Extraction of Polymeric Hemicellulloses from Spruce Wood

Shoaib Azhar

Doctoral Thesis
Wallenberg Wood Science Center
Department of Fiber and Polymer Technology
School of Chemical Science and Engineering
KTH Royal Institute of Technology
Stockholm, 2015
Principal supervisor
Professor Mikael E. Lindström

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ISSN 1654-1081
TRITA-CHE Report 2015:4

AKADEMISK AVHANDLING
Som med tillstånd av Kungliga Tekniska Högskolan i Stockholm framlägges
till offentlig granskning för avläggande av teknologie doktorsexamen
fredagen den 6 februari 2015, kl. 10.00 i sal F3, Lindstedtsvägen 26, KTH,
Stockholm. Avhandlingen försvaras på engelska.
Opponent: Professor Hasan Jameel, North Carolina State University, USA.
Dedicated to my beloved family.
ABSTRACT

Hemicelluloses are one of the three main components of spruce wood and constitute about 20% of the wood material. During mechanical pulping, 5–10% of the hemicelluloses are accumulated in waste waters, whereas during chemical pulping 70–80% of the hemicelluloses are lost in process liquors. The concept of integrated forest biorefinery involves the development of methods to extract these hemicelluloses prior to pulping in order to produce value-added products besides pulp. This thesis describes some of the feasible possibilities of extracting hemicelluloses from wood at a high molecular weight prior to pulping in addition to presenting a deeper understanding of their degradation under mild treatment conditions.

A major obstacle for the efficient extraction of hemicelluloses is the recalcitrance due to the network of lignin and polysaccharides. This network can be loosely opened by the use of enzymes and this improves the extraction of hemicelluloses. A chemical impregnation of the wood chips was performed to enhance the accessibility of the cell wall to enzymes. The ability of different additives to stabilize the hemicelluloses against peeling during the alkaline impregnation stage was also investigated in order to obtain a better yield in subsequent extraction.

Increasing the surface area and decreasing the mass transport length could also improve the yield of hemicelluloses extracted from wood. This was achieved with a mild mechanical pre-treatment of wood chips using an impressafiner and a fiberizer. Polymers mainly consisting of galactoglucomannan with an average molecular weight of 30 kDa were extracted from fiberized wood with water.

Different pre-treatment and extraction methods were combined to demonstrate the concept of material biorefinery based on wood.

The kinetics of degradation of spruce galactoglucomannan were studied under alkaline conditions. It was degraded in two phases at two different rates. A kinetic model was developed to fit the experimental data and to estimate the activation energies.
SAMMANFATTNING

Hemicellulosa är en av tre huvudkomponenter i granved, och ca 20% av veden består av denna polysackarid. Vid mekanisk massatillverkning ackumuleras 5–10% av hemicellulosan i avloppsvattnet, medan 70–80% av hemicellulosan förlorar under kemisk massatillverkning. Konceptet för integrerat bioraffinaderi baserat på ved bygger på att hemicellulosa extraheras före massatillverkningen, med syfte att producera värdefulla produkter i tillägg till massafibern. Denna avhandling beskriver några tekniskt rimliga möjligheter för att extrahera hemicellulosa från ved i högmolekylär form före massatillverkning, och studerar nedbrytning av hemicellulosa under milda förhållanden relevanta för extraktioner.

Ett dominerande problem vid extraktion av hemicellulosa är att lignin kovalent tvär-binder hemicellulosa till nätverk, vilket försvårar extraktion. Nätverken kan emellertid delvis upplösas genom enzymatisk behandling och därmed kan extraktion av hemicellulosa förbättras. Kemisk förbehandling av vedflis utfördes också för att göra cellväggen tillgänglig för enzym. Olika typer av tillsatser testades för stabilisering av hemicellulosan mot ändvis nedbrytning under alkaliska impregneringssteg i syfte att uppnå bättre utbyte i en följande extraktion.

Ett annat sätt att öka möjligheten till extraktion av hemicellulosa är att öka ytan på veden. Detta uppnåddes genom en mild mekanisk förbehandling av vedflis genom att använda en impressafiner och defibrör. Hemicellulosa med en genomsnittlig molekylvikt av 30 kDa kunde extraheras med vatten från defibrerad ved.

Olika förbehandlingar och extraktionsmetoder kombinerades för att demonstrera konceptet för materialbaserat veddioraffandianderi.

Kinetiken för alkalisk nedbrytning av galaktoglukomannan från gran studerades. Nedbrytningen skedde i två faser med olika hastighet vilket troligen förklaras genom inflytande från galaktos Leverena i polysackariden. En kinetisk modell utvecklades för att beräkna aktiveringsenergier.
PAPERS

This thesis is a summary of the following five appended papers:

I. Extraction of polymers from enzyme-treated softwood
   Azhar, S., Wang, Y., Lawoko, M., Henriksson, G., and Lindström, M. E.
   *Bioresources* 6(4), 4606–4614, 2011

II. Stabilization of polysaccharides during alkaline pretreatment of wood combined with enzyme-supported extractions in a biorefinery
    Wang, Y., Azhar, S., Henriksson, G., and Lindström, M. E.
    *Journal of Wood Chemistry and Technology* 35(2), 91–101, 2014

III. Extraction of hemicelluloses from fiberized spruce wood
     Azhar, S., Henriksson, G., Theliander, H., and Lindström, M. E.
     *Carbohydrate Polymers* 117, 19–24, 2015

IV. On the development of a material biorefinery based on wood
    *Report*

V. Kinetics of alkaline degradation of water-soluble spruce galactoglucomannan
   Azhar, S., Henriksson, G., and Lindström, M. E.
   *Manuscript*
The author’s contributions to the appended papers are as follows:

I. Designed and performed some of the experiments. Performed all the analysis and interpreted the data. Discussed and drew conclusions from the results and prepared the final manuscript.

II. Contributed in planning and performing the experiments, analysed the samples and interpreted the data. Took part in manuscript preparation.

III. Designed and performed all the experiments. Performed all the analysis and interpreted the data. Discussed and drew conclusions from the results and prepared the manuscript.

IV. Performed some of the experiments related to extraction and pre-treatment.

V. Designed and performed all the experiments. Performed all the analysis and interpreted the data. Discussed and drew conclusions from the results and prepared the manuscript.

Results relating to this work were also presented at the following conferences by the author:

  Chemoenzymatic separation of wood polymers
  Azhar, S., and Wang, Y.

- 16\textsuperscript{th} International Symposium on Wood, Fiber and Pulping Chemistry (*ISWFPC*), Tianjin, China, June 8-10, 2011
  Chemoenzymatic separation of softwood polymers
  Azhar, S., Wang, Y., Lawoko, M., Henriksson, G., and Lindström, M. E.

- 243\textsuperscript{rd} ACS National Meeting, San Diego, CA, USA, March 25-29, 2012
  Enhanced extraction of high-molecular-weight wood polymers with chemoenzymatic treatment
  Azhar, S., Wang, Y., Lawoko, M., Henriksson, G., and Lindström, M. E.
• **17th International Symposium on Wood, Fiber and Pulping Chemistry (ISWFPC), Vancouver (BC), Canada, June 12-14, 2013**
  Extraction of galactoglucomannan from spruce wood “Mild mechanical treatment and hot water extraction”
  Azhar, S., Theliander, H., and Lindström, M. E.

