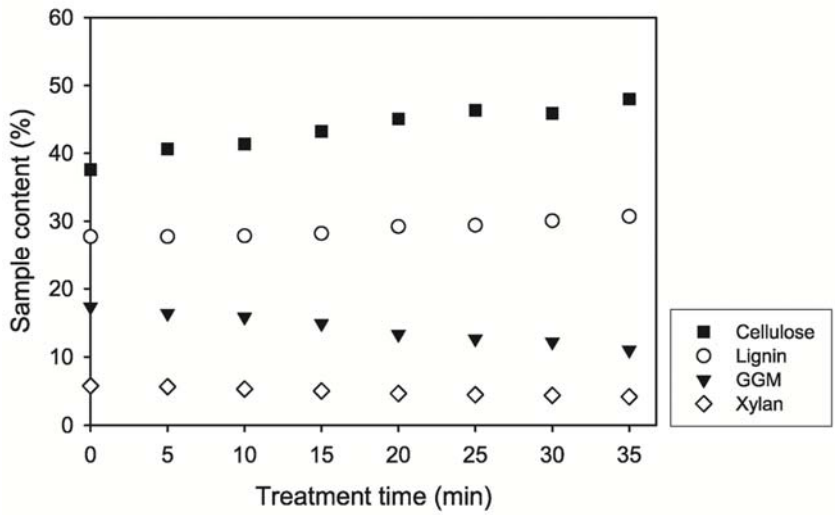

Figure 6.10 Content of the major components of wood ((w/w)% in the wood samples) after mild steam explosion at 160°C for different residence times.

The degradation/dissolution of hemicelluloses in the samples of wood powder during the mild STEX is illustrated in **Fig. 6.11**, where the results are presented as anhydro sugars and in percentage of sample weight. The degradation was most severe for mannose and arabinose. The decrease in arabinose content was most likely caused by the fact that the arabionse side-chains of the AGX are easily cleaved off in an acidic environment. The backbone of xylan, however, seemed to be quite resistant to steam explosion at these conditions.

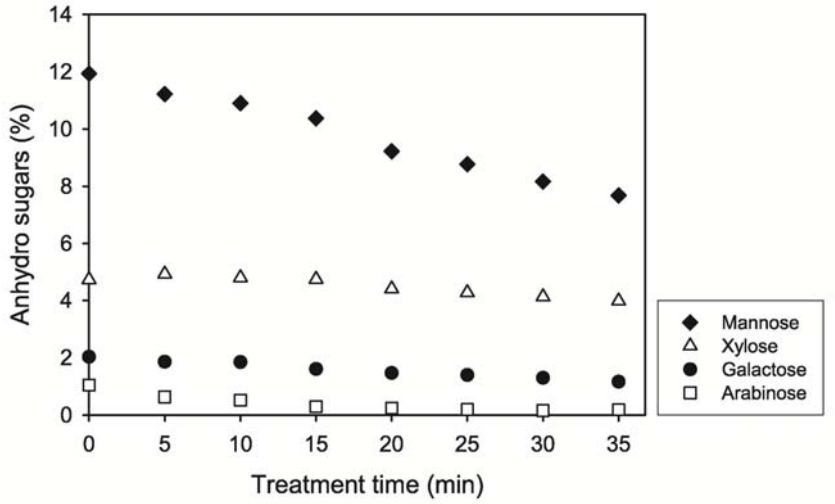

Figure 6.11 Degradation of hemicelluloses during mild steam explosion (160 °C) of wood powder at different residence times. The amounts of anhydro sugars in the samples after mild steam explosion are shown (in w/w% on sample) for the different duration times.

It was clear that the GGM was more degraded as the treatment time increased, and the degree of deacetylation was most likely quite high. Although the degradation of hemicelluloses began almost immediately during the mild STEX treatment, it is not, however, in the same range as a chemical treatment at conditions corresponding to a chemical pulping process would produce (Wigell et al., 2007).

Pre-treatment with $NaBH_4$ resulted in the presence of higher amounts of GGM in the samples of wood powder after mild STEX treatment. **Fig. 6.12** shows a comparison between pre-treated and water-impregnated samples after STEX. The findings indicated that using a reducing agent to stabilize the end-groups of the GGM was also effective during the mild steam explosion. The level of GGM retained remained almost constant, even for longer residence times, which was the reason for choosing a long residence time during the mild STEX prior to the subsequent extraction experiments in Paper I.

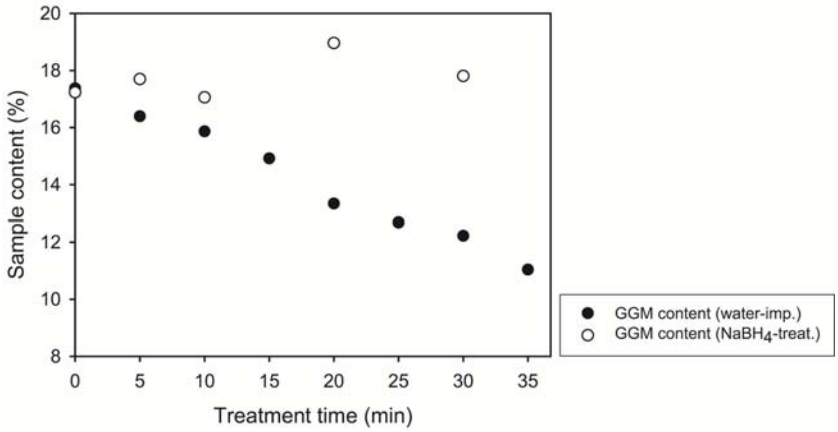

Figure 6.12 Content of GGM (% of sample) after mild steam explosion (160°C) after different residence times. Water-impregnated samples are compared with $NaBH_4$ pre-treated samples.

The stabilizing effect of GGM due to $NaBH_4$ pre-treatment might be a result of several causes. The change in the reducing end-group of the GGM could potentially make it more resistant towards degradation; it could also be an effect of pH stabilization, since the pH level is around 9-10 during the pre-treatment. Some deacetylation, due to the slightly alkaline conditions during the $NaBH_4$ pre-treatment, may also produce a more linear GGM which, in turn, will have a higher affinity to the cellulose and therefore is more likely to be retained in the wood (Annergren et al., 1961).

Measurements of wood chips samples

Analysis of the chemical composition after mild STEX treatment in the lab-scale equipment was also conducted in Paper II, this time on samples of wood chips. The analyses of carbohydrates were performed on the second set-up, with the standard deviations (based on three independent measurements of untreated wood) of the different components on this instrument being 0.05% for Klason lignin, 0.11% for cellulose, 0.05% for xylan and 0.14% for GGM. The results after treatments at different temperatures are presented in **Table 6.2**. There was virtually no mass loss during the treatments (lowest value was 99.1% of original mass), indicating that very little of the material in the wood chips was degraded/dissolved at these conditions. The values for the hemicelluloses (GGM and AGX) were also relatively constant, so the composition of the steam-exploded material was almost the same as for untreated wood. However, there is not much condensed steam present in the lab-scale equipment so it is therefore possible that

the hemicelluloses are degraded to some extent, but are retained within the structure of the wood.

Table 6.2 Results of the carbohydrate analysis of steam-exploded wood chips compared to non-exploded chips. The results are presented as percentages of sample content and the non-exploded values are averages of three samples.

Sample STEX temp.	Klason lignin (%)	Cellulose (%)	GGM (%)	AGX (%)	ASL (%)	Other (%)
Non-exploded	27.4	40.8	16.6	5.6	0.8	8.8
115°C (1.7 bar)	27.9	39.1	16.9	5.9	0.8	9.4
130°C (2.7 bar)	28.4	40.7	18.0	5.7	0.8	6.4
145°C (4.2 bar)	27.0	40.8	17.0	5.1	0.8	9.3
160°C (6.2 bar)	27.4	41.2	17.6	5.0	0.7	8.1

The small decrease in xylan content is, again, most likely due to the cleavage of arabinose side-chains. Otherwise, the effect of autohydrolysis is small for these STEX conditions in this equipment.

Similar results were also obtained for the bench-scale equipment at corresponding conditions in Papers III and V. The steam explosion conditions were set to 4 bar (i.e. 144°C) and 7 bar (165°C), with the residence time being ten minutes in both cases. The results are shown in **Table 6.3** and are presented as the percentages of the different components in the samples.

Table 6.3 Chemical composition (w/w% of the samples) of untreated and steam-exploded wood chips in Papers III and V. The values for samples treated at 4 bar (144°C) or 7 bar (165°C) for 10 min in Paper V are averages of both wood chip batches.

Sample description	Klason lignin (%)	Cellulose (%)	GGM (%)	Xylan (%)	ASL (%)	Other (%)
Paper III						
Non-exploded	28.5	40.3	16.9	5.4	0.7	8.2
STEX, 4 bar, 10 min	27.0	44.5	16.8	5.1	0.8	5.8
STEX, 7 bar, 10 min	28.9	43.2	16.2	5.3	0.8	5.6
Paper V						
Original wood material	26.6	36.8	18.2	5.9	0.7	11.9
STEX 4 bar, 10 min (avg.)	28.5	37.7	16.2	5.3	0.7	11.6
STEX 7 bar, 10 min, (avg.)	27.7	41.3	15.6	5.5	0.7	9.2
STEX 7 bar, 10 min, never-dried	29.1	38.8	16.7	5.4	0.6	9.5
STEX 7 bar, 10 min, $NaBH_4$ pre-treated	27.3	41.0	16.0	5.8	0.7	9.3

The amount of GGM decreased somewhat during treatment in the bench-scale equipment, which is most likely due to autohydrolysis. The pH of the condensed steam after the mild steam explosion treatment was measured and found to be 4.4 for mild STEX at 4 bar and 3.7 for 7 bar. The degradation was moderate: most of the wood components remained in the wood material. It could be expected that some water-soluble hemicelluloses and extractives in the wood were removed during the treatment, while the

cellulose and lignin were preserved within the wood structure. In Paper V, the never-dried wood chips and those pre-treated with $NaBH_4$ contained slightly higher amounts of GGM. The wood chips pre-treated with $NaBH_4$ also showed a variation in colour after the treatment, but this did not influence the results of the chemical composition. The change in colour could possibly be derived from structural changes of the lignin, which was not possible to observe in this analysis.

The concentrations of acetate in the hydrolysates from the wood samples in Paper V were measured and are reported in **Table 6.4**.

Table 6.4 The amount of acetate in the hydrolysates from the wood samples in Paper V.

Sample description	Acetate (mg/L)
Original wood material	46.99
STEX 4 bar, 10 min batch 1	46.17
STEX 4 bar, 10 min, batch 2	38.13
STEX 7 bar, 10 min, batch 1	38.01
STEX 7 bar, 10 min, batch 2	39.27
STEX 7 bar, 10 min, batch 2, never-dried	37.94
STEX 7 bar, 10 min, $NaBH_4$ pre-treated	15.56

The water-impregnated samples all showed values in the same range. The steam-exploded samples contained somewhat less acetate than the raw material: a higher steam explosion pressure resulted in a greater loss of acetyl groups. The samples after the $NaBH_4$ pre-treatment had considerably lower contents of acetate showing, in this case, that much acetyl had been removed during both the pre-treatment and the mild STEX.

6.2.3 Analyses of steam explosion liquor (Paper V)

The liquors (condensed steam) from mild STEX were analyzed in some detail in Paper V. The wood chips (two different batches) were steam-exploded at 4 (144°C) or 7 (165°C) bar after a treatment time of ten minutes. The characterization included analysis of the chemical composition (GC-MS, lignin-, acetate- and carbohydrate measurements and 2D-NMR) as well as determination of the molecular weight. The pH levels of the liquors are presented in **Table 6.5**.

Table 6.5 The pH level of the different liquors

STEX liquor	pH
Water imp. + STEX 4 bar	4.4-5.8
Water imp. + STEX 7 bar	3.7-4.2
$NaBH_4$ pre-treat. + STEX 7 bar	8.9

The pH values of the liquors after STEX at 4 bar were similar to those of the de-ionized water that was used for the water impregnation. The degree of deacetylation and autohydrolysis reactions was thus most likely only moderate at these conditions, although there was some variation between different runs. Steam explosion of water-impregnated wood chips at 7 bar resulted in a decrease of the pH level, while pre-treatment with $NaBH_4$ resulted in a slightly alkaline pH: one reason for this is that an aqueous solution of

$NaBH_4$ has a higher original pH. Consequently, reactions due to acidic autohydrolysis during the mild STEX can be expected to be low in this case. However, base-catalyzed reactions may occur instead. This liquor also had a darker colour, which could possibly be explained by condensation reactions of aromatic structures in the wood. Alternatively, the colour could have originated from other components of the wood, e.g. lignans and extractives.

GC-MS results

The water was evaporated from the liquors and the samples were freeze-dried prior to GC-MS analysis. The relative amounts of the carbohydrates compounds identified are summarized in **Table 6.6**.

Table 6.6 Sugar compounds identified in the freeze-dried liquors. The values are given as relative percentages (%) of the sample content and are averages of two measurements. The components marked in bold font were identified with samples from known sugars, which were used as standards. The other values were identified from the probability values from the mass spectral data base (NIST).

Component	Retention time (min)	STEX 4, batch 1	STEX 7, batch 1	STEX 4, batch 2	STEX 7, batch 2	STEX 7, batch 2, never-dried	STEX 7, batch 2, $NaBH_4$
Arabinose	34.1-34.6	26.30	12.26	25.20	14.21	14.12	3.76
Xylose	37.1	1.71	5.37	1.50	5.87	5.61	7.29
Xylose (β)	**37.6**	**0.84**	**2.75**	**0.83**	**3.08**	**2.90**	**4.62**
Galacturonic acids	40.0 + 41.0	6.05	3.79	7.44	4.12	5.02	22.33
Mannose (α)	**40.7**	**37.45**	**52.67**	**36.41**	**49.22**	**48.59**	**11.91**
Mannose	41.4	2.73	3.85	2.54	3.56	3.70	---
Galactose	41.8	5.10	3.85	6.58	4.00	4.11	19.26
Galactose (β)	**42.6**	**3.14**	**1.38**	**3.63**	**2.44**	**1.96**	**7.92**
Glucose (α)	**43.2**	**9.70**	**8.83**	**8.42**	**8.12**	**8.24**	**5.99**
Glucose (β)	**43.7**	**3.50**	**3.16**	**3.06**	**2.85**	**3.02**	**---**

The largest peak corresponded to methyl-α-D-mannopyranoside in all of the samples. It can be seen that almost 50% of the amount detected in the samples consisted of mannose, so the most abundant component in the liquor samples is GGM. Generally, the findings showed that the main constituents of the samples were compounds originating from hemicelluloses and possibly also pectins. Another large fraction of the content most likely originated from arabinose, which constituted 12-15% of the samples treated at 7 bar and approx. 25% of the samples treated at 4 bar. These probably originated from the arabinose side-chains from the AGX or potentially also from pectins. Overall, the same types of compounds were seen for all of the water-impregnated samples; it is likely that the same components of wood were dissolved during the mild STEX treatment, with a harsher treatment resulting in a higher release of GGM.

The sample pre-treated with $NaBH_4$ diverged from the other sample, since only a minor portion of the mass of the sample could be explained and identified in the analyses. This is again an indication that the $NaBH_4$ pre-treatment stabilized the hemicelluloses and retained them in the wood. Spectra from the GC-MS also showed peaks not seen in the

other samples: the NIST database recognized many of them as being various organic compounds, such as steroids and organic acids. However, the peaks were too small to make exact identifications. A larger proportion of compounds originating from extractives, lignans or lignin fragments may also be present in this sample, which could potentially also contain a higher amount of salts.

The samples also showed distinct peaks that were identified as fatty acids with approx. 17-23 carbons, which could be derived from wood extractives. The different components identified by the NIST database and their retention times are shown in **Table 6.7**.

Table 6.7 Organic fatty acids identified by the NIST database in the GC-MS spectra and their retention times.

Component	Retention time (min)
Tetradecanoic acid	41.6
Hexadecanoic acid	46.4
Trans-9-octadecanoic acid or Oleic acid	50.2 + 50.4
Octadecanoic acid	50.8
Dehydroabietic acid	53.8

Acid degradation products, such as furfurals and hydroxymethylfurfural (HMF), were not detected in the GC-MS spectra. However, the amounts of these compounds are expected to be very low at these conditions. For comparison, the amounts after a steam treatment at a higher temperature (190°C) and a residence time of ten minutes were reported in a recent study to be 0.17 mg/g for furfural and 0.38 mg/g for 5-HMF (Janzon et al., 2014).

Results from measurements of lignin and carbohydrates

The freeze-dried samples of the steam explosion liquors were also subjected to complete acid hydrolysis with sulphuric acid (72%) and subsequent analysis of their chemical composition. The results are presented in **Fig. 6.13**.

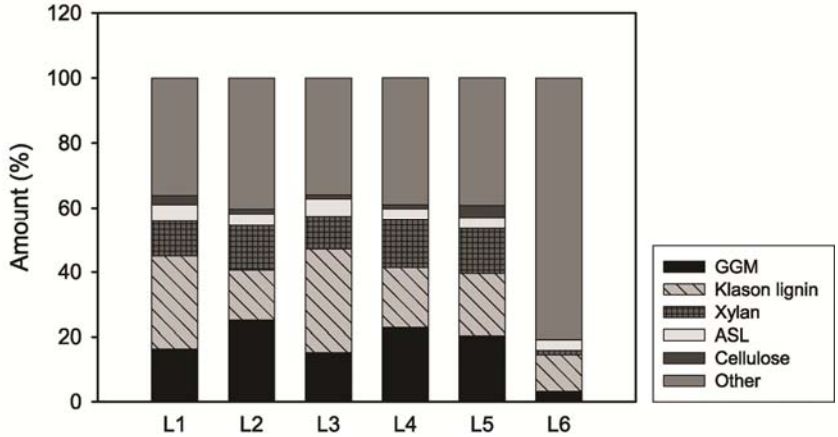

Figure 6.13 Chemical compositions of the freeze-dried samples from STEX liquors after complete acid hydrolysis. **L1** = STEX 4 bar, Batch 1, **L2** = STEX 7 bar, Batch 1, **L3** = STEX 4 bar, Batch 2, **L4** = STEX 7 bar, Batch 2, **L5** = STEX 7 bar, Batch 2, never-dried, **L6** = STEX 7 bar, Batch 2, $NaBH_4$ treated.

The samples treated at 4 bar resulted in higher amounts of Klason lignin and ASL, while the contents of GGM were correspondingly lower. This is in accordance with the results

obtained from the solid wood residues after the mild steam explosions. Again, the $NaBH_4$ pre-treated sample resulted in noticeably lower total amounts that could be explained and identified. The proportion of unidentified content was high for all the samples (approx. 34-40% for the water-impregnated samples). Some of this probably originated from the fatty acids or other organic compounds (most likely from wood extractives) observed in the GC-MS spectra.

Acetate results

The acetate concentration was also measured, both directly (condensed steam) and indirectly (hydrolysates after acid hydrolysis). The results from the hydrolysates from the different freeze-dried STEX liquor samples are presented in **Fig. 6.14**.

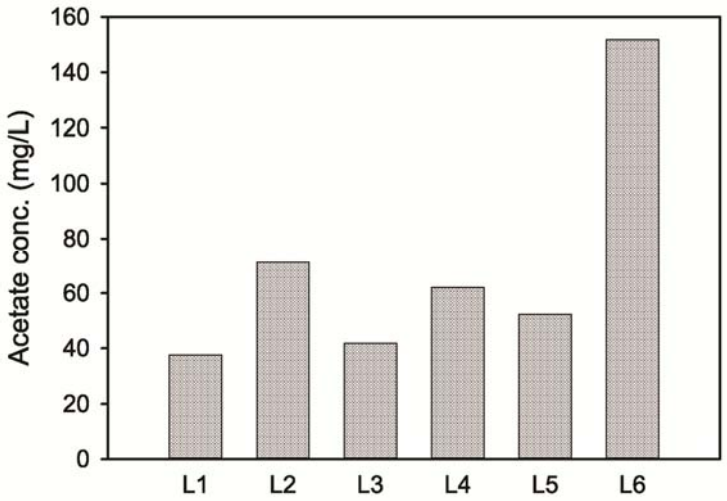

Figure 6.14 The concentration of acetate in the hydrolysates from the freeze-dried STEX liquor samples, **L1** = STEX 4, Batch 1, **L2** = STEX 7, Batch 1, **L3** = STEX 4, Batch 2, **L4** = STEX 7, Batch 2, **L5** = STEX 7, Batch 2, never-dried, **L6** = STEX 7, Batch 2, $NaBH_4$ treated.

The values of the mild STEX at 4 bar were lower than those of the samples from the 7 bar treatment; the content of acetate in the $NaBH_4$ pre-treated sample was substantially higher than the other samples, showing clearly that the degree of deacetylation was higher for this sample.

The concentration of acetate of some of the STEX liquors was also measured directly, i.e. the condensed steam was filtered and injected into the IC. The results are reported in **Table 6.8**.

Table 6.8 Concentration of acetate in the steam explosion liquors.

Sample description	Acetate conc. (mg/L)
STEX 4 bar, 10 min, Batch 2	17.86
STEX 7 bar, 10 min, Batch 2	33.34
STEX 7 bar, 10 min, $NaBH_4$ pre-treated, Batch 2	95.61

These results correspond well with the data in **Fig. 6.14** and, even though the levels of acetate differ between the two methods, the same trend can be observed. The lowest

concentration was observed for the sample from the 4 bar treatment, followed by the steam explosion at 7 bar; the highest concentration of acetate was found in the $NaBH_4$ pre-treated sample.

NMR results

The samples were also analyzed using HSQC-NMR, which is a powerful technique for characterizing lignocellulosic biomass (Komatsu and Kikuchi, 2013, Xue et al., 2012, Samuel et al., 2013). Experiments were conducted in order to compare Batch 1 with Batch 2, both of which were of STEX liquors of water-impregnated samples. The resulting spectra were divided into three regions of interest: aromatic (δ_C/δ_H 105.0-125.0/7.5-6.0 ppm), anomeric (δ_C/δ_H 110.0-90.0/5.5-4.0 ppm), and aliphatic (δ_C /δ_H 85.0-50/4.5-3.0 ppm) (Yuan et al., 2011). The aromatic region of both batches was indicative of guaiacyl-type lignin based on distinct signals attributable to C2/H2 (δ_C/δ_H 112.2/6.7 ppm), C5/H5 (δ_C/δ_H 115.5/6.7 ppm) and C6/H6 (δ_C/δ_H 119.6/6.8 ppm) of guaiacyl units, see **Fig. 6.15**.

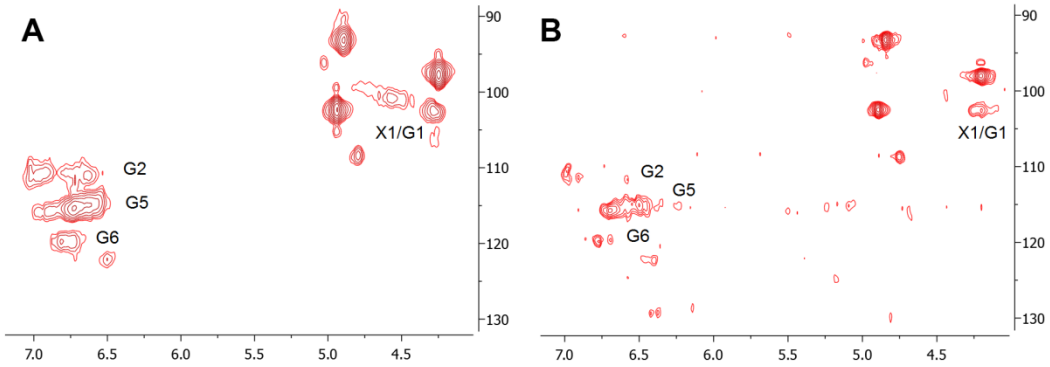

Figure 6.15 Aromatic and anomeric regions of HSQC spectra from the STEX liquor samples. **A:** Batch 1, **B:** Batch 2.

The assignment of a signal from polysaccharides was considerably more challenging due to the non-rigidity of the hemicelluloses and, the extensive variance of functional groups as well as the contaminants originating from extractives (Komatsu and Kikuchi, 2013). Thus, the anomeric cross peaks were only determined for (1→4)-β-D-Xyl*p* (δ_C/δ_H 102.7/4.20 ppm) and (1→4)-β-D-Glc*p* (δ_C/δ_H 102.6/4.24 ppm). The presence of these sugars was also observed in the chromatographic analyses. The aliphatic region was not subjected to any detailed interpretation due to overlapping signals attributed to various extractive components.

GPC results

The molecular weight distributions of the freeze-dried samples from the STEX liquors were investigated with DMSO-GPC. The chromatograms are shown (after baseline correction) in **Fig. 6.16**. A great drawback that was faced was the poor solubility of some of the samples in the eluent: the sample pre-treated with $NaBH_4$ in particular did not

dissolve at all. Therefore, this sample and the sample from STEX 4 Batch 2 were both excluded in the graph.

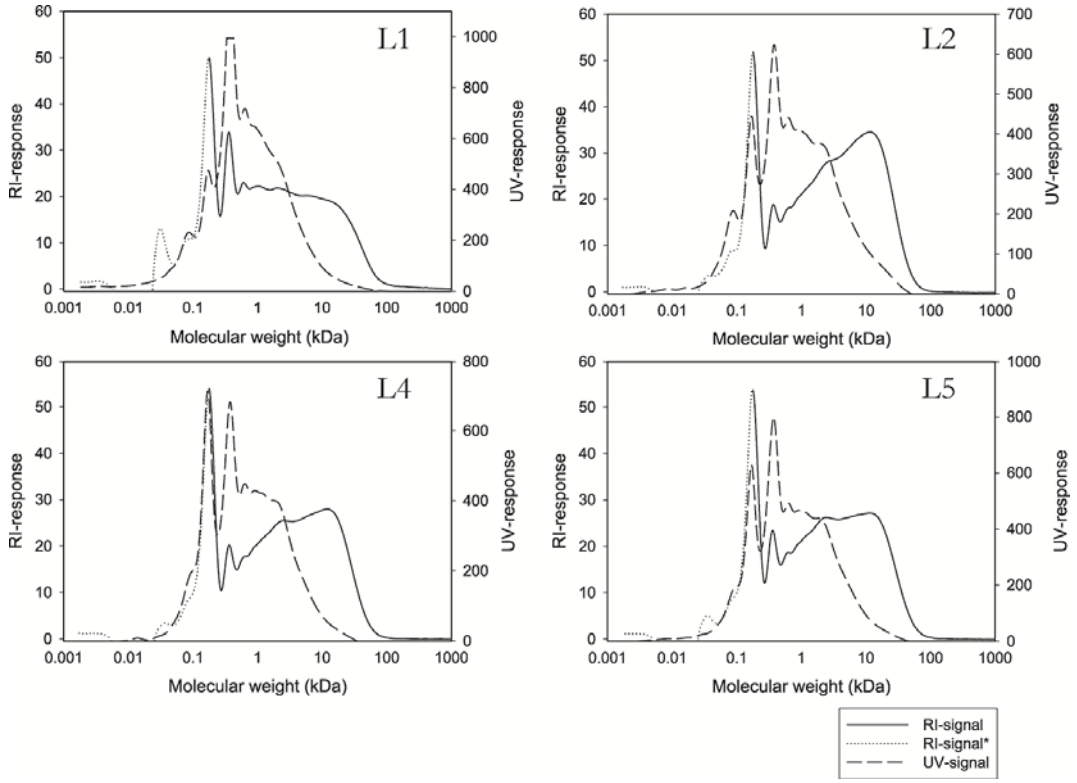

Figure 6.16 Results of the GPC measurements of the freeze-dried STEX liquors. Both the RI and UV signals are shown; the dotted lines (*) denote the RI signal below the lower limit of the calibration curve.
Sample **L1** = STEX 4 bar, Batch 1, **L2** = STEX 7 bar, Batch 1, **L4** = STEX 7 bar, Batch 2, **L5** = STEX 7 bar, Batch 2, never-dried.

There was a shift between the RI and UV signals, where the peaks corresponding to higher molecular weights were seen in the RI data, but not the UV. The UV detector was set at a wavelength of 280 nm, which is normally associated with lignin components. Thus, it could be interpreted that the higher molecular weight fraction contained more carbohydrate compounds, and that the lower molecular weight fraction contained more lignin fragments. The appearances of the GPC curves for the different samples were similar, with peaks at the same molecular weight intervals. The wide peak of the RI curve started somewhere around 20-70 kDa, which would correspond to chain lengths of about 100-350 monomer (translated into hexoses). It was difficult to draw clear conclusions about the nature of the different molecules since the samples contained a mixture of the various constituents of wood. Although the clear peaks that are visible at a molecular weight of approx. 300-500 Da in both the RI and UV curves correspond most likely to lignin or carbohydrates with 2-3 units, they could also originate from fatty acids or perhaps even LCC structures. There was also a large peak corresponding to sugar and lignin monomers (about 150 Da) in all of the measurements.

6.3 Post-treatments

6.3.1 Enzymatic treatment (Paper II)

Small pieces of wood cut from the edges and middle parts of the mild steam-exploded wood chips were used, along with some disintegrated wood chips, as samples to be subjected to enzymatic treatment. The effect of this treatment was measured in the form of reducing sugars produced due to enzymatic reactions; the results are presented in **Fig. 6.17**. The content of reducing sugars is plotted versus maximum overpressure, i.e. the pressure difference between the saturation pressure and the surrounding pressure at the moment when the pressure is released. According to the results, some reducing sugars were released even at the lowest mild STEX temperature (115°C/1.7 bar), whereas none were produced (zero absorbance) in the original, or non-exploded, wood samples. This indicates that the wood structure is more accessible to the enzymes after steam explosion even at very modest differences in pressure.

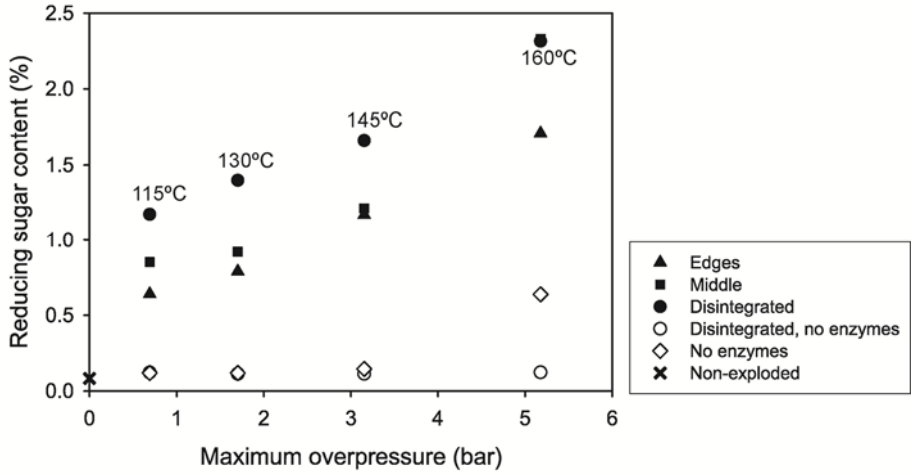

Figure 6.17 Results of measurements of reducing sugar content, recalculated from the absorbance values and glucose calibration curves.

The results obtained for the disintegrated samples showed larger contents of reducing sugar than for those taken from the different parts of the wood chips, but only up to a STEX temperature of 145°C. This is likely due to the disintegrated samples having more surfaces available for the enzymes to affect. Samples from the middle part of the wood chips resulted in consistently higher absorbance values compared to those from the edges, especially at 160°C. The mechanical effects could be expected to be greater in the former since the equalization of the pressure is slower there than at the latter. It could, however, be expected that more chemical reactions occur at the edges. Generally, there was also an increase in reducing sugars for samples treated at higher temperature: this could be expected, due to the effect of the greater difference in pressure when it was released.