• **13th European Workshop on Lignocellulosics and Pulp (EWLP), Seville, Spain, June 24-27, 2014**
  Stabilisation of polysaccharides during alkaline pretreatment of wood followed by enzyme-supported extractions
  Wang, Y., Azhar, S., Lindström, M. E., and Henriksson, G.

The author contributed in the analytical parts of the following projects:

• Recombinant expression and biochemical analyses of the *Arabidopsis thaliana* endo-β-mannanase AtMan5-2
  Wang, Y., Azhar, S., Gandini, R., Divne, C., Ezcurra, I., and Aspeborg, H.
  Submitted in Plant Science

• Investigating the functional and biochemical properties of *Arabidopsis* mannanase5-6
  Wang, Y., Azhar, S., Gandini, R., Divne, C., Ezcurra, I., and Aspeborg, H.
  Manuscript
LIST OF ABBREVIATIONS

LCC  Lignin carbohydrate complex
GGM  Galactoglucomannan
TMP  Thermomechanical pulp
ATMP Advanced thermomechanical pulp
ASAM Alkaline-sulphite-anthraquinone-methanol
MTBE Methyl tert-butyl ether
HWE  Hot water extraction
HPAEC High performance anion exchange chromatography
PAD  Pulsed amperometric detector
SEC  Size exclusion chromatography
$M_w$ Weight average molecular weight
$M_n$ Number average molecular weight
SEM  Scanning electron microscopy
EI   Extended impregnation
UV   Ultraviolet
RI   Refractive index
STEX Steam explosion
CMC  Carboxymethyl cellulose
NMR Nuclear magnetic resonance
odt  Oven dry ton
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INTRODUCTION

Increasing global environmental concerns and the decreasing competitiveness of the paper industry have led the research to focus on utilizing the different components of wood in a more effective way. In recent years, the concept of an integrated forest biorefinery has become common and presents an attractive alternative for producing novel value-added products from lignocellulosic side-streams.

Wood is composed of three main polymeric components viz. cellulose, hemicelluloses and lignin. During pulping, most of the hemicelluloses are accumulated in the process liquors. These hemicelluloses are heavily degraded and they are either used for producing energy in the recovery boiler together with lignin or partially lost in the waste waters. However, if the hemicelluloses can be extracted prior to pulping, the high molecular weight can be preserved and they might therefore have a potential for valuable applications. This work presents methods to extract polymeric hemicelluloses from spruce prior to pulping and the extractability of these hemicelluloses.

1.1 Wood structure and composition

Wood is a highly hierarchical composite material consisting of several layers of living and dead cells each performing their own function. 90-95 % of softwood material consists of cells aligned in the longitudinal direction called
tracheids (Fengel & Wegener, 1983) and also referred to as fibers. The fiber wall is composed of two different layers around the lumen: the primary layer and the secondary layer which is subdivided into S1, S2 and S3 layers. All these layers differ from one another in structure and chemical composition (Fig. 1). The wood cell is mainly composed of three polymeric components: cellulose, hemicelluloses and lignin. A typical composition of softwood cell wall is 43% cellulose, 35% hemicelluloses and 29% lignin (Rydholm, 1965).

Cellulose is a linear polysaccharide composed of anhydroglucose units linked together by 1,4-β-glucosidic bonds. The degree of polymerization of the native spruce cellulose is unknown but it is often stated between 500–1000 units (Fengel & Wegener, 1983). The cellulose content in the cell wall increases from the outer to the inner layer and is highest in the secondary cell wall, especially in the S2 and S3 layers. The linear structure of cellulose polymers leads to strong intra- and intermolecular hydrogen bonds, in

![Figure 1](Composition of the fiber wall (Panshin & Zeeuw, 1980)).
addition to van der Waal forces and hydrophobic interactions, which then form cellulose microfibrils which are also assumed to be largely crystalline (O’Sullivan, 1997).

Hemicelluloses are amorphous with relatively short chains of branched hetero-polysaccharides and are composed of both hexoses and pentoses. Most of the hemicelluloses have a degree of polymerization of only 200. Main hemicelluloses in wood are glucomannan and xylan. Unlike cellulose, hemicelluloses often contain O-acetyl groups substituting the hydroxyl groups at certain positions. The degree of substitution by acetyl groups affects the solubility of hemicelluloses. Hemicelluloses are also relatively easily hydrolysed to monomers by acids or alkalis. The principal sugar components of these hemicelluloses are D-glucose, D-mannose, D-galactose, D-xylose and L-arabinose. There are also some pectins consisting of D-galacturonic acid among others (Sjöström, 1993).

Lignin is a highly complex and amorphous polymeric molecule consisting of phenyl-propane-based units linked together with ethers and carbon-carbon bonds (W. G. Glasser & Glasser, 1974). It has also been shown that the lignin polymers crosslink polysaccharides at different points making a complex network referred as lignin-carbohydrates complex (LCC) (Lawoko, Henriksson, & Gellerstedt, 2006). The lignin concentration is highest in the middle lamellae and it functions as an adhesive to bind the fibers together. In addition, the hydrophobic nature of lignin makes cell walls impermeable to allow water transportation through the cells.

1.2 Spruce hemicelluloses: solubility and vulnerability

The principle hemicellulose in Norway spruce is galactoglucomannan (GGM), which consists of a backbone of randomly distributed (1→4)-linked mannose and glucose units with side groups of (1→6)-linked galactose units attached to mannose (Fig. 2) (Sjöström, 1993; Timell, 1967). There are two types of GGM in softwood, one rich and one poor in galactose. The molar ratio of galactose : glucose : mannose in the GGM is approximately 3–4 : 1 : 0.5–1 (Sjöström, 1993; Stålbrand et al., 2004; Timell, 1967). GGM is also
acetylated at mannose units with a degree of substitution of 0.28-0.37 (Hannuksela & Hervé du Penhoat, 2004; Lundqvist et al., 2002). The average molecular weight of the isolated polysaccharides, mainly containing GGM, is about 30 kDa, although some of these may have a molecular weight as high as 60 kDa (Lundqvist et al., 2002; Willför et al., 2003b). Other hemicelluloses present in noticeable amounts in spruce include arabinoglucuronxylan and arabinogalactan.

![Figure 2](image)

**Figure 2** Representative structure of O-acetyl GGM.

The relatively low molecular weight and the simple structure allow a fraction of the hemicelluloses to dissolve in hot water or even water at room temperature by auto-hydrolysis (Casebier, Hamilton, & Hergert, 1969; Örså, Holmbom, & Thornton, 1997; Willför & Holmbom, 2004), but the dissolved amount is not large enough to be adapted into an industrial process. In the fiber wall, hemicelluloses are enclosed within layers of crystalline cellulose and amorphous lignin, which obstruct the diffusion of hemicelluloses through the cell wall. The aforementioned LCC network could also be a possible reason for the limited solubility of hemicelluloses (Tunc, Lawoko, & van Heinningen, 2010).

Hemicelluloses are also highly vulnerable to degradation. High temperature hydrolyzes acetyl groups from GGM generating acetic acid causing hemicelluloses to undergo autohydrolysis (Fengel & Wegener, 1983; Lundqvist et al., 2002; Song, Pranovich, Sumerskiy, & Holmbom, 2008). Hemicelluloses also depolymerize under alkaline conditions at high temperatures or even at 100 °C due to hydrolysis and peeling reactions (Casebier & Hamilton, 1965; Panshin & Zeeuw, 1980; Wigell, Breid, & Theliander, 2007a).
1.3 Pre-treatment of wood

Because of the limited solubility of hemicelluloses from wood chips and their high vulnerability under acidic or basic conditions, a pre-treatment of the wood is required in order to quantitatively isolate polymeric hemicelluloses under mild conditions. This pre-treatment may either break down the complex matrix structure of lignin and carbohydrates to a certain extent or create cracks in the fiber wall so that the hemicelluloses can diffuse through the cell wall when subjected to extraction. Enzymatic and mechanical pre-treatment methods were used in this study, as described below.