6.3.2 Extraction/Leaching (Papers I and II)

The extraction experiments on the samples of wood powder in Paper I were performed after steam explosion at 160°C for 25 min. The aim was to investigate the stability of the

wood components after mild steam explosion, as well as to evaluate methods for isolating GGM from the wood. Some of the experiments were conducted at a high liquor-to-wood ratio (100:1) with aqueous liquors of different levels of pH (i.e. 2.5, 4.5 and 8). Water-impregnated samples and samples pre-treated with $NaBH_4$ were compared after being subjected to STEX and subsequent extraction. Analysis of the chemical composition was performed on the solid residues after extraction. The results of the content of GGM are shown in **Fig. 6.18**, in which the different sets of bar represent different experimental conditions. The extraction at acidic conditions, with the most extreme pH of 2.5, showed the greatest effect on the release of GGM. Thus, quite harsh conditions or prolonged extraction times may be required in order to extract large amounts of GGM in aqueous solution. These conditions will, on the other hand, also lead to the greatest degree of degradation, with the consequence that there is a trade-off between high yield and high molecular weights.

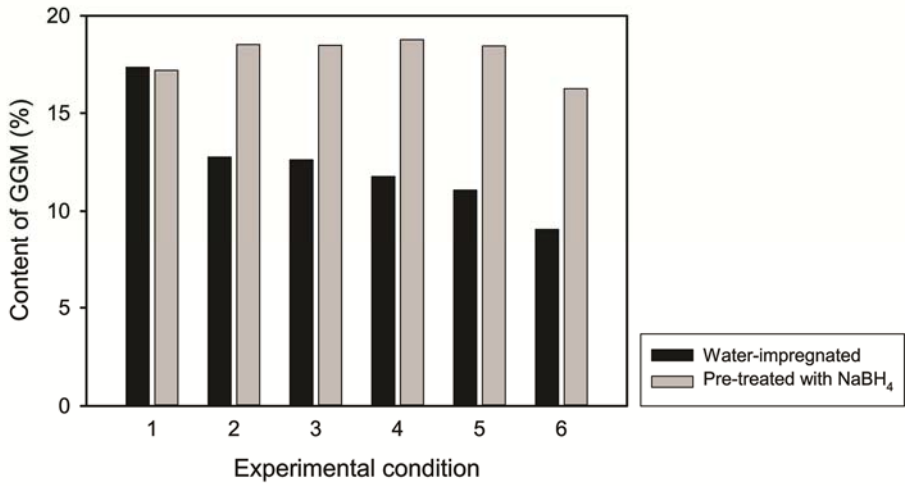

Figure 6.18 GGM content (% of sample) in the solid residues for **1.** Raw material, **2.** Mild steam explosion only, **3.** After STEX + extraction at pH 8, 1 h, **4.** After STEX + extraction at pH 4.5, 1 h, **5.** After STEX + extraction at pH 4.5, 2 h and **6.** After STEX + extraction at pH 2.5, 1 h.

The effect of pre-treatment with $NaBH_4$ was also seen clearly in the results of these experiments, where the amount of GGM in the pre-treated samples was noticeably higher than in the water-impregnated samples. The chemical compositions prior to the extractions (but after STEX) were also measured. The amount of GGM was between 12.7-12.9% for the water-impregnated samples and 18.4-18.8% for the $NaBH_4$ pre-treated samples (as % of sample content). The narrow intervals (the standard deviations based on all of the samples were 0.12 and 0.14%) indicate that the samples had the same composition before the extraction experiments, so the pre-treatment had good reproducibility. The other carbohydrate components and the content of lignin were similar for both water-impregnated and $NaBH_4$ pre-treated samples, and were almost constant after extraction at the conditions investigated.

A few of the experiments in Paper I (see **Table 5.3**) were conducted with a larger amount of wood material and at a lower liquor-to-wood ratio with the aim of obtaining liquors with a higher concentration of extracted wood components. The solid residues were analyzed for chemical composition; the findings showed once again that the samples pre-treated with $NaBH_4$ resulted in higher concentrations of GGM being retained in the wood. There was no large difference between the two extraction temperatures (110°C and 130°C) for either the water-impregnated samples (GGM content of 12.5-12.6%) or the $NaBH_4$ pre-treated samples (GGM content of 19.0-19.4%).

The extraction liquors in these experiments were also analyzed: chemical composition, molecular weights and molecular weight distributions were all investigated after rotary evaporation and freeze-drying. The results from the GPC were baseline corrected and are presented in **Fig. 6.19**. Here, it can be seen that the extracted compounds were only in the range of monomers for the $NaBH_4$ pre-treated sample treated with extraction liquor of pH 2.5 (C). Three peaks were detected with molecular weights (Mw) from 160 to 600 Da and polydispersity (PD) between 1.02 and 2.37. These findings show that extraction at such a low pH was too harsh to extract wood components in polymeric form, even after $NaBH_4$ pre-treatment. The formation of degradation products (e.g. furfurals) due to dehydration reactions also occurs at this really low level of pH.

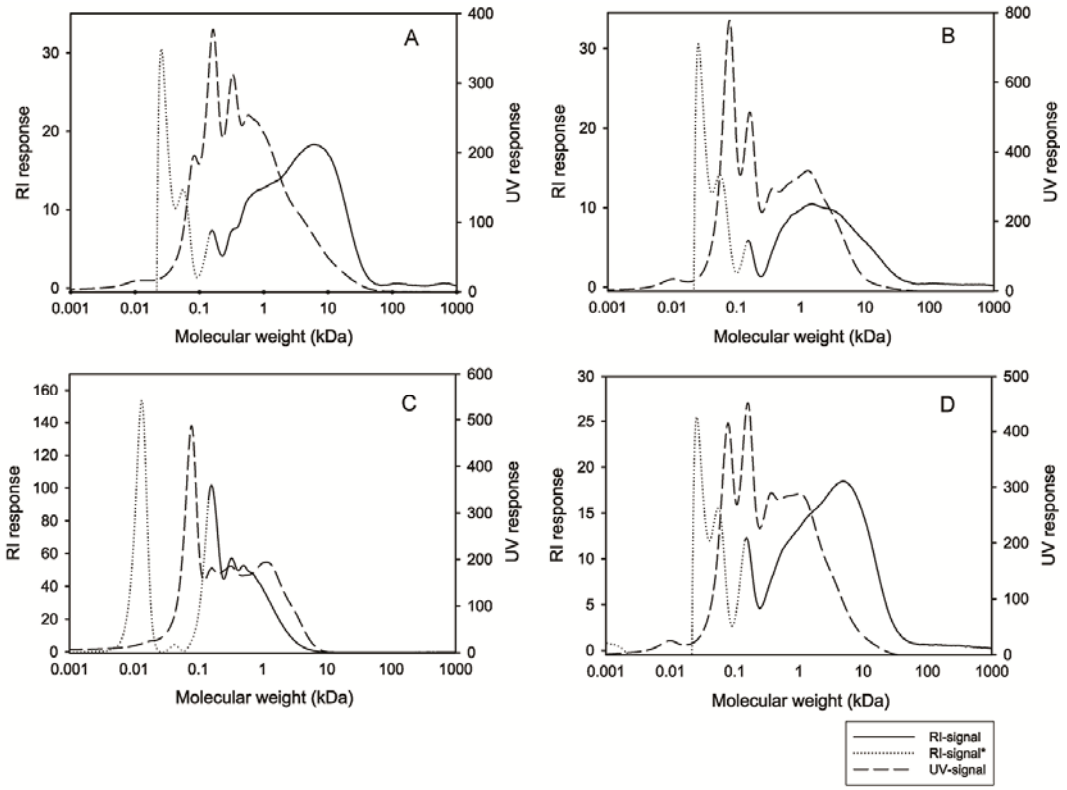

Figure 6.19 GPC chromatograms for the extraction liquor from: **A:** water-impregnated sample extracted at pH 11, 110°C, **B:** $NaBH_4$ pre-treated sample extracted at pH 11, 110°C, **C:** $NaBH_4$ pre-treated sample extracted at pH 2.5, 160°C and **D:** $NaBH_4$ pre-treated sample extracted at pH 11, 130°C.
Both the RI and UV signals are displayed; the dotted lines (*) denote the RI signal below the lower limit of the calibration curve.

The molecular weight of the samples treated at pH 11 (both at 110°C and 130°C) ranged between 3300 and 5700 Da, see **Table 6.9**, which corresponds to sugar chains of about 20 to 30 monomers (translated into hexoses).

Table 6.9 Molecular weight averages and polydispersities for the samples treated at pH 11, 1 h.

Extraction Condition	Mw (Da)	Mn (Da)	PD
pH 11, 110 °C, water	5688	908	6.26
pH 11, 110 °C, $NaBH_4$	3310	577	5.74
pH 11, 130 °C, $NaBH_4$	5426	1909	2.84

In **Fig. 6.19**, the GPC curves from the $NaBH_4$ pre-treated samples extracted at pH 11 at 110°C and 130°C are shown (B and D). The distributions of molecular weight were very broad for these samples and it is therefore reasonable to assume that these samples consisted of a great variation of compounds of various sizes. The largest peaks in the RI curve in Graph A and D correspond to higher molecular weights than in the corresponding UV curves, which indicate that the larger molecules originated from polysaccharides. However, it was difficult to draw clear conclusions as to whether the larger molecules originated from lignin or carbohydrates. It is most likely that the samples also contained LCC fragments with both lignin and carbohydrate parts.

The samples of extraction liquors were also analyzed for carbohydrate and Klason lignin content after complete acid hydrolysis. There were some uncertainties here, since only part of the total weight of the samples could be explained by the analyses. The detection levels also differed between the different samples (45-73%). The results of the samples extracted at pH 11 are compared in **Table 6.10**.

Table 6.10 Results of chemical composition analysis of wood residues and freeze-dried samples of extraction liquors. The results are presented as (w/w)% of the sample content.

Sample type	GGM (%)	AGX (%)	Klason lignin (%)
Water-impregnated samples, pH 11, 110°C			
Untreated wood powder	17.4	5.8	27.9
Wood powder after STEX	12.8	4.6	29.9
Wood powder after extraction	12.6	4.4	29.7
Extraction liquor	20.8	6.2	9.4
$NaBH_4$ pre-treated samples, pH 11, 110°C			
Untreated wood powder	17.2	5.8	27.5
Wood powder after STEX	19.2	5.7	27.2
Wood powder after extraction	18.9	5.4	26.7
Extraction liquor	4.7	5.7	12.7
$NaBH_4$ pre-treated samples, pH 11, 130°C			
Untreated wood powder	17.2	5.8	27.9
Wood powder after STEX	19.9	5.7	28.2
Wood powder after extraction	19.4	5.2	28.2
Extraction liquor	7.5	15.0	9.0

The results show that the amount of GGM extracted in the water-impregnated sample was considerably higher than for the $NaBH_4$ pre-treated samples: this is reasonable, since a larger amount was shown to remain in the solid residue of these samples. It was also of

interest that the amount of xylan in the material extracted from the 130°C experiment seemed to be very large, and that the molecular weight in this case was also higher than in the 110°C experiment. Thus, a higher temperature may potentially result in the dissolution of larger fragments of xylan.

In Paper II, the extraction experiments were performed on wood chips in alkaline conditions at 130°C for two hours. Water-impregnated samples were again compared with $NaBH_4$ pre-treated samples, and the wood chips were steam-exploded at either 140°C or 160°C. All of the samples were treated with alkali after steam explosion. The amounts of GGM (% of sample content) in the solid residues of wood are shown in **Fig. 6.20**.

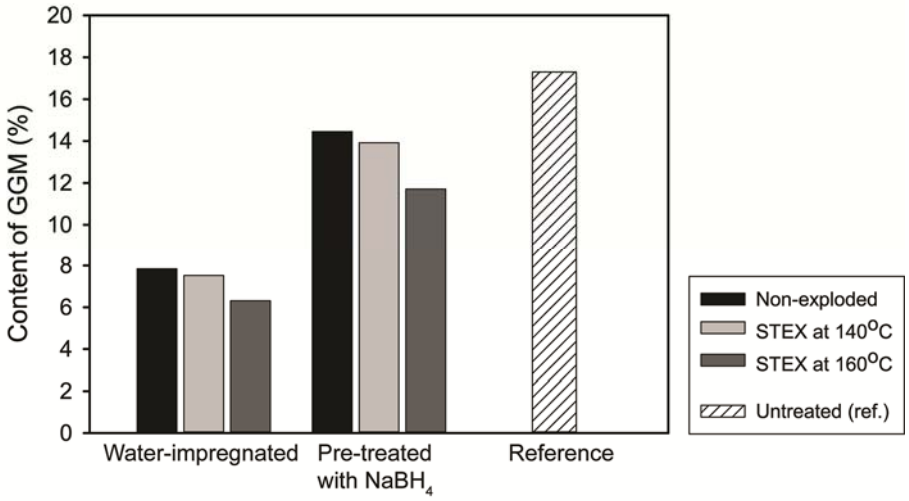

Figure 6.20 A comparison of the effect of mild STEX on water-impregnated and $NaBH_4$ pre-treated samples steam-exploded at different temperatures. Content of GGM is presented as (w/w)% of the sample content.

As can be seen in **Fig. 6.20**, there is a large difference in the amount of GGM remaining in the water-impregnated samples compared to the $NaBH_4$ pre-treated samples. This is most likely due to the stabilization of the end-groups of the GGM during the $NaBH_4$ pre-treatment, which protects the GGM from peeling reactions in the alkaline conditions. The GGM is most likely also deacetylated, which lowers its solubility. The steam-exploded samples contained a lower amount of GGM than those not subjected to STEX, which could be explained either by a faster release of GGM due to a more open wood structure, or the degradation of GGM as an effect of autohydrolysis.

6.3.3 Kraft cooking (Papers II, III and VI)

Initial study (Paper II)

The first kraft cooking experiments were performed in an initial study in Paper II. Untreated wood chips were compared with wood chips that were steam exploded in the lab-scale equipment at 160°C with a residence time of ten minutes. The kappa numbers

after the subsequent kraft cook are shown in **Fig. 6.21** for different times at the cooking temperature. It can be seen that a slightly lower kappa number was reached for the steam-exploded wood chips and, consequently, the overall delignification rate was higher in this particular case. A plausible reason for this is the mass transfer of cooking chemicals and lignin fragments that occurs more rapidly inside steam-exploded wood chips; it also indicated that the morphology of these wood chips was altered to some extent.

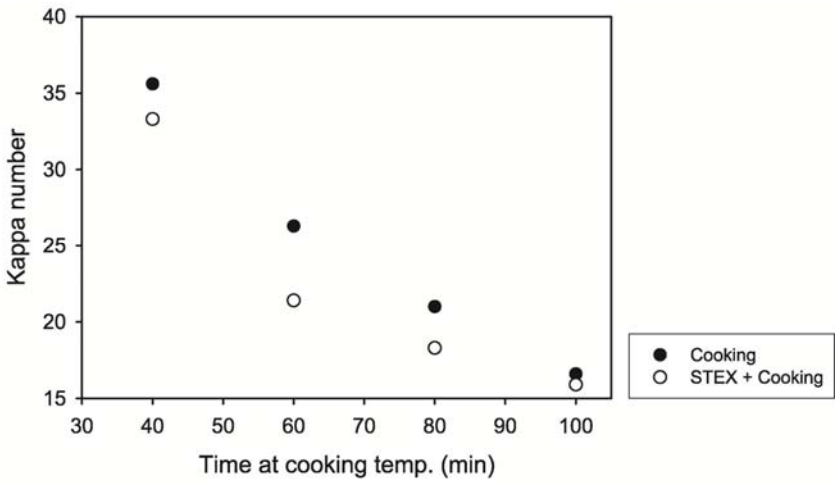

Figure 6.21 The kappa numbers of the pulps after 40, 60, 80 and 100 min at cooking temperature (170°C) for both mild steam-exploded and reference samples.

The properties of the resulting pulps were also measured; the findings showed that there were no large differences between the reference pulp and the pulp produced from the steam-exploded wood chips. The latter had slightly higher values of ISO-brightness at the same kappa number and the fibre length was maintained (with an average fibre length of ca 2.5 mm for all of the pulps). The results from the small initial study formed the basis of a more detailed study using steam-exploded wood chips from the bench-scale equipment in Paper III.

Kraft cooking and oxygen delignification after STEX in the bench-scale equipment (Paper III)

In Paper III, two different batches of wood chips, Samples 1 and 2, were subjected to STEX (at 4 and 7 bar for 10 min) followed by kraft cooking and oxygen delignification. The difference between the two batches was the storage time (at RT): more than two years for Sample 1 and only a couple of weeks for Sample 2. The chemical composition and properties of the pulps were investigated and compared with pulps from reference (untreated) wood chips.

The lignin in the pulps was measured both as content of Klason lignin and kappa number. The results of the Klason lignin measurements are shown in **Fig. 6.22** for both samples of wood chips. The trends are as expected, i.e. a decrease in lignin content after longer cooking times and after oxygen delignification. There were only small differences between the two samples; the steam-exploded wood chips showed somewhat lower lignin

values for the shorter times at the cooking temperature, while this difference levelled out after longer cooking times.

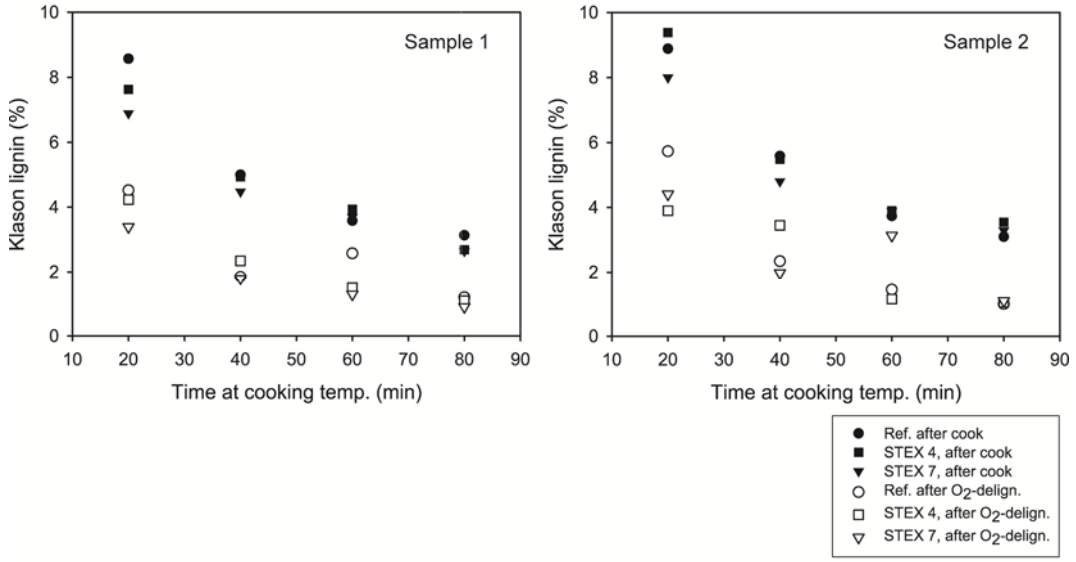

Figure 6.22 Content of Klason lignin (% of sample) vs. time at cooking temperature for Samples 1 and 2 after both kraft cooking and oxygen delignification.

Similar trends were seen in the results of the kappa numbers, see **Fig. 6.23**. For both samples, the STEX at 7 bar decreased the cooking time required to reach a certain kappa number: e.g. a kappa number of 30 could be reached after about 50 min for the steam-exploded wood chips but 60 min for the reference chips. This could probably be explained by a change in the structure of the wood, which increases its accessibility to the cooking chemicals and lowers initial mass transport resistances. There may also be some effect caused by the reduced consumption of cooking chemicals due to the removal of some of the components of wood during mild STEX treatment. However, this effect is most likely modest since there were no noteworthy differences in the content of residual alkali measured in the black liquors after the cooks, which was determined after each kraft cook according to Wilson (1968). The values for the concentration of NaOH ranged from 10.1 to 15.2 g/L, the lower values corresponding to longer times at cooking temperature. There were no observable differences in the residual alkali content for the reference cooks compared to the experiments with the steam-exploded wood chips.

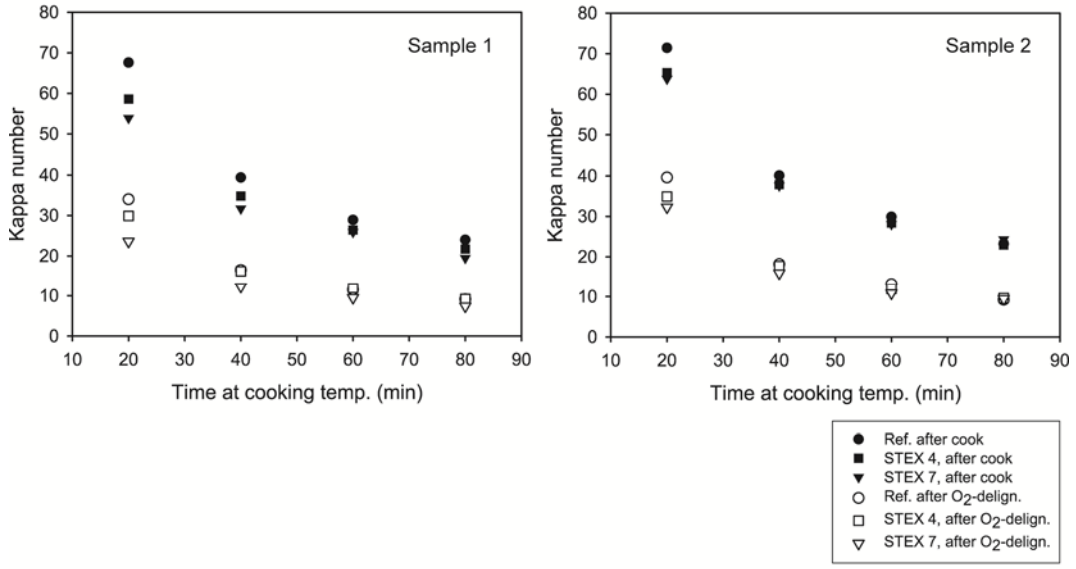

Figure 6.23 Kappa number vs. time at the cooking temperature for Samples 1 and 2 after kraft cooking and oxygen delignification.

The kappa numbers and contents of Klason lignin were both generally slightly higher for Sample 2. The mild STEX treatments as well as the cooking and oxygen delignification processes were performed at the exact same conditions, so it is reasonable to conclude that the differences derive from variations in the raw materials. The wood chips came from the same pulp mill but with a time interval of two years, and were stored in room temperature for different lengths of time. It is also likely that they originated from different cutting areas. For longer cooking times, the content of lignin showed similar values, and it was no longer possible to distinguish between the steam-exploded and reference materials. This could be interpreted as the initial impregnation rate of the cooking liquor being faster due to the mild STEX treatment, which is levelled out after longer cooking times. The effect could also, at least partially, be explained by the fact that the alkali-consuming reactions with carbohydrates were intense at the beginning of the cook, and the less alkali-consuming reactions with lignin were initiated later on.

Carbohydrate analyses were also performed on samples taken from all of the pulps after complete acid hydrolysis. The relative content of cellulose in the samples increased expectedly after longer times at the cooking temperature and after oxygen delignification. The amounts of xylan were fairly stable; although the samples taken from the mild STEX treatment at 7 bar consistently contained a somewhat lower amount of xylan, see **Table 6.11**. The values in the table are from both Sample 1 and Sample 2 and ranges across all of the different cooking times; the lower values for hemicelluloses (and correspondingly higher values for cellulose) were observed after longer times at the cooking temperature.

Table 6.11 Carbohydrate composition of the pulps. The ranges cover all times at the cooking temperature (20-80 minutes). The values are presented as percentages of the sample content.

Treatment	Cellulose (%)	GGM (%)	AGX (%)
Ref., Kraft	68.8-80.3	10.8-8.5	7.4-6.5
Ref., Kraft + O_2 delign.	76.0-82.7	11.0-8.5	7.9-6.4
STEX 4 bars, Kraft	71.4-81.5	11.1-7.7	7.9-6.5
STEX 4 bars, Kraft + O_2 delign.	75.9-83.8	11.8-7.7	8.2-6.5
STEX 7 bars, Kraft	74.6-83.3	7.6-6.7	6.2-5.6
STEX 7 bars, Kraft + O_2 delign.	80.2-84.9	10.0-6.9	7.0-5.7

The content of GGM was also slightly lower for the samples steam-exploded at 7 bar. Mild STEX could have led to degradation of GGM due to autohydrolysis as well as making the wood structure more accessible to the cooking liquors, which would also result in a lower content of GGM after kraft cooking. There were no large differences in the composition of carbohydrates after oxygen delignification, indicating that most of the losses of hemicelluloses occurred during the kraft cook.

The pulp yield and various pulp properties were investigated after both kraft cooking and oxygen delignification. The values of the pulp yield are presented in **Fig. 6.24** and were within the range expected for these kappa numbers (Rydholm, 1965). There was a minor difference between the two wood chips samples, where Sample 2 resulted in somewhat higher yield values. However, there was no difference evident in yield between the pulps made from the steam-exploded wood chips and the reference pulps.

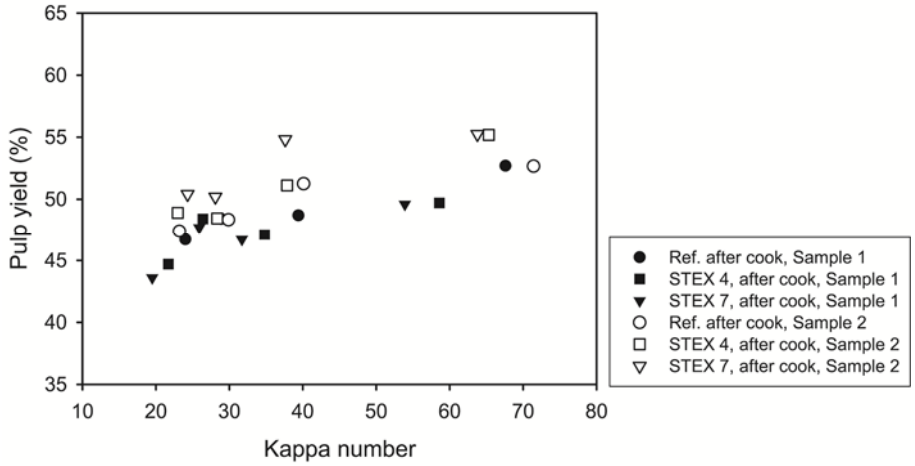

Figure 6.24 Pulp yield after kraft cooking vs. kappa number for both sample series.

The losses in mass during oxygen delignification were also measured. The values of the material recovered varied between 94 and 101%, which could be explained by minor losses of material during the actual treatment and the subsequent washing steps.

The intrinsic viscosities of the pulps were measured in order to investigate the extent of cellulose degradation; the results are presented versus kappa numbers in **Fig. 6.25**. The trend shows a lower viscosity at lower kappa numbers: this is expected and is due to degradation reactions of carbohydrates during both kraft cooking and oxygen

delignification. The intrinsic viscosity values for kappa numbers above kappa 50 were not reliable: the method is not adapted to such high viscosity values and they were therefore removed from the graphs.

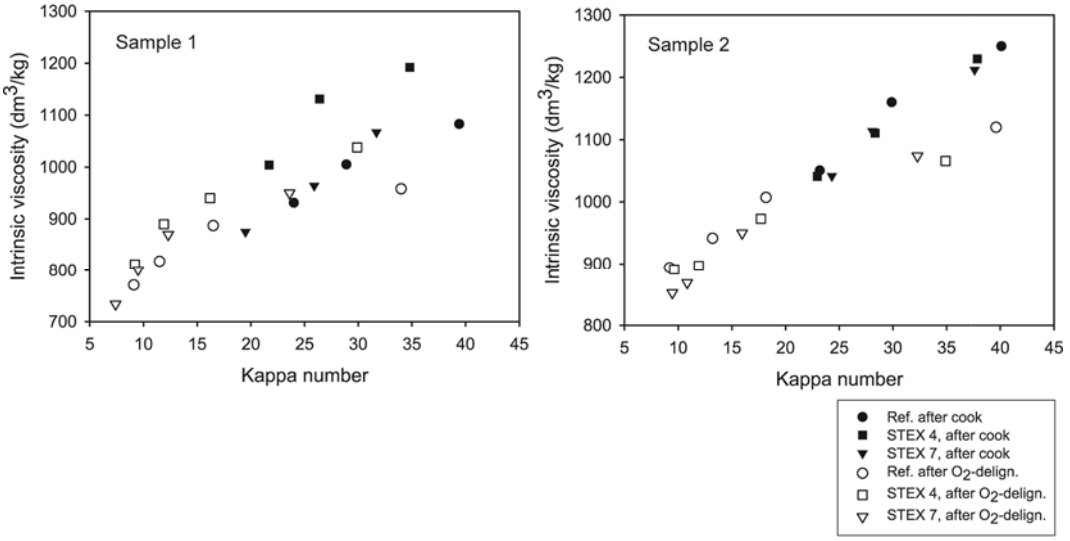

Figure 6.25 Intrinsic viscosity vs. kappa number of Sample 1 and Sample 2 after kraft cooking and oxygen delignification.

The intrinsic viscosity values of Sample 1 were generally slightly lower than those of Sample 2: this is in accordance with, and probably related to, the results of the pulp yield, content of Klason lignin and kappa numbers. A slightly lower DP of the wood components will probably result in lower values of intrinsic viscosity. The samples from the mild STEX at 4 bar for Sample 1 resulted in somewhat higher intrinsic viscosities than was expected, whilst those for Sample 2 seem to relate to kappa number only, irrespective of the previous mild STEX treatment. This was true both after kraft cooking and after oxygen delignification.

Laboratory sheets were prepared from each sample of pulp and measurements of the ISO brightness were made; the results are shown in **Fig. 6.26**. All of the ISO brightness values were found to be in the same range, with the values of Sample 1 being slightly higher than those of Sample 2. The brightness of the pulps seemed to be nearly identical at the same kappa number independent of the previous STEX treatment.

The lengths of the fibres in Sample 2 were also measured. The fibres were almost unaffected by any of the treatments: the values were all between 2.3 and 2.7 mm, where the shorter fibre lengths correspond to the pulps with lower kappa numbers.

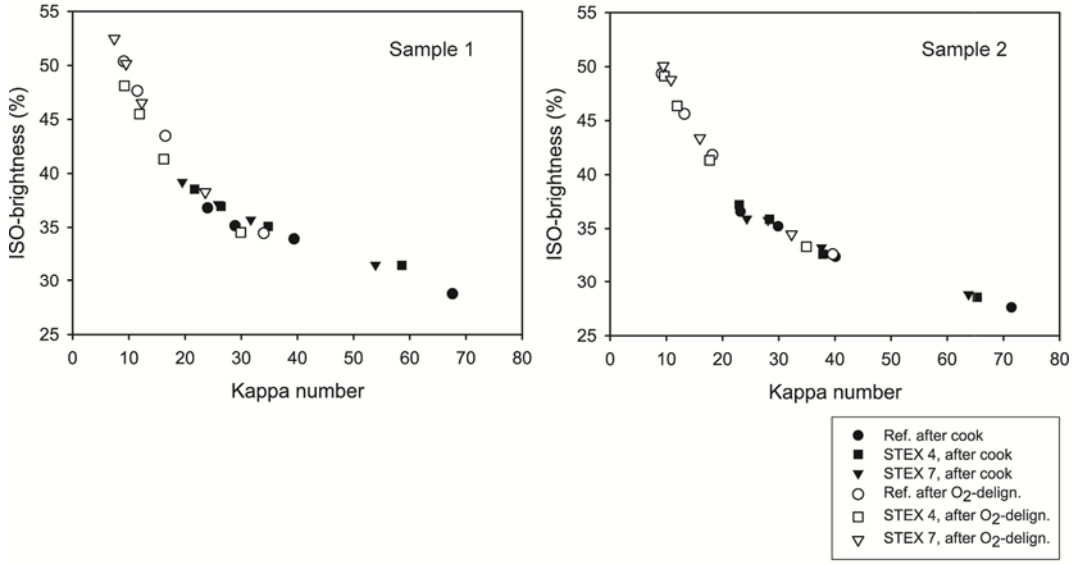

Figure 6.26 ISO brightness vs. kappa number for Samples 1 and 2 after kraft cooking and oxygen delignification.

The results of the measurements of intrinsic viscosity, where the pulp samples had similar values for the same kappa numbers, indicated that, despite concerns of the contrary, the mild STEX treatment did not affect the cellulosic fibres to any great extent. The same brightness values at the same kappa numbers also indicates that the properties of the final pulps are not affected negatively by a mild STEX treatment at these conditions.