**Enzymatic**

Enzymes specific for cellulose and hemicelluloses are produced by different microorganisms (Have & Teunissen, 2001; Rabinovich, Melnick, & Bolobova, 2002; Rydholm, 1965; Shallom & Shoham, 2003) and can be used as tools to break down the network structure of lignocelluloses. However, a problem with this approach is that the wood structure is quite compact and large molecules such as enzymes cannot penetrate into the cell wall (Blanchette, Krueger, Haight, Akhtar, & Akin, 1997; O'Sullivan, 1997). On the other hand, treating the wood with solution of sodium hydroxide and sodium sulphide (NaOH/Na₂S) can “open up” the wood structure, subsequently allowing the enzymes to penetrate and degrade the wood polymers to some extent (Sjöström, 1993; Wang, Lindström, & Henriksson, 2011). During the alkaline treatment hemicelluloses are degraded mainly due to peeling but they can be stabilized by the addition of a suitable stabilizing agent e.g. sodium borohydride (NaBH₄), polysulphide (PS) and anthraquinone (AQ). These stabilizing agents reduce or oxidize the unstable carbonyl groups to stable end groups (Courchene, 1998; Fleming, Kubes, MacLeod, & Bolker, 1978; W. G. Glasser & Glasser, 1974).

A mild steam explosion at 115–160 °C for 10 min has also been shown to be a good way of opening the wood structure for subsequent enzymatic treatment (Jedvert et al., 2012; Lawoko et al., 2006). There are several other techniques to overcome the recalcitrance in the plant cell walls and separate
the crystalline cellulose from the matrix of lignin and hemicellulose polymers to make them more accessible for enzymatic hydrolysis (Brodeur et al., 2011; Sjöström, 1993; Timell, 1967). However, the main goal in most of the techniques is to obtain a high yield of monomeric sugars for the production of fuels and chemicals.

**Mechanical**

A mild mechanical treatment of wood chips separates the wood fibers and creates cracks in the S1 and S2 fiber walls, and this increases the surface area and decreases the mass transport length (Kure, Dahlqvist, Sabourin, & Helle, 1999; Sjöström, 1993; Stålbrand et al., 2004; Timell, 1967). This type of mechanical pre-treatment can be performed using a fiberizer, which is essentially a small moderately pressurized refiner operating at a low energy input which defibrates the wood chips into fiber bundles called fiberized wood (Hannuksela & Hervé du Penhoat, 2004; Hill, Sabourin, Johansson, & Aichinger, 2009; Hill et al., 2010; Lundqvist et al., 2002). The addition of a fiberizer has recently been used in a novel process called advanced thermomechanical pulping (ATMP) (L. Johansson, Hill, Gorski, & Axelsson, 2011; Lundqvist et al., 2002; Willför et al., 2003b) which reduces the energy demand by 20% compared to conventionally produce thermomechanical pulp (TMP) (Casebier et al., 1969; Örså et al., 1997; Sabourin, Aichinger, & Wiseman, 2003; Willför & Holmbom, 2004). In this work, the fiberizer played a key role for pre-treating the wood when hot water was used as an extraction medium.

1.4 Methods of extraction

Several methods have been studied to extract hemicelluloses from spruce. Some of them lead to the extraction of polymeric hemicelluloses, but most of them focus on obtaining low molecular weight saccharides for the production of fuels such as ethanol (Bozell, 2010; Tunc et al., 2010). Water has been widely used in different studies as a simple solvent to dissolve non-cellulosic polysaccharides from the wood chips, ground wood and thermomechanical pulp (TMP). When subjected to hot water extraction
Hemicelluloses (HWE), a larger amount of hemicelluloses can be extracted from ground wood than from wood chips (Song et al., 2008), but at the cost of a low molecular weight of the dissolved carbohydrates and a wet solid low-value residue. The severe depolymerisation of hemicelluloses caused by autohydrolysis can be controlled by the addition of an alkaline buffer such as sodium bicarbonate \((\text{NaHCO}_3)\) (Song, Pranovich, & Holmbom, 2011). Hemicelluloses dissolved from spruce TMP with HWE at mild conditions have been shown to have a rather high degree of polymerization (DP) ranging up to 340 (Willför et al., 2003b), considerably higher than the previously reported DP of 100 for GGM (Sjöström, 1993).

Other methods of isolating hemicelluloses from lignocelluloses include steam treatment (Biermann, Schultz, & Mcginnia, 1984; Puls, Poutanen, Körner, & Viikari, 1985) and microwave irradiation (Magara, Ueki, Azumu, & Koshijima, 1988). These methods are often combined with chemicals in order to dissolve the fractionated hemicelluloses, although the demand of high energy and complexity has restricted these methods to a small scale.

Another way to separate wood into its components is by using organic solvents. Organic solvents have been of great interest for the production of pulp during the 20th century since they are environmentally benign processes, which also yield more valuable by-products (Bannani, Rigal, & Gaset, 1991; Dahlmann & Schroeter, 1990; Pye & Lora, 1991). The most well known organosolv pulping processes include Organocell and ASAM (alkaline-sulphite-anthraquinone-methanol) using methanol with an alkaline catalyst (Muurinen, 2000). The by-products of organosolv pulping are less degraded and can be further fractionated into hemicelluloses and lignin (Li, Sun, Xu, & Sun, 2012; McDonough, 1993). The molar mass of the hemicelluloses dissolved by the ASAM process is higher than that dissolved in Kraft pulping, but the pulp produced is of comparable quality and has a higher yield (Jacobs & Dahlman, 2001; Sixta, 1998).
1.5 Alkaline degradation of GGM

The degradation kinetics of GGM are of great importance for the factors which affect the fractionation and isolation of polymeric GGM from the wood cell wall. A number of studies related to the degradation of hemicelluloses during alkaline Kraft cooking have been published of which some include a modeling of hemicellulose reactions during cooking (e.g. Andersson, Wilson, & Germgard, 2003; Aurell & Hartler, 1965; Casebier & Hamilton, 1965; Gustavsson & Al-Dajani, 2000; D. Johansson & Germgard, 2008; Kondo & Sarkanen, 1984; Wigell, Brelid, & Theliander, 2007b). Nevertheless, only a few studies have been devoted to the degradation of polymeric GGM originally isolated from softwood (e.g. C. Xu et al., 2008; Young & Liss, 1978). These studies concluded that the rate of degradation of GGM increased with increasing temperature, whereas no obvious effect of alkali concentration was observed.

Under alkaline conditions, the GGM undergoes a primary peeling reaction due to reducing end-groups even at low temperatures (Wigell, Brelid, & Theliander, 2007a) and the reaction continues until a competitive stopping reaction occurs, primarily a rearrangement of the reducing end-groups (Whistler & BeMiller, 1958). Peeling reactions continue at new reducing ends formed as a result of the alkaline hydrolysis of the GGM chains. The activation energy for the alkaline hydrolysis has been found to be lower than that of the peeling and stopping reactions.