Kraft cooking after dithionite impregnation and STEX (Paper VI)

Paper VI compared water-impregnated wood chips with wood chips pre-treated with dithionite ($Na_2S_2O_4$). The chips were subsequently subjected to kraft cooking with or without a prior mild STEX treatment. Two different STEX conditions were used: 4 bar (144°C) for 10 min and 7 bar (165°C) for 10 min. A further cook was also performed in which the raw material was subjected to kraft cooking (without prior treatments) and where $Na_2S_2O_4$ was added to the white liquor (sample 0_0).

The content of lignin decreased after pre-impregnation and mild STEX, shown by lower kappa numbers and lower values in the content of Klason lignin; the results of the different pulp analyses are presented in **Table 6.12**. The lowest kappa numbers were observed for the $Na_2S_2O_4$ pre-treated samples, which indicated that the presence of dithionite contributed to delignification during kraft cooking. Pre-impregnation with $Na_2S_2O_4$ also resulted in pulps with noticeably higher values of brightness. There are several possible explanations for this: the dithionite reactions could lead to the formation of sulphite ions that could sulphonate the lignin and increase its solubility; the sulphite ions could also catalyze the cleavage of bonds to polysaccharides. $Na_2S_2O_4$ may also react in a similar manner as when it is used for bleaching of mechanical pulps. The main purpose of using dithionite as a bleaching agent for mechanical pulp is supposed to be the reduction or sulphonation of coloured structures, e.g. quinones, quinone methides, coniferyl aldehyde side-chain units and oxidized transition metal ions (Ellis, 1996).

Reducing quinones into phenols could restrict a potential stopping reaction in the delignification process. Phenols could react with the sulphide ions in the white liquor, whereas quinones are more stable at these conditions (Carreira et al., 2012).

Table 6.12 Summary of the results of the different cooks. W = water-impregnated, D = $Na_2S_2O_4$ impregnated, 0 = no mild steam explosion, S4 = steam explosion at 4 bar, 10 min, S7 = steam explosion at 7 bar, 10 min.

Sample	Klason lignin (%)	Kappa number	Cellulose (%)	GGM (%)	Xylan (%)	Brightness (%)	Viscosity (mL/g)	Tensile index (N·m/g)
0_0	7.67	43.2	66.9	7.2	6.3	23.7	1324	74.8
W_0	5.14	35.0	71.5	7.3	6.7	21.1	1284	81.0
W_S4	4.88	34.3	71.2	6.9	5.8	22.8	1325	92.1
W_S7	5.94	39.3	76.4	5.6	4.5	20.4	1238	83.0
D_0	3.04	20.9	73.4	7.9	6.9	28.5	992	85.3
D_S4	3.13	22.6	74.0	7.7	6.9	25.4	1066	85.3
D_S7	2.68	18.9	74.3	7.8	6.6	26.4	939	83.3

The cook in which $Na_2S_2O_4$ was added into the white liquor (0_0) resulted in considerably higher values of Klason lignin and kappa number. This is probably due to the wood being poorly impregnated by the white liquor, as the addition of $Na_2S_2O_4$ causes an increase in its viscosity. Therefore, it may be more effective to use a pre-impregnation step with $Na_2S_2O_4$ than adding it to the white liquor. Furthermore, positive effects of $Na_2S_2O_4$ on the brightness values were also observed in the cook where dithionite was added to the white liquor. A mild STEX treatment appears to influence delignification to some extent at these cooking conditions; the highest degree of delignification was observed for D_S7. This, together with the results of Paper III, where the mild STEX treatment led to a slightly reduced cooking time, indicates that a combination of pre-impregnation with $Na_2S_2O_4$ and mild STEX could lead to an improvement in the kraft cooking process.

Compared to the cook with $Na_2S_2O_4$ added into the white liquor, the content of cellulose was substantially higher in the pre-impregnated and mild steam-exploded samples, see **Table 6.12**. The same was true for the hemicelluloses, especially for the samples pre-impregnated with $Na_2S_2O_4$. The explanation is most likely that the dithionite acts as a reducing agent that stabilizes the reducing end-group of the GGM, thereby protecting the carbohydrates from peeling reactions during the cook. Moreover, a higher content of GGM might protect parts of the xylan and cellulose from being affected by alkaline hydrolysis.

In the case of samples of pulp, the value of viscosity gives an indication of the average length of the cellulose chains (DP); it is typically a very important factor for the tensile strength and, correspondingly, the strength of the pulp. The viscosity of the samples of pulp used in this study was lower for the $Na_2S_2O_4$ pre-treated and mild steam-exploded samples than for the cook with $Na_2S_2O_4$ added into the white liquor. This did not lead to a decrease in tensile index which, on the contrary, actually increased. The reason for this

may be due to the pre-impregnated pulp samples having a higher content of hemicelluloses, which could result in stronger fibre-fibre interactions. The lower viscosity values might also indicate more flexible fibres, which also could lead to stronger fibre-fibre interactions and, consequently, a higher tensile index. The tensile index of the paper sheets from the cook with $Na_2S_2O_4$ added to the white liquor was considerably lower than the other pulps. The kappa number of this pulp, on the other hand, was much higher than the other pulps so it is therefore irrelevant to compare these samples.

The mild STEX treatment at 7 bar of the water-impregnated samples resulted in lower contents of hemicelluloses, while the results from the 4 bar treatment were in the same range as the non-exploded samples. It is interesting that pre-impregnation with $Na_2S_2O_4$, just as with pre-treatment with $NaBH_4$, leads to an increased amount of hemicelluloses being retained after mild STEX and kraft cooking. This could be due to a shift in the pH level towards more alkaline conditions during pre-treatment, which leads to deacetylation and a more linear GGM. It could also be due to their effect as reducing agents, which protects the hemicelluloses from peeling reactions during the alkaline kraft cook.

The chemical composition and molecular weight distribution of the STEX liquors were analyzed in order to obtain additional information. The content of Klason lignin and different anhydro sugars are presented in **Table 6.13**.

Table 6.13 Results of the carbohydrate analysis and measurements of Klason lignin content of the STEX liquor samples.

Liquor sample	Ara (%)	Gal (%)	Glu (%)	Xyl (%)	Man (%)	Klason lignin (%)	Total amount of sample (g/L)
D_S7	0.33	0.74	0.46	0.54	0.32	0.046	19.3
D_S4	0.13	0.37	0.15	0.19	0.18	0.024	11.0
W_S7	7.15	3.03	4.96	4.05	16.1	17.76	5.95
W_S4	11.8	4.37	7.99	2.01	14.9	30.35	1.86

The total amount of sample was higher for the pre-impregnated samples than for the water-impregnated samples, while the amounts of compounds detected were lower. This may be due to these samples having a higher content of inorganic salts. It is also reasonable that the amounts of hemicelluloses in these liquors are lower since a greater amount of hemicelluloses were retained in the wood chips from which these samples originate.

7. Concluding remarks

The application of mild steam explosion treatment to Norway spruce led to the wood being affected both morphologically and chemically. The changes that occurred to the wood chips steam-exploded at different pressures were clear and visible. A more intensive treatment (i.e. higher temperature or longer residence time) led to the wood chips becoming darker in colour, as well as having more visible cracks and damages and a higher degree of hemicellulose degradation.

Arabinose side-chains on the arabinoglucuronoxylan were cleaved off at relatively mild conditions (after only a slight decrease in pH) and were released into the condensed steam. Acetyl groups were also sensitive to the hydrothermal treatment and the bonds were easily cleaved, leading to the formation of acetic acid. The (galacto)glucomannan was also very sensitive to the steam explosion treatment: there was a clear trend towards a higher degree of degradation of GGM at higher steam explosion temperatures or prolonged steam treatment times. The lignin and cellulose in the wood were not affected by the steam explosion treatments to any substantial extent in the conditions investigated. Analyses of the steam explosion liquors also showed that the compounds released during the STEX treatments were mainly from the hemicelluloses (possibly also pectins) and wood extractives. Analyses of the molecular weight distribution in samples from steam explosion liquors indicated that the compounds with higher molecular weights originated most likely from carbohydrate components rather than lignin, shown by a shift between the RI and the UV signals. However, the samples contained a mixture of many different compounds and possibly also some LCC structures, making clear conclusions regarding the molecular weights of the wood components in the condensed steam difficult to draw.

The morphological study found that the penetration of mercury into the wood chips occurred mainly through the pits in the cell wall of the wood. The pore sizes increased after steam explosion treatments in the experimental conditions investigated. It was shown that the explosion step (i.e. the pressure release) was not the main cause of the wood material disintegrating into small pieces. It was, instead, the impact of highly softened wood chips with the walls of the equipment that appeared to have a great influence on the mechanical damage afflicted on the wood chips. Moreover, small changes in the internal structure were also observed after the explosion step. It can therefore be concluded that chemical changes, the expansion of vapour within the wood chips and the impact of softened wood chips with the equipment and other material all contribute to structural changes of the wood structure.

In order to protect the GGM component in the wood from extensive degradation, a stabilization of the reducing end-group with a reducing agent (such as $NaBH_4$) was shown to be effective. Changing from an aldehyde group to a primary alcohol stopped degradation caused by peeling reactions that occur in alkaline conditions. The pre-treatment with $NaBH_4$ also seemed to protect the GGM during mild steam explosions. It was shown that the pre-treatment itself not only led to deacetylation of the GGM but also caused increased deacetylation during the subsequent mild steam explosion. This could be one reason for the greater amount of GGM being retained in the wood in these samples: the deacetylated GGM would be more linear and have a higher affinity to the cellulose fibres. The pre-treatment with $NaBH_4$ also resulted in the condensed steam from the mild steam explosion having a higher pH, so the chemical reactions during the steam treatment would be altogether different in this case. The analysis of the STEX liquor from pre-treated wood chips proved to be more difficult and only part of the sample could be identified.

A mild steam explosion treatment of spruce wood chips also influenced subsequent procedures, such as enzymatic treatment, extraction/leaching and kraft cooking. The results of the investigation using enzymes showed that mild steam explosion, also at low temperatures, allowed the enzymes to react with the components of the wood. There was a clear difference between untreated and steam-exploded wood chips and, since enzymes are generally rather large molecules and need to be positioned in a specific way to act on a substrate, the findings suggest that the structure of the wood had become more accessible. There was also a trend towards the presence of a higher amount of reducing sugars, i.e. more enzymatic reactions, after STEX at higher temperatures.

The mild steam explosion treatment also resulted in a faster alkaline extraction and somewhat faster delignification during the first part of a kraft cook. The properties of the resulting pulps (e.g. intrinsic viscosity, brightness and fibre lengths) after steam explosion followed by kraft cooking and oxygen delignification had values similar to those of the

reference pulps at the same kappa numbers. The results therefore indicate that a mild steam explosion treatment at these conditions does not influence either the cellulose fibres or the pulping process negatively. Instead, there could be some positive effects of lowering the initial mass transport resistances, potentially giving the wood chips a more even chemical profile. The experiments on dithionite pre-impregnation followed by mild STEX and kraft cooking were also shown to have interesting effects. Dithionite, like $NaBH_4$, is a reducing agent but is less expensive. Treatment with dithionite seemed to protect the hemicelluloses during both mild STEX and kraft cooking. The resulting pulps after kraft cooking also showed lower contents of Klason lignin and higher values of brightness. The dithionite pre-treated samples had a small decrease in intrinsic viscosity, but this did not appear to have an impact on the strength of the pulp, since the values for the tensile index of the paper sheets from this pulp remained high.

In this work, damage to the hemicelluloses and fibres was undesirable so the steam explosion treatments were kept at relatively low pressures. However, in order to provide chemicals and enzymes access to the wood structure, and be able to extract and isolate hemicellulosic components, certain pressures and temperatures are probably needed. There is obviously a trade-off between retaining molecular weights and obtaining higher yields of hemicelluloses in the steam effluents. The conditions of a mild steam explosion therefore need to be optimized, depending on the purpose of the process. Future work should include examining the optimization of mild steam explosion treatment in combination with methods of fractionating the various components of the steam-exploded spruce wood material as well as from the liquor fraction. Future studies should also involve further scaling-up of the process.

Finally, taking into account all the results obtained in the studies conducted during this work, it seems that mild steam explosion is an interesting process step that has potential in a future wood-based biorefinery. Mild steam explosion is a process that is technically feasible on an industrial scale. Making the structure of the wood more accessible, it may be beneficial as a first step in a biorefinery where it could facilitate the separation of the different components of wood based on, for instance, enzymatic treatments. It could also be advantageous as a pre-treatment step prior to subsequent process steps, e.g. traditional pulping, where the effect of a mild steam explosion may result in somewhat faster delignification and, possibly, a more homogeneous pulp.

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9. References

ALLISON, R. W. 1985. *Oxidative pretreatment with hydrogen peroxide prior to alkaline pulping.* Doctorial, North Carolina State University, Raileigh, USA.

ALVIRA, P., TOMAS-PEJO, E., BALLESTEROS, M. & NEGRO, M. J. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresour. Technol.,* 101(13)**,** 4851-4861.

ANDERSSON, A., PERSSON, T., ZACCHI, G., STAALBRAND, H. & JOENSSON, A.-S. 2007. Comparison of diafiltration and size-exclusion chromatography to recover hemicelluloses from process water from thermomechanical pulping of spruce. *Appl. Biochem. Biotechnol.,* 137-140**,** 971-983.

ANGLÈS, M. N., REGUANT, J., GARCIA-VALLS, R. & SALVADÓ, J. 2003. Characteristics of lignin obtained from steam-exploded softwood with soda/anthraquinone pulping. *Wood Sci. Technol.,* 37(3-4)**,** 309-320.

ANNERGREN, G. E., CROON, I., ENSTRÖM, B. F. & RYDHOLM, S. A. 1961. On the stabilization of spruce glucomannan in wood and holocellulose. *Sven. Papperstidn.,* 64**,** 386-393.

ANNERGREN, G. E. & RYDHOLM, S. A. 1960. The stabilization of glucomannan in the pulping processes. *Sven. Papperstidn.,* 63**,** 591-600.

ARESKOGH, D., LI, J., GELLERSTEDT, G. & HENRIKSSON, G. 2010. Structural modification of commercial lignosulfonates through laccase catalysis and ozonolysis. *Ind. Crops Prod.,* 32(3)**,** 458-466.

BABCOCK, L. W. 1932. *Method of producing fermentable sugars and alcohol from wood.* USA patent application: US1855464.

BACK, E. L. & ALLAN, L. H. (eds.) 2000. *Pitch control, Wood resin and Deresination,* Atlanta: Tappi Press.

BARGIGIA, I., NEVIN, A., FARINA, A., PIFFERI, A., D'ANDREA, C., KARLSSON, M., LUNDIN, P., SOMESFALEAN, G. & SVANBERG, S. 2013. Diffuse optical techniques applied to wood characterisation. *J. Near Infrared Spectrosc.,* 21(4)**,** 259-268.

BIERMANN, C. J. 1993. *Essentials of Pulping and Papermaking,* San Diego, Academic Press Inc.

BLANCHETTE, R. A., KRUEGER, E. W., HAIGHT, J. E., AKHTAR, M. & AKIN, D. E. 1997. Cell wall alterations in loblolly pine wood decayed by the white-rot fungus, Ceriporiopsis subvermispora. *J. Biotechnol.,* 53(2,3)**,** 203-213.

BOEHM, R. 1930. The Masonite Process. *Ind. Eng. Chem.,* 22(5)**,** 493-497.

BOOKER, R. E. & SELL, J. 1998. The nanostructure of the cell wall of softwoods and its functions in a living tree. *Holz als Roh- und Werkstoff, Springer-Verlag,* 56**,** 1-8.

BORREGAARD. 2014. *Borregaard - Products* [Online: www.borregaard.com]. Sarpsborg: Borregaard. [Accessed 2014-03-27].

BOZELL, J. J. 2010. Fractionation in the biorefinery. *Bioresources,* 5(3)**,** 1326-1327.

BRASCH, B. J. & FREE, K. W. 1965. Prehydrolysis-kraft pulping of Pinus radiata grown in New Zealand. *Tappi,* 48(4)**,** 245-248.

BRELID, H. 2002. Chip leaching before cooking. 7th International Conference on new available technologies, The World Pulp and Paper Week, June 4-6, Stockholm. SPCI, pp. 17-21.

BROWNELL, H. H. & SADDLER, J. N. 1987. Steam pretreatment of lignocellulosic material for enhanced enzymatic hydrolysis. *Biotechnol Bioeng,* 29(2)**,** 228-35.

BRUNOW, G. & LUNDQUIST, K. 2010. Chapter 7. Functional Groups and Bonding Patternes in Lignin (Including the Lignin-Carbohydrate Complexes). *In:* HEITNER, C., DIMMEL, D. R. & SCHMIDT, J. A. (eds.) *Lignin and lignans - Advances in Chemistry.* CRC Press.

CAPRETTI, G. & FOCHER, B. 1988. Cell components of steam exploded poplar wood: Morphological and structural characterization. *In:* FOCHER, B., MARZETTI, A. & CRESCENZI, V. (eds.) *Steam Explosion Techniques, Fundamentals and Applications*, Milan, Italy. Gordon and Breach Science Publishers.

CARRASCO, J. E., SÁIZ, M. C., NAVARRO, A., SORIANO, P., SÁEZ, F. & MARTINEZ, J. M. 1994. Effects of dilute acid and steam explosion pretreatments on the cellulose structure and kinetics of cellulosic fraction hydrolysis by dilute acids in lignocellulosic materials. *Appl. Biochem. Biotech.,* 45/46**,** 23-34.

CARREIRA, H. J. M., LOUREIRO, P. E. G., CARVALHO, M. G. V. S. & EVTUGUIN, D. V. 2012. Reductive degradation of residual chromophores in kraft pulp with sodium dithionite. *Tappi J.,* 11(3)**,** 59-67.

CARVALHEIRO, F., DUARTE, L. C. & GIRIO, F. M. 2008. Hemicellulose biorefineries: a review on biomass pretreatments. *J. Sci. Ind. Res.,* 67(11)**,** 849-864.

CHIRAT, C., LACHENAL, D. & DUFRESNE, A. 2010. Biorefinery in a kraft pulp mill: from bioethanol to cellulose nanocrystals. *Cellul. Chem. Technol.,* 44(1-3)**,** 59-64.

CÖPÜR, Y. & TOZLUOGLU, A. 2007. The effect of AQ and $NaBH_4$ on bio-kraft delignification (*Ceriporiopsis subvermispora*) of brutia pine chips. *Int. Biodeter. Biodegr.,* 60(2)**,** 126-131.

CULLIS, I. F., SADDLER, J. N. & MANSFIELD, S. D. 2004. Effect of initial moisture content and chip size on the bioconversion efficiency of softwood lignocellulosics. *Biotechnol. Bioeng.,* 85(4)**,** 413-421.

DEMIRBAS, A. 2009. Biorefineries: Current activities and future developments. *Energy Convers. Manage.,* 50(11)**,** 2782-2801.

DENCE, C. W. & REEVE, D. W. (eds.) 1996. *Pulp bleaching: principles and practice,* Atlanta, GA, USA: Tappi Press.

DOMSJÖ. 2014. *History - Domsjö frabriker* [Online: www.domsjo.adityabirla.com]. Örnsköldsvik: Domsjö Fabriker AB. [Accessed 2014-03-27].

DONALDSON, L. A., WONG, K. K. Y. & MACKIE, K. L. 1988. Ultrastructure of steam-exploded wood. *Wood Sci. Technol.,* 22(2)**,** 103-114.

DU, X., GELLERSTEDT, G. & LI, J. 2013. Universal fractionation of lignin-carbohydrate complexes (LCCs) from lignocellulosic biomass: an example using spruce wood. *Plant J.,* 74(2)**,** 328-338.

EBRINGEROVA, A., HROMADKOVA, Z. & HEINZE, T. 2005. Hemicellulose. *Adv. Polym. Sci.,* 186**,** 1-67.

EDLUND, U. & ALBERTSSON, A. C. 2008. A microspheric system: hemicellulose-based hydrogels. *J. Bioact. Compat. Polym.,* 23(2)**,** 171-186.

ELANDER, R. T., DALE, B. E., HOLTZAPPLE, M., LADISCH, M. R., LEE, Y. Y., MITCHINSON, C., SADDLER, J. N. & WYMAN, C. E. 2009. Summary of findings from the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI): corn stover pretreatment. *Cellulose (Dordrecht, Neth.),* 16(4)**,** 649-659.

ELLIS, M. E. 1996. The technology of mechanical pulp bleaching. Hydrosulfite (dithionite) bleaching. *In:* DENCE, C. W., REEVE, D. W. (eds.) *Pulp bleaching*, Atlanta, GA, USA, Tappi Press, 491-512.

EXCOFFIER, G., PEGUY, A., RINAUDO, M. & VIGNON, M. R. 1988. Evolution of lignocellulosic components during steam explosion. Potential applications. *In:* FOCHER, B., MARZETTI, A. & CRESCENZI, V. (eds.) *Steam Explosion Techniques, Fundamentals and Applications*, Milan, Italy. Gordon and Breach Science Publishers.

FATEHI, P. 2013. Recent advancements in various steps of ethanol, butanol, and isobutanol productions from woody materials. *Biotechnol. Prog.,* 29(2)**,** 297-310.

FATIH, D. M. 2009. Biorefineries for biofuel upgrading: A critical review. *Appl. Energy,* 86(suppl. 1)**,** S151-S161.

FENGEL, D. & WEGENER, G. 1989. *Wood - Chemistry, Ultrastructure, Reactions,* Berlin, Walter de Gruyter.

FERNANDO, S., ADHIKARI, S., CHANDRAPAL, C. & MURALI, N. 2006. Biorefineries: Current Status, Challenges, and Future Direction. *Energ. Fuel.,* 20(4)**,** 1727-1737.

FOGARTY, W. M. & WARD, O. P. 1972. Pectic substances and pectinolytic enzymes. *Process Biochem.,* 7(7)**,** 13-17.

FRANZON, O. & SAMUELSON, O. 1957. Degradation of Cellulose by Alkali Cooking. *Sven. Papperstidn.,* 60(23)**,** 872-877.

GATENHOLM, P., BODIN, A., GROENDAHL, M., DAMMSTROEM, S. & ERIKSSON, L. 2004. *Polymeric film or coating comprising hemicellulose.* PCT Int. Appl. WO 2004083286.

GELLERSTEDT, G., SJOEHOLM, E. & BRODIN, I. 2010. The wood-based biorefinery: a source of carbon fiber? *Open Agric. J.,* 4**,** 119-124.

GIERER, J. 1980. Chemical aspects of kraft pulping. *Wood Sci. Technol.,* 14(4)**,** 241-66.

GIERER, J. 1997. Formation and involvement of superoxide ($O_2^{\cdot-}/HO_2^{\cdot}$) and hydroxyl ($OH^{\cdot}$) radicals in TCF bleaching processes: A review. *Holzforschung,* 51(1)**,** 34-46.

GIERTZ, H. W. & MCPHERSON, J. 1956. Brightness reversion of bleached pulps. *Sven. Papperstidn.,* 59(3)**,** 93-97.

GLASSER, W. G. & WRIGHT, R. S. 1998. Steam-assisted biomass fractionation. II. Fractionation behavior of various biomass resources. *Biomass Bioenerg.,* 14(3)**,** 219-235.

GNANSOUNOU, E. & DAURIAT, A. 2005. Ethanol fuel from biomass: a review. *J. Sci. Ind. Res.,* 64(11)**,** 809-821.

GRETHLEIN, H. E. & CONVERSE, A. O. 1991. Common aspects of acid prehydrolysis and steam explosion for pretreating wood. *Bioresource Technol.,* 36(1)**,** 77-82.

GROUS, W. R., CONVERSE, A. O. & GRETHLEIN, H. E. 1986. Effect of steam explosion pretreatment on pore size and enzymic hydrolysis of poplar. *Enzyme Microb. Technol.,* 8(5)**,** 274-280.

HAMELINCK, C. N., VAN HOOIJDONK, G. & FAAIJ, A. P. C. 2005. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. *Biomass Bioenerg.,* 28(4)**,** 384-410.

HANNUKSELA, T. & HERVE, D. P. C. 2004. NMR structural determination of dissolved O-acetylated galactoglucomannan isolated from spruce thermomechanical pulp. *Carbohydr. Res.,* 339(2)**,** 301-312.

HARTLER, N. 1959. Sulphate Cooking with the Addition of Reducing Agents - Part 1. Preliminary report on the addition of sodium borohydride. *Sven. Papperstidn.,* 62(13)**,** 467-470.

HEITZ, M., CAPEK-MÉNARD, E., KOEBERLE, P. G., GAGNÉ, J., CHORNET, E., OVEREND, R. P., TAYLOR, J. D. & YU, E. 1991. Fractionation of Populus tremuloides at the pilot plant scale: Optimization of steam pretreatment conditions using the STAKE II technology. *Bioresource Technol.,* 35(1)**,** 23-32.

HENRIKSSON, G., BRÄNNVALL, E. & LENNHOLM, H. 2004. *Chapter 2 The Trees, Wood Chemistry, The Ljungberg Textbook,* Stockholm, Department of Pulp and Paper Chemistry and Technology, KTH.

HUANG, H.-J., RAMASWAMY, S., AL-DAJANI, W. W. & TSCHIRNER, U. 2010. Process modeling and analysis of pulp mill-based integrated biorefinery with hemicellulose pre-extraction for ethanol production: a comparative study. *Bioresource Technol.,* 101(2)**,** 624-631.

HULTHOLM, T. 2004. *Impregnation behaviour of the active ions in the kraft process.* Doctorial, Åbo Akademi University, Åbo, Finland.

IBRAHIM, M. M., AGBLEVOR, F. A. & EL-ZAWAWY, W. K. 2010. Isolation and characterization of cellulose and lignin from steam-exploded lignocellulosic biomass. *Bioresources,* 5(1)**,** 397-418.

JAIN, R. K., IBRAHIM, M., WRIGHT, R. S. & GLASSER, W. G. 1999. Biopolymers by steam explosion/fractionation: Cellulose, lignin, xylan and derivatives. Book of Abstracts, 218th ACS National Meeting, Aug. 22-26, New Orleans. American Chemical Society, Washington D.C.

JANSON, J. 1974. Analytik der Polysaccharide in Holz und Zellstoff. *Faserforschung und Textiltechnik,* 25(9)**,** 375.

JANZON, R., SCHUETT, F., OLDENBURG, S., FISCHER, E., KOERNER, I. & SAAKE, B. 2014. Steam pretreatment of spruce forest residues: Optimal conditions for biogas production and enzymatic hydrolysis. *Carbohydr. Polym.,* 100**,** 202-210.

JEFFRIES, T. W. 1990. Biodegradation of lignin-carbohydrate complexes. *Biodegradation,* 1(2-3)**,** 163-76.

JOSEFSSON, T., LENNHOLM, H. & GELLERSTEDT, G. 2002. Steam explosion of aspen wood. Characterisation of reaction products. *Holzforschung,* 56(3)**,** 289-297.

KALLAVUS, U. & GRAVITIS, J. 2009. A Comparative Investigation of the Ultrastructure of Steam Exploded Wood With Light, Scanning and Transmission Electron Microscopy. *Holzforschung,* 49(2)**,** 182-188.

KAMIDE, K. (ed.) 2005. *Cellulose and Cellulose Derivatives - Molecular Characterization and its Applications,* Amsterdam, The Netherlands: Elsevier.

KAMM, B., GRUBER, P. R. & KAMM, M. (eds.) 2006. *Part 1. Background and Outline - Principles and Fundamentals,* Weinheim, Germany: Wiley-VCH.

KAMM, B. & KAMM, M. 2004. Principles of biorefineries. *Appl. Microbiol. Biotechnol.,* 64(2)**,** 137-145.

KLEMM, D., HEUBLEIN, B., FINK, H.-P. & BOHN, A. 2005. Cellulose: Fascinating biopolymer and sustainable raw material. *Angew. Chem., Int. Ed.,* 44(22)**,** 3358-3393.

KLEPPE, P. J. 1970. Kraft pulping. *Tappi,* 53(1)**,** 35-47.

KOKTA, B. & AHMED, A. 1998. Steam explosion pulping. *In:* YOUNG, R. A., AKHTAR, M. (ed.) *Environmentally Friendly Technologies for the Pulp and Paper Industry.* Madison: John Wiley & Sons, Inc.

KOMATSU, T. & KIKUCHI, J. 2013. Comprehensive Signal Assignment of 13C-Labeled Lignocellulose Using Multidimensional Solution NMR and 13C Chemical Shift Comparison with Solid-State NMR. *Anal. Chem. (Washington, DC, U. S.),* 85(18)**,** 8857-8865.

LANGAN, P., PETRIDIS, L., O'NEILL, H. M., PINGALI, S. V., FOSTON, M., NISHIYAMA, Y., SCHULZ, R., LINDNER, B., HANSON, B. L., HARTON, S., HELLER, W. T., URBAN, V., EVANS, B. R., GNANAKARAN, S., RAGAUSKAS, A. J., SMITH, J. C. & DAVISON, B. H. 2014. Common processes drive the thermochemical pretreatment of lignocellulosic biomass. *Green Chem.,* 16(1)**,** 63-68.

LAW, K. N. & VALADE, J. L. 1990. The myth of fiber liberation during explosion pulping. *J. Pulp Pap. Sci.,* 16(1)**,** 41-42.

LAWOKO, M., HENRIKSSON, G. & GELLERSTEDT, G. 2006. Characterization of lignin-carbohydrate complexes (LCCs) of spruce wood (Picea abies L.) isolated with two methods. *Holzforschung,* 60(2)**,** 156-161.

LESKINEN, T., KING, A. W. T., KILPELAINEN, I. & ARGYROPOULOS, D. S. 2011. Fractionation of Lignocellulosic Materials with Ionic Liquids. 1. Effect of Mechanical Treatment. *Ind. Eng. Chem. Res.,* 50(22)**,** 12349-12357.

LI, H., SAEED, A., JAHAN, M. S., NI, Y. & VAN HEININGEN, A. 2010. Hemicellulose Removal from Hardwood Chips in the Pre-Hydrolysis Step of the Kraft-Based Dissolving Pulp Production Process. *J. Wood Chem. Technol.,* 30(1)**,** 48-60.

LI, J., GELLERSTEDT, G. & TOVEN, K. 2009. Steam explosion lignins; their extraction, structure and potential as feedstock for biodiesel and chemicals. *Bioresource Technol.,* 100(9)**,** 2556-2561.

LI, J., HENRIKSSON, G. & GELLERSTEDT, G. 2005. Carbohydrate reactions during high-temperature steam treatment of aspen wood. *Appl. Biochem. Biotechnol.,* 125(3)**,** 175-188.

LIN, S. Y. & DENCE, C. W. 1992. The Determination of Lignin. *Methods in Lignin Chemistry.* Berlin Heidelberg, Germany: Springer-Verlag.

LINDSTRÖM, M. & TEDER, A. 1995. The effect of polysulfide treatment when kraft pulping to very low kappa numbers. *Nord. Pulp Pap. Res. J.,* 10(1)**,** 8-11.

LIU, H., FU, S., ZHU, J. Y., LI, H. & ZHAN, H. 2009. Visualization of enzymatic hydrolysis of cellulose using AFM phase imaging. *Enzyme Microb. Technol.,* 45(4)**,** 274-281.

LIU, Z., FATEHI, P. & NI, Y. 2011. Isolation of hemicelluloses from the pre-hydrolysis of kraft pulping method using two alternative approaches. 16th International Symposium on Wood, Fiber and Pulping Chemistry, June 8-10, Tianjin, China. 813-816.

LJUNGGREN, S. 1980. The significance of aryl ether cleavage in kraft delignification of softwood. *Sven. Papperstidn.,* 83(13)**,** 363-369.

LUNDQVIST, J., JACOBS, A., PALM, M., ZACCHI, G., DAHLMAN, O. & STALBRAND, H. 2002. Characterization of galactoglucomannan extracted from spruce (Picea abies) by heat-fractionation at different conditions. *Carbohydr. Polym.,* 51(2)**,** 203-211.

LUO, L., VAN, D. V. E. & HUPPES, G. 2010. Biorefining of lignocellulosic feedstock--Technical, economic and environmental considerations. *Bioresource Technol.,* 101(13)**,** 5023-5032.