1.6 Integrated forest biorefinery (IFBR)

The concept of an integrated biorefinery includes the use of all fractions of the biomass for the production of biofuel, bioenergy and biomaterials (Ragauskas et al., 2006). In an integrated forest biorefinery, the three major components of wood can be allocated to those applications that make the best use of their specific characteristic i.e. cellulose for pulp production and lignin and hemicelluloses for other high-value products (Heiningen, 2006). Lignin can be used as a source of energy with a relatively high heat-value or as a raw material for high-value chemicals (Grierson, Knight, & Maharaj,
It can also be purified via the LignoBoost process (Ohman, Wallmo, & Theliander, 2007) for producing carbon fiber (Gellerstedt, Sjöholm, & Brodin, 2010). Hemicelluloses can be either fermented to bioethanol or further derivatized for other potential applications. Hemicelluloses have so far been tested for making films for use as gas barriers with promising mechanical properties (Edlund, Ryberg, & Albertsson, 2010; Höije, Gröndahl, Tømmeraas, & Gatenholm, 2005; Mikkonen, Heikkilä, Willför, & Tenkanen, 2012), as an additive in paper making to improve the paper strength properties (Hannuksela, Fardim, & Holmbom, 2003; Hartmans et al., 2009), and as hydrogels (Gabrielii, Gatenholm, Glasser, Jain, & Kenne, 2000; Lindblad, Ranucci, & Albertsson, 2001).

The recovery of hemicelluloses prior to pulping has been demonstrated for both Kraft pulping (Al-Dajani & Tschirner, 2008) and TMP (Willför, Rehn, Sundberg, Sundberg, & Holmbom, 2003a). Integrating an extraction system prior to pulping will create new value-added products from extracted hemicelluloses which would otherwise be degraded and/or dissolved in the processing liquors (Heiningen, 2006; Lisboa, Evtuguin, Neto, & Goodfellow, 2005).

1.7 Aim of the thesis

The overall goal of this study was to develop economically feasible methods for the selective extraction of polysaccharides without degradation of the cellulose remaining in the fiber. The main objective was to study ways of extracting high molar mass hemicelluloses from Norway spruce prior to pulping while keeping the cellulose in the main stream for pulp and paper making. The aim was also to increase the yield of extracted hemicelluloses using chemo-enzymatic treatments without degrading the polysaccharides. The kinetics of degradation of isolated GGM were also studied in order to understand the factors affecting the fractionation and isolation of GGM from spruce wood.
EXPERIMENTAL

2.1 Materials and pre-treatment methods

Norway spruce (*Picea abies*) wood chips were used in all the experiments. The chips, obtained from Holmen Paper AB in Norrköping, Sweden, were air-dried and stored in the dark at room temperature.

GGM used for the kinetic studies was isolated from spruce TMP obtained from a Scandinavian pulp mill. The TMP was subjected to HWE and the dissolved GGM was purified with ethanol and methyl tert-butyl ether (MTBE). The precipitated GGM was then dried in a vacuum drier.

*Enzymatic treatment*

Prior to enzymatic treatment, the wood chips were impregnated with a solution of NaOH and Na₂S in order to open up the wood structure. The impregnation was performed at two different temperatures with and without additives. The additives (NaBH₄, PS or AQ) were used to stabilize the hemicelluloses against peeling reactions and to preserve them during the impregnation stage. The impregnated wood was filtered and washed with water to remove residual alkali, and it was then disintegrated and incubated with enzymes at 60 °C for 24 h. The incubation was terminated by increasing the temperature to above 90 °C. More details about the chemical impregnation of wood chips can be found in papers I and II. Fig. 3 shows a schematic diagram of the stages included in the enzymatic treatment.
followed by extraction.

**Figure 3** Schematic of the steps involved in enzymatic pre-treatment followed by extraction.

**Mechanical treatment**

Spruce chips were impressafined at the paper mill, Holmen Paper AB in Norrköping, Sweden. The impressafiner is a screw-press where chips are compressed to a high strain in a pressurized environment. Impressafined chips were steamed for 5 min with atmospheric steam and fiberized using a pilot scale 12” disc refiner (Sprout-Waldron) at Chalmers University of Technology, Sweden. The net energy input to the refiner was adjusted to 300 kWh/odt wood by adjusting the speed of the conveyer belt delivering wood to the refiner in a single pass mode. Fiberized wood was stored in the dark at –24 °C and thawed overnight at room temperature prior to further use.
2.2 Extractions

The enzymatically treated wood material was extracted with a mixture of methanol and alkali, whereas fiberized wood was subjected to hot water extractions with different combinations of time and temperature. The two extraction procedures are explained below.

**Methanol-alkali extraction**

The extractions were conducted in 2 L stainless steel autoclaves rotating in a high-pressure vessel filled with polyethylene glycol as the heating medium. The enzyme-treated samples were subjected to extraction and a non-enzyme-treated sample was extracted as a reference. A 10 g o.d. aliquot of each sample was extracted with a mixture of 50% w/w methanol containing 5% w/w alkali charge on wood. The liquid-to-wood ratio was 10:1. The material was heated at 130 °C for two hours, and the autoclaves were then quenched in a cold-water bath for 30 minutes. The liquid containing the dissolved solids was separated from the wood via vacuum filtration using a fiber cloth sieve (Monodur PA-71, Mesh opening 71 µm) and washed with deionized water. Aliquots of the residual wood were dried at 105 °C to determine the quantity of material extracted. The liquids containing the dissolved solids were lyophilized.

**Hot water extraction**

Specific batches of fiberized wood were extracted with water at 30, 60 and 90 °C for different times ranging from 5 to 120 min. For comparison, frozen spruce wood chips were thawed overnight and subjected to hot water extraction at 90 °C for different times. For each batch, 500 ml of deionized water was first heated in a beaker to the target temperature and 10 g (dry weight) of fiberized wood or wood chips were then added. The temperature drop due to the addition of wood was recovered in 2–3 min, but a variation of ±2 °C was noted throughout the treatment. The water–wood mixture was stirred with a mechanical stirrer using an axial flow impeller rotating at ≈400 rpm, and the liquid was then separated from the wood using a fiber cloth sieve (Monodur PA-71, Mesh opening 71 µm) under vacuum, followed by washing of the wood with 100 ml deionized water. The liquid was again
vacuum-filtered through a glass fiber filter (Whatman GF/A, 1.6 µm) to remove any particles or colloids. The filtrate was allowed to cool and the pH was recorded at room temperature. Filtrate containing dissolved solids from the wood was concentrated by vacuum evaporation using a water bath at 50 °C and lyophilized.

To reuse the extraction liquor, six consecutive extractions were performed at 90 °C for 120 min. The liquid containing dissolved solids from the first extraction was reused for extraction with a new batch of fiberized wood and was further used for four more batch-wise extractions. 10 g (dry weight) of fiberized wood was used in each batch. The extraction liquid in each stage was filtered as mentioned above before being subjecting to extraction (Fig. 4).

![Figure 4](image_url)  
**Figure 4**  Schematic diagram showing the mechanical treatment of spruce wood chips followed by hot water extraction.

### 2.3 Integrating different procedures into a wood biorefinery

Mechanical and enzymatic treatments were combined prior to the extraction stage with several other techniques (more details in the appended report IV) in order to develop a material biorefinery based on wood. An overview of the whole process used in this biorefinery concept is presented in Fig. 5.
In this project, a mild steam explosion (STEX) was used for a first separation of the hemicelluloses. The material dissolved (4) during the steam explosion was either upgraded by polymerization (8) or fractionated and purified by ultrafiltration (6) and chromatography (9). The remaining wood chips (3) were mechanically treated (10) using the same procedure as described earlier. The mechanically treated wood was treated with enzymes (11) and subjected to methanol-alkali extraction. The residual wood and the steam-exploded wood were cooked and subjected to delignification after extraction (3). Ionic liquids were also tested on portions of the extracted wood residue (14).
2.4 Alkaline degradation of GGM

Spruce GGM with the following sugar composition (Table 1), was dissolved in 0.5 mol/L sodium hydroxide (NaOH) at a concentration of 2 mg/ml at room temperature. 5 ml aliquots of the solution were transferred to 12 ml glass tubes, which were sealed with PTFE caps and heated at 90, 100 and 110 °C in an oil bath. The heating times were 5, 10, 20, 30, 40, 50, 60, 70, 80 and 90 min. After the treatment, the solutions were allowed to cool to room temperature. A model compound, carboxymethyl cellulose (CMC) was also treated with alkali for comparison. The CMC was dissolved in 0.5 mol/L NaOH and heated at 100 °C for 5, 10 30, 60, 90 and 120 minutes.

Table 1  Sugar composition of the dried material.

<table>
<thead>
<tr>
<th>Anhydrous monosaccharides</th>
<th>mol %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannose</td>
<td>55</td>
</tr>
<tr>
<td>Glucose</td>
<td>25</td>
</tr>
<tr>
<td>Galactose</td>
<td>12</td>
</tr>
<tr>
<td>Arabinose</td>
<td>2.1</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.3</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>0.5</td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>4.0</td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td>0.9</td>
</tr>
</tbody>
</table>

2.5 Analysis

**Klason lignin**

The Klason lignin content was determined on the wood material, after enzymatic treatment and after extraction. The wood was dried at 105 °C and ground to pass a 40-mesh screen. The ground wood was hydrolysed according to a standard method (SCAN-CM 71:09), the hydrolysates were filtered and the insoluble fraction on the filter paper was dried to determine the amount of Klason lignin.
**Carbohydrate composition**

The carbohydrate composition was determined by acid hydrolysis using the procedure described in (SCAN-CM 71:09) and the monosaccharide content of the hydrolysates was analysed with a high-performance anion exchange chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a pulsed amperometric detector (HPAEC-PAD). Uronic acids were quantified with HPAEC-PAD after the samples had been hydrolysed with trifluoroacetic acid at 121 °C for 3 h, following the method presented by De Ruiter, Schols, Voragen, & Rombouts, (1992).

**Molecular weight determination**

Size exclusion chromatography (SEC) was employed to estimate the molecular weight of the extracted material and of the GGM treated with NaOH. The SEC system was equipped with ultraviolet (UV) at 280 nm and refractive index (RI) detectors. The samples were eluted with 10 mM NaOH at 1 ml/min through three columns connected in series and maintained at 40 °C or at room temperature. Pollulan standards ranging from Mp = 342 to 708,000 or 250,000 Da (PSS, Germany) were used for calibration.

**Acetyl content**

The acetyl content in the hot water extracts from fiberized wood was estimated from the spectra obtained using $^1$HNMR, the integral response of the acetyl peaks being compared with that of Trimesic acid, was used as an internal standard. About 3 mg of the dried sample was dissolved in 0.7 ml of deuterated dimethyl sulfoxide (DMSO-d$_6$) together with a known amount of the internal standard. $^1$HNMR spectra were recorded at room temperature on a Bruker Avance 400 MHz instrument.

**Scanning electron microscopy (SEM)**

Freeze-dried samples of wood chips and fiberized wood were cut transverse to the fiber direction using Cold Laser ablation. SEM images of the cross sections were taken using a tabletop Hitachi TM-1000 SEM with an acceleration voltage of 15 kV.
RESULTS AND DISCUSSION

This chapter discusses the effects of enzymatic and mechanical pre-treatments on the structure of wood and on the subsequent extraction of lignocelluloses. The extraction yields were calculated and the extracted materials were thoroughly characterized and compared. These methods were also integrated with several other techniques in order to demonstrate a wood biorefinery concept, briefly reviewed herein. Finally, the kinetics of the alkaline degradation of isolated galactoglucomannan at low temperatures were discussed.

3.1 Extraction of enzymatically pre-treated wood

Wood chips were impregnated with a solution of NaOH and Na₂S prior to enzymatic treatment in order to open up the compact wood structure. Two impregnation methods were tested in this study: one in which the wood was impregnated at 150 °C for 30 min, herein designated as “I–150”, and the another in which impregnation was carried at 110 °C for 120 min, herein referred to as extended impregnation (EI). EI performed with the addition of NaBH₄, PS or AQ, is referred to as EI–NaBH₄, EI–PS and EI–AQ respectively. Two different enzymes, gamanase and xylanase, were used for the enzymatic pre-treatment of I–150 whereas only gamanase was used in the case of EI, EI–NaBH₄, EI–PS and EI–AQ. Organic solvents were used for the extraction with the possibility of obtaining better yields of non-degraded polymers including
both hemicelluloses and lignin.

The extraction of enzyme pre-treated I–150 showed that the amount of extracted material could be increased by the use of enzymes, as is evident in Fig. 6. Though different types of enzymes showed different efficacies therefore more material was observed to be extracted from gamanase-treated wood than from xylanase-treated wood. Pre-treating the wood with xylanase enhanced the extraction by 2 percentage points, whereas gamanase pre-treatment increased the extraction by almost 9 percentage points.

![Figure 6](image)

**Figure 6** Wood residues after extraction of I-150 with or without enzymatic treatment.

The carbohydrate composition of the residual wood material left after the extraction is shown in Fig. 7. The liquid extracts were concentrated using ultrafiltration and dialfiltration (1000 Da cellulose membrane) and purified from low molecular weight substances. The retentates were lyophilized and the weight average molecular weight ($M_w$) was assessed with SEC. As illustrated in Fig. 8, the $M_w$ of the material obtained from xylanase-pre-treated wood was higher than the $M_w$ of the extract obtained from wood without any enzymatic pre-treatment. Xylanase is a mono-component enzyme with activity specific towards xylan. The xylan content in softwood is
relatively small, but the specific degradation of xylan by xylanase nevertheless reduced the recalcitrance caused by the LCC network to some extent and allowed the extraction of a small amount of high molecular weight material from the wood. On the other hand, gamanase is a culture filtrate and contains several enzyme activities. This may be the reason for the greater degradation of the hemicelluloses and greater extraction yield of highly degraded material (Fig. 8). The removal of other wood components in addition to the component specific to the enzyme has previously been reported (Salmén & Olsson, 1998). Correspondingly the removal of other wood components than xylan and mannan was observed when the residual wood was examined after xylanase and gamanase treatment (Fig. 7), but the cellulose chains were almost unaffected by the enzymes and gave intrinsic viscosity similar to the references.

![Figure 7](image-url)  
*Figure 7* Amounts and compositions of lignocelluloses in the extracted residues of enzyme-treated wood in comparison with the references a) xylanase-treated b) gamanase-treated.

The specific enzymes led to the extraction of high molecular weight material whereas the non–specific enzymes degraded the hemicellulloses to a large extent. The impregnation stage was a pre-requisite for the enzymes to penetrate into the wood structure, but a drawback was that a large fraction of
the wood hemicelluloses was lost during this impregnation stage carried out at 150 °C for 30 min (I–150), especially GGM (Table 2). A mild impregnation of spruce wood chips at 110 °C for 120 min was therefore tested.