MABEE, W. E., GREGG, D. J., ARATO, C., BERLIN, A., BURA, R., GILKES, N., MIROCHNIK, O., PAN, X., PYE, E. K. & SADDLER, J. N. 2006. Updates on softwood-to-ethanol process development. *Appl. Biochem. Biotech.,* 129-132**,** 55-70.

MALKOV, S., TIKKA, P., KUZMIN, V. & BALTAKHINOV, V. 2002. Efficiency of chip presteaming -- result of heating and air escape processes. *Nord. Pulp Pap. Res. J.,* 17(4)**,** 420-426.

MARCHESSAULT, R. H. 1988. Steam Explosion: A Refining Process for Lignocellulosics. *In:* FOCHER, B., MARZETTI, A. & CRESCENZI, V. (eds.) *Steam Explosion Techniques, Fundamentals and Applications*, Milan, Italy. Gordon and Breach Science Publishers.

MARTIN-SAMPEDRO, R., EUGENIO, M. E., REVILLA, E., MARTIN, J. A. & VILLAR, J. C. 2011. Integration of kraft pulping on a forest biorefinery by the addition of a steam explosion pretreatment. *BioResources,* 6(1)**,** 513-528.

MARTIN, R. S., PEREZ, C. & BRIONES, R. 1995. Simultaneous production of ethanol and Kraft pulp from pine (*Pinus radiata*) using steam explosion. *Bioresource Technol.,* 53(3)**,** 217-223.

MASON, W. H. 1928. *Process and apparatus for disintegration of fibrous material.* USA patent application: US1824221.

MATTHEWS, C. H. 1974. Carbohydrate losses at high temperature in kraft pulping. *Sven. Papperstidn.,* 77(17)**,** 629-635.

MCPHERSON, J. A. 1958. The fractionation of spruce wood. *Acta Chem. Scand.,* 12(4)**,** 779-780.

MEIER, H. 1958. Barium Hydroxide as a Selective Precipitating Agent for Hemicelluloses. *Acta Chem. Scand.,* 12(1)**,** 144-146.

MELLER, A. 1953. Reduction of Chemically Modified Cellulose by Sodium Borohydride. *Tappi J.,* 36(8)**,** 366-367.

MIKKONEN, K. S., HEIKKILAE, M. I., HELEN, H., HYVOENEN, L. & TENKANEN, M. 2010. Spruce galactoglucomannan films show promising barrier properties. *Carbohydr. Polym.,* 79(4)**,** 1107-1112.

MILLER, G. L. 1959. Dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.,* 31**,** 426-428.

MONTANÉ, D., FARRIOL, X. & SALVADÓ, J. 1998. Fractionation of wheat straw by steam-explosion pretreatment and alkali delignification. Cellulose pulp and byproducts from hemicellulose and lignin. *J. Wood Chem. Technol.,* 18(2)**,** 171-191.

MOREIRA, L. & FILHO, E. 2008. An overview of mannan structure and mannan-degrading enzyme systems. *Appl. Microbiol. Biotechnol.,* 79(2)**,** 165-178.

MOURA, M. J., FERREIRA, P. J. & FIGUEIREDO, M. M. 2005. Mercury intrusion porosimetry in pulp and paper technology. *Powder Technol.,* 160(2)**,** 61-66.

NAES, T., ISAKSSON, T., FEARN, T. & DAVIES, T. 2002. *A User-friendly Guide to Multivariate Calibration and Classification,* Chichester, UK, NIR Publications.

NOGUEIRA, J. M. F. 1996. Refining and separation of crude tall-oil components. *Sep. Sci. Technol.,* 31(17)**,** 2307-2316.

O'SULLIVAN, A. C. 1997. Cellulose: the structure slowly unravels. *Cellulose,* 4(3)**,** 173-207.

ÖHMAN, F., WALLMO, H. & THELIANDER, H. 2007. A novel method for washing lignin precipitated from kraft black liquor - laboratory trials. *Nord. Pulp Pap. Res. J.,* 22(1)**,** 9-16.

OVEREND, R. P. & CHORNET, E. 1987. Fractionation of lignocellulosics by steam-aqueous pretreatments. *Phil. Trans. R. Soc. Lond. A,* 321(1561)**,** 523-536.

PALM, M. & ZACCHI, G. 2003. Extraction of Hemicellulosic Oligosaccharides from Spruce Using Microwave Oven or Steam Treatment. *Biomacromolecules,* 4(3)**,** 617-623.

PARAWIRA, W. & TEKERE, M. 2011. Biotechnological strategies to overcome inhibitors in lignocellulose hydrolysates for ethanol production: review. *Crit. Rev. Biotechnol.,* 31(1)**,** 20-31.

PEKKALA, O. & PALENIUS, I. 1973. Hydrogen sulfide pre-treatment in alkaline pulping. *Paperi ja Puu,* 55(9)**,** 659-662.

PEREZ, D. D. S., GUILLEMAIN, A., JANODET, A., SORANZO, A., SCHELCHER, M., HUBER, P. & PETIT-CONIL, M. 2011. Softwoods hemicelluloses: Impact of the extraction conditions and wood species on their chemical compositiion. 16th International Symposium on Wood, Fiber and Pulping Chemistry, June 8-10, Tianjin, China. 1069-1074.

PETERS, R. 2000. *Woodworker's Guide to Wood*, Sterling Publishing Company.

PETTERSEN, R. C. (ed.) 1984. *The Chemistry of Solid Wood: Ch. 2. The chemical composition of wood,* Madison: American Chemical Society.

PEW, J. C. & CONNORS, W. J. 1971. Color of coniferous lignin. *Tappi,* 54(2)**,** 245-51.

PFRIEM, A., ZAUER, M. & WAGENFUEHR, A. 2009. Alteration of the pore structure of spruce (Picea abies (L.) Karst.) and maple (Acer pseudoplatanus L.) due to thermal treatment as determined by helium pycnometry and mercury intrusion porosimetry. *Holzforschung,* 63(1)**,** 94-98.

RAGAUSKAS, A. J., WILLIAMS, C. K., DAVIDSON, B. H., BRITOVSEK, G., CAIRNEY, J., ECKERT, C. A., JR., W. J. F., HALLETT, J. P., LEAK, D. J., LIOTTA, C. L., MIELENZ, J. R., MURPHY, R., TEMPLER, R. & TSCHAPLINSKI, T. 2006. The Path Forward for Biofuels and Biomaterials. *Science,* 311(5760)**,** 484-489.

RIBE, E., SÖDERQVIST-LINDBLAD, M., DAHLMAN, O. & THELIANDER, H. 2010. Xylan sorption kinetics at industrial conditions - Part 1 Experimental results. *Nord. Pulp Pap. Res. J.,* 25(2)**,** 138-149.

RICHTZENHAIN, V. H., LINDGREN, B. O., ABRAHAMSSON, B. & HOLMBERG, K. 1954. Über den alkalischen Abbau von Polysacchariden. *Sven. Papperstidn.,* 57(10)**,** 363-366.

ROWELL, R. M. (ed.) 2005. *Handbook of wood chemistry and wood composites*: Boca Raton, London, New York, Singapore, Taylor & Francis: CRC Press.

RYDHOLM, S. A. 1965. *Pulping Processes,* New York, London, Sydney, Interscience Publishers, John Wiley & Sons Inc.

SADDLER, J. N., BROWNELL, H. H., CLERMONT, L. P. & LEVITIN, N. 1982. Enzymatic hydrolysis of cellulose and various pretreated wood fractions. *Biotechnol. Bioeng.,* 24(6)**,** 1389-1402.

SAMUEL, R., CAO, S., DAS, B. K., HU, F., PU, Y. & RAGAUSKAS, A. J. 2013. Investigation of the fate of poplar lignin during autohydrolysis pretreatment to understand the biomass recalcitrance. *RSC Adv.,* 3(16)**,** 5305-5309.

SATHITSUKSANOH, N., ZHU, Z., ROLLIN, J. & ZHANG, Y. H. P. 2010. Solvent fractionation of lignocellulosic biomass. *Woodhead Publ. Ser. Energy,* 3**,** 122-140.

SAVITZKY, A. & GOLAY, M. J. E. 1964. Smoothing and differentiation of data by simplified least squares procedures. *Anal. Chem.,* 36(8)**,** 1627-1639.

SCHELL, D. J., TORGET, R., POWER, A., WALTER, P. J., GROHMANN, K. & HINMAN, N. D. 1991. A technical and economic analysis of acid-catalyzed steam explosion and dilute sulfuric acid pretreatments using wheat straw or aspen wood chips. *Appl. Biochem. Biotech.,* 28/29**,** 87-97.

SCHUETT, F., WESTERENG, B., HORN, S. J., PULS, J. & SAAKE, B. 2012. Steam refining as an alternative to steam explosion. *Bioresource Technol.,* 111**,** 476-481.

SCHULTZ, T. P., RUGHANI, J. R. & MCGINNIS, G. D. 1989. Comparison of the pretreatment of sweetgum and white oak by the steam explosion and RASH processes. *Appl. Biochem. Biotech.,* 20/21**,** 9-27.

SCHULTZ, T. P., TEMPLETON, M. C., BIERMANN, C. J. & MCGINNIS, G. D. 1984. Steam explosion of mixed hardwood chips, rice hulls, corn stalks and sugar cane bagasse. *J. Agric. Food Chem.,* 32(5)**,** 1166-1172.

SENTHILKUMAR, V. & GUNASEKARAN, P. 2005. Bioethanol production from cellulosic substrates: engineered bacteria and process integration challenges. *J. Sci. Ind. Res.,* 64(11)**,** 845-853.

SHEVCHENKO, S. M., BEATSON, R. P. & SADDLER, J. N. 1999. The nature of lignin from steam explosion/ enzymatic hydrolysis of softwood. *Appl. Biochem. Biotech.,* 77-79, 867-876.

SHEVCHENKO, S. M., CHANG, K., ROBINSON, J. & SADDLER, J. N. 2000. Optimization of monosaccharide recovery by post-hydrolysis of the water-soluble hemicellulose component after steam explosion of softwood chips. *Bioresource Technol.,* 72(3)**,** 207-211.

SHIMIZU, K., SUDO, K., ONO, H., ISHIHARA, M., FUJII, T. & HISHIYAMA, S. 1998. Integrated process for total utilization of wood components by steam-explosion pretreatment. *Biomass Bioenerg.,* 14(3)**,** 195-203.

SIXTA, H. (ed.) 2006. *Handbook of Pulp,* Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA.

SJÖSTRÖM, E. 1993. *Wood chemistry - Fundamentals and Applications,* San Diego, Academic Press.

SKOGSINDUSTRIERNA 2013. Skogsindustrin - En faktasamling. *In:* LUNDBERG, C. (ed.) *2012 års branschstatistik*. Växjö: Grafiska Punkten.

SLU. 2013. *Riksskogstaxeringen - Virkesförrådet* [Online: www.slu.se/Documents/externwebben/webbtjanster/statistik-om-skog/Tabeller/0812/Tab24.pdf]. Sveriges lantbruksuniversitet [Accessed 2013-07-17].

SÖDERQVIST-LINDBLAD, M., RANUCCI, E. & ALBERTSSON, A.-C. 2001. Biodegradable Polymers from Renewable Sources. New Hemicellulose-Based Hydrogels. *Macromol. Rapid Comm.,* 22(12)**,** 962-967.

SÖDERSTRÖM, J., PILCHER, L., GALBE, M. & ZACCHI, G. 2003. Two-step steam pretreatment of softwood by dilute H_2SO_4 impregnation for ethanol production. *Biomass Bioenerg.,* 24(6)**,** 475-486.

SÖDRA. 2014. *Södra-Textilmassa* [Online: www.sodra.com]. Södra. [Accessed 2014-03-27].

SONG, T., PRANOVICH, A. & HOLMBOM, B. 2011a. Characterisation of Norway spruce hemicelluloses extracted by pressurised hot-water extraction (ASE) in the presence of sodium bicarbonate. *Holzforschung,* 65(1)**,** 35-42.

SONG, T., PRANOVICH, A. & HOLMBOM, B. 2011b. Effects of pH control with phtalate buffers on hot-water extraction of hemicelluloses from spruce wood. 16th International Symposium of Wood, Fiber and Pulping Chemistry, June 8-10, Tianjin, China. 408-412.

SONG, T., PRANOVICH, A., SUMERSKIY, I. & HOLMBOM, B. 2008. Extraction of galactoglucomannan from spruce wood with pressurised hot water. *Holzforschung,* 62(6)**,** 659-666.

SUN, Y. & CHENG, J. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technol.,* 83(1)**,** 1-11.

SUNDBERG, A., SUNDBERG, K., LILLANDT, C. & HOLMBOM, B. 1996. Determination of hemicelluloses and pectins in wood and pulp fibers by acid methanolysis and gas chromatography. *Nord. Pulp Pap. Res. J.,* 11(4)**,** 216-219, 226.

TANAHASHI, M. 1990. Characterization and degradation mechanisms of wood components by steam explosion and utilization of exploded wood. *Wood Research,* 77**,** 49-117.

THEANDER, O. & WESTERLUND, E. A. 1986. Studies on Dietary Fiber. 3. Improved Procedures for Analysis of Dietary Fiber. *J. Agric. Food Chem.,* 34(2)**,** 330-336.

THELIANDER, H. 2008. Withdrawing lignin from black liquor by precipitation, filtration and washing. NWBC, March 11-14, Stockholm, pp. 36-42.

TIMELL, T. E. 1967. Recent Progress in the Chemistry of Wood Hemicelluloses. *Wood Sci. Technol.,* 1(1)**,** 45-70.

TOFIGHI, A., AZIN, M., MAZAHERI ASSADI, M., ASSADI-RAD, M. H. A., NEJADSATTARI, T. & FALLAHIAN, M. R. 2010. Inhibitory effect of high concentrations of furfural on industrial strain of Saccharomyces cerevisiae. *Int. J. Environ. Res.,* 4(1)**,** 137-142.

VAN HEININGEN, A. 2006. Converting a kraft pulp mill into an integrated forest biorefinery. *Pulp Pap. Can.,* 107(6)**,** 38-43.

VARSHNEY, A. K. & PATEL, D. P. 1989. Biomass delignification-organosolv approach. *J. Sci. Ind. Res.,* 47(6)**,** 315-319.

VENEMARK, E. 1964. Some Ideas on Polysulfide Pulping *Sven. Papperstidn.,* 67(5)**,** 157-166.

VIOLA, E., ZIMBARDI, F., CARDINALE, M., CARDINALE, G., BRACCIO, G. & GAMBACORTA, E. 2008. Processing cereal straws by steam explosion in a pilot plant to enhance digestibility in ruminants. *Bioresource Technol.,* 99(4)**,** 681-689.

VIT, R. & KOKTA, B. V. 1986. *Method and apparatus for converting wood, wood residue, vegetable fiber and biomass into pulp*. Eur. Pat. Appl., EP 1985-810326

WASHBURN, E. W. 1921. Note on a Method of Determining the Distribution of Pore Sizes in a Porous Material. *Proc Natl Acad Sci U S A,* 7(4)**,** 115-116.

WIGELL, A., BRELID, H. & THELIANDER, H. 2007. Degradation/dissolution of softwood hemicellulose during alkaline cooking at different temperatures and alkali concentrations. *Nord. Pulp Pap. Res. J.,* 22(4)**,** 488-494.

WILLFÖR, S., SJOHOLM, R., LAINE, C., ROSLUND, M., HEMMING, J. & HOLMBOM, B. 2003. Characterisation of water-soluble galactoglucomannans from Norway spruce wood and thermomechanical pulp. *Carbohydr. Polym.,* 52(2)**,** 175-187.

WILLFÖR, S., SUNDBERG, K., TENKANEN, M. & HOLMBOM, B. 2008. Spruce-derived mannans - A potential raw material for hydrocolloids and novel advanced natural materials. *Carbohydr. Polym.,* 72(2)**,** 197-210.

WILSON, K. 1968. Bestämning av effektivt alkali i svartlut. *Sven. Papperstidn.,* 71(11)**,** 446-447.

XUE, B.-L., WEN, J.-L., XU, F. & SUN, R.-C. 2012. Structural characterization of hemicelluloses fractionated by graded ethanol precipitation from Pinus yunnanensis. *Carbohydr. Res.,* 352**,** 159-165.