**Figure 8** SEC chromatograms showing RI signals for the extracts obtained from the wood pre-treated with xylanase and with gamanase, and without any enzymatic pre-treatment.

**Table 2** Composition of wood impregnated with or without additives, presented as percentages based on the original wood.

<table>
<thead>
<tr>
<th>% of wood</th>
<th>Yield</th>
<th>K.L.</th>
<th>Ara</th>
<th>Gal</th>
<th>Glu</th>
<th>Xyl</th>
<th>Man</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spruce wood</td>
<td>100</td>
<td>28.4</td>
<td>1.4</td>
<td>2.0</td>
<td>45.7</td>
<td>6.3</td>
<td>12.7</td>
</tr>
<tr>
<td>I-150</td>
<td>67.9</td>
<td>13.4</td>
<td>0.9</td>
<td>1.0</td>
<td>43.7</td>
<td>5.3</td>
<td>3.6</td>
</tr>
<tr>
<td>EI</td>
<td>76.6</td>
<td>22.4</td>
<td>0.9</td>
<td>0.8</td>
<td>44.1</td>
<td>4.8</td>
<td>4.0</td>
</tr>
<tr>
<td>EI–NaBH₄</td>
<td>89.5</td>
<td>23.2</td>
<td>1.0</td>
<td>1.4</td>
<td>48.5</td>
<td>5.9</td>
<td>10.2</td>
</tr>
<tr>
<td>EI–PS</td>
<td>80.0</td>
<td>22.8</td>
<td>0.9</td>
<td>1.1</td>
<td>45.9</td>
<td>5.5</td>
<td>5.8</td>
</tr>
<tr>
<td>EI–AQ</td>
<td>81.3</td>
<td>22.7</td>
<td>0.8</td>
<td>0.7</td>
<td>45.2</td>
<td>5.7</td>
<td>5.5</td>
</tr>
</tbody>
</table>
NaBH₄, PS and AQ were added during the extended impregnation in order to preserve the wood and stabilize the carbohydrates against peeling. NaBH₄ was found to be the most efficient of the various additives tested giving a yield of more than 89% w/w of residual wood after impregnation, retaining most of the hemicelluloses in the wood during impregnation (Table 2). The greater efficiency of the reducing agent: NaBH₄, compared to the oxidizing agents: PS and AQ, can be explained that the reduction of the reducing end of the polysaccharides results in a hydroxyl group and stops the peeling reaction, whereas oxidation of reducing end generates a carboxylic group. The carboxylic group can create intermediates like enols that initiate the peeling reaction (Klien, 2012). However, the resonance stabilization of carboxylic acid slows the peeling reaction and the oxidative agents nevertheless help to preserve the hemicelluloses against peeling (Istek & Özkan, 2008) (Fig. 9). The efficiency of the reactions or the chemical dosage may also be additional factors influencing the reactions. The degradation of hemicelluloses under alkaline conditions proceeds through two main reactions: hydrolysis and peeling. Severe hydrolysis of polysaccharides occurs at temperatures higher than those used in the mild impregnation performed here and peeling reactions were reduced with additives, so the content of hemicelluloses was high in the residual wood impregnated with NaOH and Na₂S, in the presence of additives.
**Figure 9**  Mechanism for the stabilization of hemicelluloses by reduction using NaBH₄ and by oxidation using PS and AQ.

Fig. 10 shows the amount of wood remaining after the extractions. More material could be extracted from the wood which has been impregnated in the presence of additives. The amount of material extracted from each of the stabilized woods was 11–12%, compared to 9% from the wood without any additive. Gamanase treatment could enhance the extraction yield up to 14% w/w only in the EI–NaBH₄, whereas no significant increase in the extraction
was observed from EI–PS or EI–AQ after gamanase treatment. One explanation for this could be the higher yield of hemicelluloses in the impregnated wood, and there were large amounts of easily extractable material available, i.e. the LCC was no longer a limiting factor.

The amounts of lignin and anhydro-monosugars extracted from the extended impregnated wood samples with or without additives are shown in Table 3. About 13% of the total mannose present in the impregnated wood was extracted and this value increased to 20% following the gamanase treatment. NaBH₄ preserved a significant quantity of mannose in the wood, so that a comparatively larger quantity of 25% of mannose could be extracted, which increased to 30% after gamanase treatment. The material extracted from the non-enzyme-treated wood had a molecular weight ranging up to 20 kDa, indicating that the wood structure was opened up after extended impregnation enabling high-molecular-weight materials to be extracted. However, the molecular weight of the material extracted from the gamanase-treated wood was not high, and most of the extracted hemicelluloses appear in the oligomeric region. This was because the non-specific degradation of polysaccharides by gamanase, as described earlier.

![Figure 10](image-url)

**Figure 10** Yield of extracted wood residues after enzymatic or non-enzymatic treatment of wood impregnated with or without additives.
Table 3  Amounts of lignocelluloses extracted from extended impregnated wood.

<table>
<thead>
<tr>
<th>% of wood</th>
<th>KL</th>
<th>Ara</th>
<th>Gal</th>
<th>Glu</th>
<th>Xyl</th>
<th>Man</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-enzyme treated</td>
<td>5.77</td>
<td>0.07</td>
<td>0.09</td>
<td>2.62</td>
<td>0.20</td>
<td>0.53</td>
</tr>
<tr>
<td>Gamanase treated</td>
<td>6.77</td>
<td>0.08</td>
<td>0.15</td>
<td>2.10</td>
<td>0.20</td>
<td>0.88</td>
</tr>
<tr>
<td>EI-NaBH₄</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-enzyme treated</td>
<td>4.64</td>
<td>0.26</td>
<td>0.48</td>
<td>2.98</td>
<td>1.20</td>
<td>2.51</td>
</tr>
<tr>
<td>Gamanase treated</td>
<td>5.86</td>
<td>0.11</td>
<td>0.52</td>
<td>4.36</td>
<td>0.50</td>
<td>3.02</td>
</tr>
<tr>
<td>EI-PS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-enzyme treated</td>
<td>5.76</td>
<td>0.02</td>
<td>0.53</td>
<td>3.64</td>
<td>1.41</td>
<td>1.54</td>
</tr>
<tr>
<td>Gamanase treated</td>
<td>7.27</td>
<td>0.10</td>
<td>0.57</td>
<td>4.08</td>
<td>0.62</td>
<td>1.81</td>
</tr>
<tr>
<td>EI-AQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-enzyme treated</td>
<td>5.01</td>
<td>0.11</td>
<td>0.09</td>
<td>2.18</td>
<td>1.32</td>
<td>2.18</td>
</tr>
<tr>
<td>Gamanase treated</td>
<td>6.40</td>
<td>0.10</td>
<td>0.18</td>
<td>1.16</td>
<td>0.58</td>
<td>2.25</td>
</tr>
</tbody>
</table>

3.2 Extraction of mechanically pre-treated wood

Mechanical pre-treatment was tested as an alternative to enzymatic pre-treatment of wood with the aim of preserving 100% of the wood material during the pre-treatment stage prior to the extraction. The fiberizer used in the mechanical pre-treatment was operated at a much low energy input of 300 kWh/odt. The fiberizer disintegrated the wood chips to fiber bundles and possibly induced cracks in the fiber walls. The average length of the fiber bundles produced was about 10 mm. SEM micrographs of the cross section of fiberized wood (Fig. 11c, d) show that the cell walls were separated in the middle lamellae, which increased the rate of diffusion of soluble hemicelluloses through the cell wall. Freeze drying of the fiberized wood might have enlarged the cracks in the samples prepared for SEM analysis. However, the reference wood samples were prepared in similar way.

The purpose of the mild mechanical pre-treatment was to partially separate the wood cells without destroying the basic morphological structure of the wood so that the material could be used for pulping after the extraction stage. The extractions were performed with water at temperatures below 90 °C in order to avoid the depolymerization of dissolved hemicelluloses due to auto-hydrolysis.
RESULTS & DISCUSSION

![SEM micrographs of wood chips](image)

**Figure 11** Cross-sectional SEM micrographs of wood chips (a, b) and fiberized wood (c, d). The effect of the fiberizer can be seen as a disrupted wood structure with cracks in the middle lamellae compared to the aligned structure in the case of the wood chips.

The amount of carbohydrates extracted from fiberized wood increased with increasing temperature and longer treatment time (Table 4). This increase in the extraction amount was believed to be due to a softening of the wood fibers at high temperatures that enabled the dissolution of hemicelluloses through the cracks in the cell wall created by the defibration. A large amount of carbohydrates (8.9 mg/g wood) was extracted at 90 °C/120 min, whereas only 4.5 mg/g was extracted from wood chips under similar conditions. This difference in the amounts of extracted carbohydrates was due to the larger available surface area in the fiberized wood compared to the wood chips.
Table 4  Amounts and composition of carbohydrates (mg/g wood) extracted with water from fiberized wood and wood chips.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>30 °C</th>
<th>90 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Man</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Glu</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Gal</td>
<td>0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Ara</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Xyl</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Sum</td>
<td>3.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Dissolved GGM(^1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.0</td>
<td>2.9</td>
</tr>
<tr>
<td>% Yield(^2)</td>
<td>1.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>90 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Man</td>
<td>2.6</td>
</tr>
<tr>
<td>Glu</td>
<td>1.3</td>
</tr>
<tr>
<td>Gal</td>
<td>1.0</td>
</tr>
<tr>
<td>Ara</td>
<td>0.3</td>
</tr>
<tr>
<td>Xyl</td>
<td>0.1</td>
</tr>
<tr>
<td>Sum</td>
<td>5.2</td>
</tr>
<tr>
<td>Dissolved GGM(^1)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.9</td>
</tr>
<tr>
<td>% Yield(^2)</td>
<td>2.4</td>
</tr>
</tbody>
</table>

\(^1\) GGM = (Gal+(1+1/3.5)×Man)×0.9

\(^2\) GGM in spruce = 163 mg/g wood (Sjöström, 1993)
Figure 12  
$^1$H NMR spectrum of acetyl-GGM extracted from fiberized wood at 90 °C/15 min. The acetyl content was calculated from the integrated area of the acetyl peak relative to the peak for the internal standard.

Figure 13  
SEC chromatogram of material extracted at 60 °C/60 min. The RI signal represents both lignin and carbohydrates whereas the UV signal represents only lignin.

More than 3.6% of the spruce GGM was dissolved at 90 °C/120 min, comprising about 66% of the total extracted carbohydrates. Other carbohydrates present in the extracts were mainly arabinose and glucose.
Arabinose was probably derived from water-soluble arabinogalactans and glucose from xyloglucan and/or other glucans present in minor amounts in spruce. The extracted GGM was found to be acetylated to 25–40% (Fig 12), which was in agreement with previous observations of water-soluble acetylated-GGM (Capek, Alföldi, & Lišková, 2002). The weight average molecular weight ($M_w$) of the extracted material was greater than 30 kDa and the shoulder in the SEC chromatogram (Fig. 13) at about 22 min was interpreted as indicating the presence of a pure lignin-free fraction. The extracted liquor containing dissolved carbohydrates could be reused many times without any significant decrease in the extraction yields of high $M_w$ material from fiberized wood.

### 3.3 A material biorefinery based on wood

A wood biorefinery concept was developed based on the combination of a number of pre-treatment, purification and extraction techniques. It was possible to dissolve a total of about 29% of wood material at rather harsh conditions before subjecting the wood material to cooking and delignification stages (Fig. 14).

During the steam explosion (7 bar) of spruce wood chips, about 7% of the wood material, mainly hemicelluloses, was dissolved in the liquid. These hemicelluloses were purified from low $M_w$ material by ultrafiltration, and lignin was removed by chromatography. In addition to the removal of a part of the wood material, the STEX treatment made the wood structure more porous and hence accessible for a subsequent enzymatic treatment. A mild mechanical treatment was however performed on the STEX material in order to increase the available surface area and decrease the mass transfer length.
Figure 14  Cumulative yields for a 7 bar STEX pre-treatment followed by cooking (60 min at $T_{\text{max}} = 170^\circ\text{C}$).

The enzymatic treatment performed here had no significant effect and the material was dissolved mainly by diffusion into the buffer solution. The reason could be the mechanical treatment on STEX. The porous wood material with an enhanced fiber surface area facilitated the greater dissolution of wood material into the buffer. The material extracted with the methanol-alkali mixture had a yield of about 8% with a relatively low $M_W$. The high $M_W$ material was probably already dissolved either during the steam explosion step or later in the buffer. However if the STEX treatment was performed under milder conditions (4 bar), less material was dissolved during the STEX and it was later possible to extract more material after the enzymatic treatment with relatively high molar mass.

The pulp produced after the extraction stages had a higher brightness and a lower kappa number than the reference material (without pre-treatment), but with relatively low pulp viscosity in case of STEX 7. Since the hemicelluloses can protect the cellulose chain towards alkaline hydrolysis (Lindström & Teder, 1995), the low pulp viscosities in this study could be due to the removal of hemicelluloses before kraft cooking. On the other hand, at mild treatment conditions (STEX 4) the pulp properties were similar to those of the untreated wood. Consequently, there would be possibilities to optimize the pulping conditions and/or pre-treatment stages that need to be considered in order to further develop this biorefinery concept.
3.4 Alkaline degradation of GGM

The degradation under alkaline conditions and mild temperatures of GGM isolated from spruce TMP was studied. The number average molecular weight \((M_n)\) of the GGM was 10.4 kDa as determined by SEC. This value decreased with increasing time of the alkaline treatment, and the decrease was observed to take place in two phases, a fast first phase and a slow second phase (Fig. 15). The molecular weight of the GGM decreased rapidly in the first few minutes and then levelled off. The initial drop in \(M_n\) was observed to be faster at higher temperatures. The two phases were assigned to first order reactions in order to acquire more information by fitting a model curve to the experimental data. A first order rate of reaction can be derived to give the following equation.

\[
M^t = M^0_f e^{-k_f^t} + M^0_s e^{-k_s^t} \quad (1)
\]

\[
M^t = M^f_t + M^s_t = 10.4 \quad (2)
\]

\[
k_f = k_f^{100} e^{E_a \left(\frac{1}{T} - \frac{1}{373.15}\right)} \quad (3)
\]

\[
k_s = k_s^{100} e^{E_a \left(\frac{1}{T} - \frac{1}{373.15}\right)} \quad (4)
\]

where the subscripts \(f\) and \(s\) refer to fast and slow phases respectively, \(M^t\) and \(M^0\) are the molecular weights at times \(t\) and 0 respectively, \(E_a\) is the activation energy, \(R\) is the universal gas constant (8.314 J/mol K), \(T\) is the treatment temperature and \(k^{100}\) is the rate constant at the average temperature i.e. 100 °C.