YLLNER, S. & ENSTRÖM, B. 1956. Studies on the Adsorption of Xylan on Cellulose Fibres During the Sulphate Cook. Part 1. *Sven. Papperstidn.,* 59(6)**,** 229-232.

YOUNG, J. 1992. Commercial Organocell process comes online at Kelheim mill. *Pulp Pap.,* 66(9)**,** 99-102.

YUAN, T.-Q., SUN, S.-N., XU, F. & SUN, R.-C. 2011. Characterization of Lignin Structures and Lignin-Carbohydrate Complex (LCC) Linkages by Quantitative 13C and 2D HSQC NMR Spectroscopy. *J. Agric. Food Chem.,* 59(19)**,** 10604-10614.

ZHANG, Y. & CAI, L. 2006. Effects of steam explosion on wood appearance and structure of sub-alpine fir. *Wood Sci. Technol.,* 40(5)**,** 427-436.

ZHU, R. Y. Z., EDLUND, U. & ALBERTSSON, A. C. 2011. Conceptual approach to renewable barrier film design based on wood hydrolysate. *Biomacromolecules,* 12(4)**,** 1355-1362.

Appendix

A. Chemicals

The sodium borohydride pre-treatments in Papers I and V used $NaBH_4$ (98% powder, Alfa Aesar).

The enzymes used in Paper II was a cellulolytic culture filtrate, Novozym 342, which is a mixture of cellulases and hemicellulases containing mainly endoglucanase, exoglucanase and β-glucosidase (Liu et al., 2009). The original enzymatic activity was 90 ECU/mL. The DNS reagent solution for measuring the content of the reducing sugars contained 1% dinitrosalicylic acid, 1% NaOH, 10% NaKtartate, 0.05% Na_2SO_3 and 0.2% phenol dissolved in water.

The cooking chemicals in Papers II, III and VI were sodium hydroxide, NaOH, (reagent grade) and sodium sulphide, NaS_2 (analytic grade). In Papers II-III, sodium carbonate Na_2CO_3 (analytic grade) was also used. The chemicals for the oxygen delignification were NaOH, $MgSO_4$ and oxygen gas. For the dithionite impregnations in Paper VI, $Na_2S_2O_4$ (85%) was used.

The chemicals used for preparation and analysis of the samples in Paper V were anhydrous methanol, acetyl chloride, pyridine, chlorotrimethylsilane (TMCS) and hexamethyldisilazane (HMDS), all of analytical grade. For GC-MS standards, D-Sorbitol (99%) was used as the internal standard, and methyl β-D-xylopyranoside (>99%) and synthesized glucose, galactose and mannose for peak identification.

The pullulan standards for the DMSO-GPC (Papers I and V) were prepared from a Pullulan Polysaccharide Calibration Kit SAC-10 (by Varian). The acetate standards for IC (Paper V) were prepared from "Acetate std for IC", Fluka Analytical, Sigma-Aldrich.

B. Analysis of carbohydrates

The contents of cellulose, (galacto)glucomannan (GGM) and arbinoglucuronoxylan (AGX) were calculated after carbohydrate analysis had been carried out using the following assumptions/corrections:

The amounts of sugars analyzed were corrected for the acid hydrolysis yield. Anhydro sugars were calculated from sugar monomers by the withdrawal of water, i.e. multiplication by 0.88 in the case of pentosans and 0.90 in the case of hexosans. GGM was calculated as the sum of galactan, mannan and part of the glucan. The molar ratio between the mannose and the glucose in GGM was assumed to be 3.5:1 (Meier, 1958); all galactan measured was included in the GGM. Acetyl groups were not, however, included. AGX was calculated as the sum of xylan and arabinan: all arabinan measured was included in the xylan. Cellulose was calculated as the content of glucan after withdrawal for the contribution of glucan to GGM.

Cellulose = glucose – (1/3.5) * mannose
GGM = galactose + (1 + (1/3.5)) * mannose
AGX = xylose + arabinose

C. Calibration curve: GPC

An example of a calibration curve, with pullulan of ten different molecular weights, is shown in **Fig. C1**. The molecular weights of pullulan and their retention times are shown in **Table C1**.

Table C1: The molecular weights of the pullulan standards with corresponding retention times.

Data point	Peak max at RT (min)	MW (Da)	Log MW
1	20.20	708000	5.850
2	21.12	375000	5.574
3	22.33	200000	5.301
4	23.57	107000	5.029
5	25.43	47100	4.673
6	26.88	21100	4.324
7	27.93	11100	4.045
8	29.08	5900	3.771
9	33.35	667	2.824
10	35.52	180	2.255

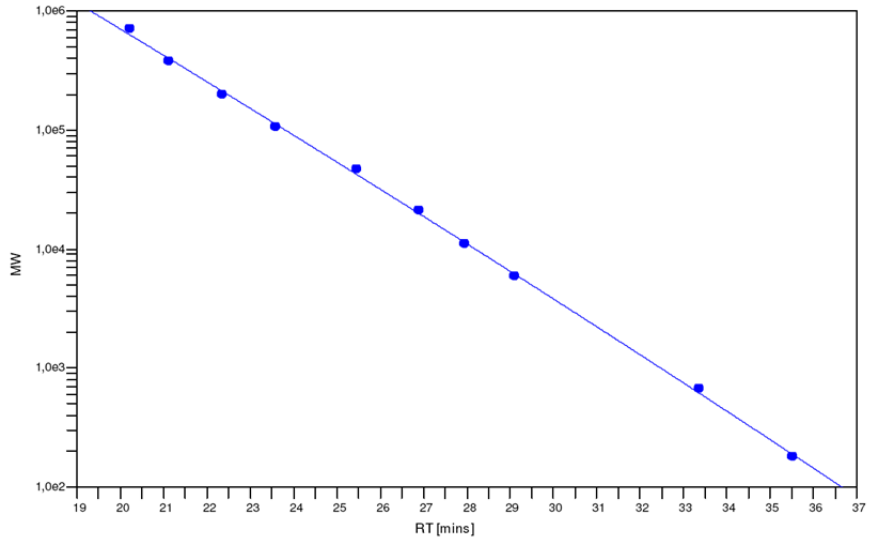

Figure C1 Calibration curve with the ten data points from the pullulan standards, with calibration curve: $10.01-0.1953x-0.0006313x^2$. The residual sum of squares was 0.008682, the coeff. of determination was 0.999298 and the linear correlation coeff. was -0.999570.

D. Calibration curve: analysis of reducing sugars

The absorbance was measured at 575 nm for all of the samples in the analysis of reducing sugar content. The absorbance values were recalculated into a content of reducing sugars using the glucose calibration curves, see **Figs. D1 and D2**.

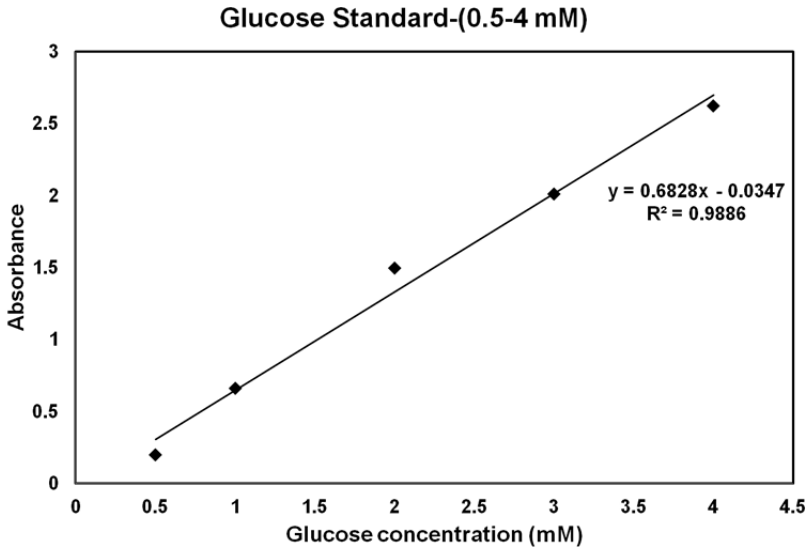

Figure D1 Calibration curve for the glucose standards with concentrations between 0.5 and 4 mM.

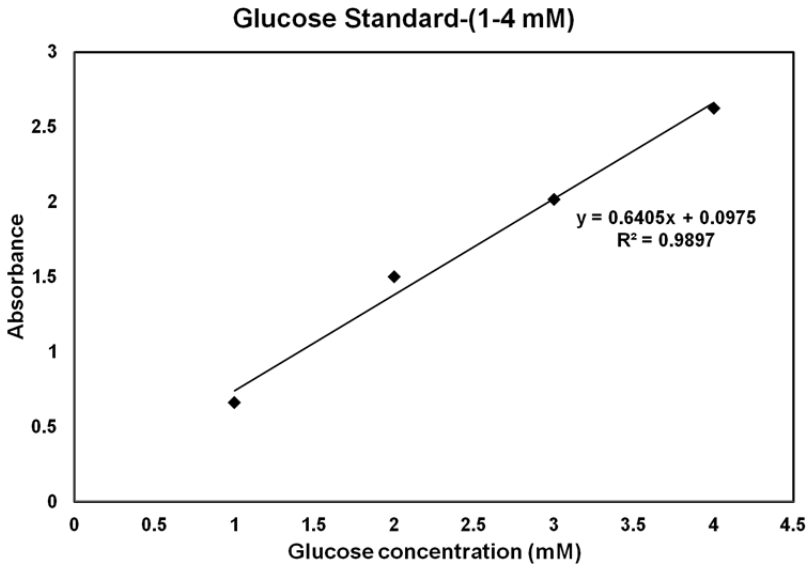

Figure D2 Calibration curve for the glucose standards with concentrations between 1 and 4 mM.

E. Chemical compositions: reference study

The results of the analyses of chemical composition, obtained after treatment, of wood powders in aqueous solutions with different levels of pH are presented in this section. The results from the alkaline treatment, for various temperatures and treatment times, are shown in **Table E1**. The results are presented as w/w% of the sample weights, and water-impregnated samples are compared to $NaBH_4$ pre-treated samples.

Table E1. Chemical composition after alkaline treatment (W = water impregnated, P = pre-treated with $NaBH_4$)

Sample	Klason lignin (%)	ASL (%)	Cellulose (%)	GGM (%)	Xylan (%)	Other (%)
Reference	28.5	0.7	40.3	16.9	5.4	8.2
W_90°C_10	26.8	0.9	39.4	17.1	5.2	10.6
W_90°C_30	28.0	0.7	42.4	16.2	5.3	7.5
W_90°C_60	28.7	0.7	43.6	14.8	5.4	6.9
W_90°C_120	30.8	1.0	43.8	12.2	5.4	6.8
W_110°C_10	30.4	0.7	41.8	17.3	5.7	4.1
W_110°C_30	29.3	0.6	45.2	12.0	5.9	6.9
W_110°C_60	30.9	0.6	45.4	9.4	5.5	8.2
W_110°C_120	30.8	0.8	48.2	8.1	5.9	6.1
W_130°C_10	30.4	0.6	39.8	15.0	5.5	8.7
W_130°C_30	30.5	0.6	46.9	8.7	5.5	7.7
W_130°C_60	31.7	0.6	47.6	7.8	5.4	6.9
W_130°C_120	29.5	0.6	47.7	7.7	5.9	8.6
P_90°C_10	28.1	0.6	40.1	18.7	5.8	6.7
P_90°C_30	27.0	0.6	40.0	18.8	5.9	7.6
P_90°C_60	27.0	0.6	40.8	19.0	5.8	6.8
P_90°C_120	27.0	0.5	39.0	18.4	5.8	9.3
P_110°C_10	27.3	0.6	41.6	18.9	5.8	5.8
P_110°C_30	26.8	0.5	41.4	18.4	5.7	7.1
P_110°C_60	26.7	0.5	40.6	17.9	5.5	8.7
P_110°C_120	26.7	0.5	40.6	18.3	5.5	8.4
P_130°C_10	27.2	0.5	41.2	18.2	5.7	7.2
P_130°C_30	26.5	0.8	39.6	18.6	5.7	8.9
P_130°C_60	26.5	0.9	42.8	18.4	5.5	6.0
P_130°C_120	25.8	0.7	44.7	18.6	5.5	4.8

The results (w/w% of sample) from the chemical composition analyses for water-impregnated samples treated at acidic conditions are shown in **Table E2**.

Table E2. Contents of the different wood components after acidic treatment (W = water impregnated)

Sample	Klason lignin (%)	ASL (%)	Cellulose (%)	GGM (%)	Xylan (%)	Other (%)
Reference	28.5	0.7	40.3	16.9	5.4	8.2
W_90°C_10	27.9	0.5	38.4	17.1	5.6	10.5
W_90°C_30	28.1	0.5	39.6	17.3	5.6	8.8
W_90°C_60	28.1	0.5	40.2	17.1	5.4	8.8
W_90°C_120	28.2	0.5	40.0	17.2	5.2	9.1
W_110°C_10	28.2	0.4	39.0	17.0	5.5	9.8
W_110°C_30	28.1	0.4	39.6	17.0	5.3	9.7
W_110°C_60	28.3	0.5	40.8	16.3	5.1	9.0
W_110°C_120	28.9	0.4	41.1	14.5	4.7	10.4
W_130°C_10	28.0	0.4	39.0	16.7	5.4	10.4
W_130°C_30	28.9	0.4	42.3	13.1	4.6	10.7
W_130°C_60	30.3	0.3	43.5	10.3	3.9	11.7
W_130°C_120	32.5	0.3	47.5	7.9	3.3	8.5

F. NIR

The NIR spectra collected for all of the samples treated in the reference study are shown in **Fig. F1**.

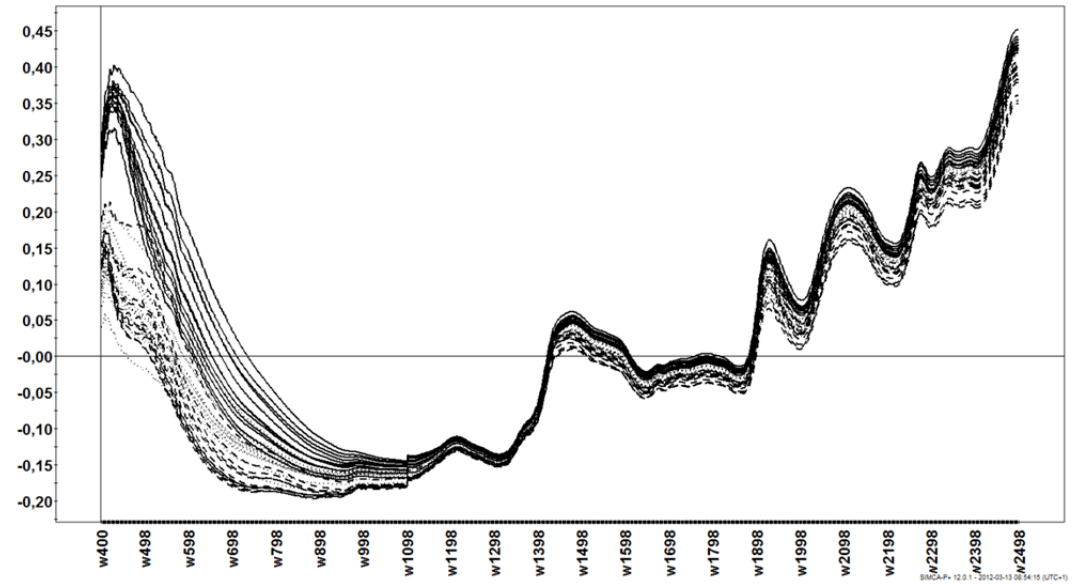

Fig. F1 The figure shows the NIR spectra, with wavelengths on the x-axis. The solid black lines represent the samples treated at alkaline conditions, long dashed lines represent the samples treated at acidic conditions and the dotted lines are the spectra from the samples that were pre-treated with $NaBH_4$.

References

LIU, H., FU, S., ZHU, J. Y., LI, H. & ZHAN, H. 2009. Visualization of enzymatic hydrolysis of cellulose using AFM phase imaging. *Enzyme Microb. Technol.,* 45(4)**,** 274-281.

MEIER, H. 1958. Studies on Hemicelluloses from Pine (*Pinus silvestris* L.). *Acta Chem. Scand.,* 12(10)**,** 1911-1918.