The model suggests that the \(M_n\) was reduced to 4.9 kDa after the first phase, and then slowly decreased to 4.1 kDa after 90 min. The calculated activation energy from the model for the first fast phase, the quick degradation of GGM at 90–110 °C, was 65.8 kJ/mol, indicating a temperature dependence of the first phase.
Figure 15  Degradation of GGM with 0.5 mol/L NaOH as a function of time at different temperatures with curves fitted by the least squares method, using the solver function. The symbols represent the experimental data. The mean error of estimate was at 90, 100 and 110 °C was 0.32, 0.20 and 0.15 kDa respectively.

Representative samples were dialysed through a 100–500 Da membrane and the retentates were analysed with respect to their sugar composition. The existence of galactose side-groups suggested that treatment conditions were not adequate to cleave the galactose side-groups from the main chain.

The main reactions in GGM degradation are peeling and hydrolysis. Peeling causes one monosaccharide unit to be removed from the reducing end, whereas hydrolysis may break the chain in the middle. One explanation for the fast degradation of GGM in the first phase could be that it is due to a
peeling reaction. Peeling may have continued until the $M_n$ of GGM was reduced to 4.8 kDa when the stopping reaction occurred. The degradation subsequently continued slowly in the second phase due to hydrolysis.

Timell (1964) studied the hydrolysis of the glucose–glucose, glucose–mannose and mannose–mannose bonds. He reported that the glucose–glucose bond is the most difficult to hydrolyse and that mannose–mannose is the most easily hydrolysable among the three. GGM contains two types of glycosidic bonds, glucose–mannose and mannose–mannose. The two phases of GGM degradation can alternatively be explained that some of the glycosidic bonds present in GGM are readily hydrolysed in the first phase whereas the remaining bonds are either difficult to hydrolyse under these conditions or are protected by the remaining galactose groups, as shown in Fig. 16. The sandwich structure presented in the figure 16 is an obstacle for the separation of the chain ends from each other in intermediate II, and this stimulates the recoupling reaction. The sandwich structure can also hinder alkaline hydrolysis by holding the chain ends together in the intermediate II.

**Figure 16** a) A galactose side-group in GGM forms a sandwich structure with a monosaccharide residue in the main chain. b) The presence of galactose residues may stimulate two chains to form a temporary “dimer” complex.
CMC is a straight chain unbranched polymer and, in contrast to GGM, was degraded in a single phase under similar alkaline conditions at 100 °C (Fig. 17). Glucose–glucose bonds present in CMC are more difficult to hydrolyse than the glycosidic bonds present in GGM, and GGM therefore degrades faster than CMC in the first phase. In the second slow phase, the galactose side-groups or the sandwich structures are probably stabilized to some extent making it more difficult for GGM to degrade than CMC.

![Figure 17](image)

**Figure 17** Alkaline degradation of CMC when treated with 0.5 mol/L NaOH at 100 °C as a function of treatment time.
Conclusions

It has been shown that a mild mechanical or an enzymatic pre-treatment of spruce wood chips could successfully enhance the subsequent extraction of polymeric hemicelluloses.

A chemical impregnation of wood chips was performed in order to open the compact wood structure so that the enzymes can penetrate through the cell wall. Hemicelluloses were degraded during the impregnation stage, but they could be stabilized against peeling using an oxidizing or a reducing agent. Among various agents tested, NaBH₄ was the most efficient and preserved more than 89% of the total wood, compared to 76% without the agent. The quality and quantity of the extracted material depended on the type of the enzyme used; a mono-component enzyme led to the extraction of high $M_w$ material, whereas a non-specific enzyme with multiple hemicellulose activities gave a higher extraction yield of a relatively degraded material.

A mechanical pre-treatment of wood with a low energy input of 300 kWh/odt led to the creation of cracks in the fiber walls. This facilitated a partial dissolution of wood polymers into water due to the large available surface area and the shorter mass transport length. The hot-water-extracted material from fiberized wood was rich in GGM with an average molecular weight of more than 30 kDa. A maximum of about 6 mg/g-wood of GGM could be extracted at 90 °C/120 min from fiberized wood without lowering the $M_w$. The yield of extracted GGM can be increased, but at the cost of a low
molecular weight. It was also shown that the water containing wood extracts could be reused multiple times without any significant decrease in the extraction yield. This technique showed a potential to be applied in a TMP mill to extract high molecular weight hemicelluloses prior to refining.

In a wood biorefinery concept, the pre-treatment methods and extraction techniques can be combined to extract polymeric hemicelluloses from wood material before subjecting the wood to pulping processes. However, more work is needed to optimize certain stages.

The degradation of GGM under alkaline conditions at low temperatures could be described as the sum of the two first order reactions. In the first fast phase, the $M_n$ of GGM was reduced from 10.4 to 4.9 kDa, whereas the degradation of GGM was very slow in the second phase. The quick degradation of GGM might be due to peeling in the first phase followed by slow degradation caused by hydrolysis in the second phase. Alternatively, galactose side-groups were not selectively cleaved off under the applied conditions and thus may have stabilized the GGM against degradation in the second slow phase.
I would like to express my deep appreciation and gratitude to my supervisor, Prof. Mikael Lindström for his patient guidance all over these years. His intellectual gifts are matched only by his genuinely good nature and down-to-earth humility, and I am fortunate to have had the opportunity to work with him.

I would also like to thank my co-supervisors, Prof. Hans Theliander for his rigorous and thought-provoking suggestions, Prof. Gunnar Henriksson for his enthusiastic ideas, and Assoc. Prof. Martin Lawoko for fruitful discussions.

Furthermore, I would like to acknowledge all my colleagues and friends, both present and past, at the Department of Fiber and Polymer Technology and at the Wallenberg Wood Science Center, especially Inga, Pia, Mia, Thérèse and Jan-Erik for dealing with the administrative tasks; Mona, Anders and Ramiro for keeping the laboratories functional; Lars B. and Monica for being wonderful heads of each division; Yan and Yang for successful collaborations; Christofer, Francisco and Lauren for their intellectual help in some projects; Mikaela, Nicholas, Anna and Emil for a memorable start of the tenure in the Ljungbergrummet; Kerstin, Susanne, Niklas and Tuve for the nice discussions during Theme-1 meetings; and Petri, Ran, Myriam, Dimitri, Helena, Stefan, Jiebing, Liming, Olena, Elisabet, Daniel, Dongfang, Yujia, Xueyu, Michaela, Kasinee, Gisela, Farhan, Andreas, Anas, Joby, Ngesa, Selda and Jennie for all the chatting and for making it a pleasant workplace.
The Knut and Alice Wallenberg Foundation is acknowledged for financial support within the Wallenberg Wood Science Center.

Back in Pakistan, I would like to thank my beloved parents for teaching me the basics of life and for always remembering me in their prayers that have enabled me to achieve this level.

I cannot forget to mention my lovely children, Talha, Ayaan and Minal, whose smiles and hugs helped me erase the frustration that came from the futile projects and whose morning kisses reinforce me in my daily challenges.

Finally, I would like to acknowledge the innumerable sacrifices made by my wife, Noor, in shouldering more than her fair share of parenting and household burdens while I pursued my work towards this final degree.


Courchene, C. E. (1998). The tried, the true, and the new-getting more pulp from chips-modifications to the kraft process for increased yield (pp. 11–20). Presented at the *Breaking Pulp Yield Barrier Symp.*, Atlanta, Georgia: the Institute.


