Åbo Akademi University

Pressurized hot water flow-through extraction of birch wood

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Academic Dissertation

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Preface

Most of the experimental work was done at the Finnish Forest Research Institute (Metla) in Jokiniemi, Vantaa. The chemical analyses were done in Vantaa and at the Laboratory of Wood and Paper Chemistry at Åbo Akademi University. Professor Stefan Willför, Professor Hannu Ilvesniemi, Docent Andrey Pranovich and Docent Veikko Kitunen supervised my work.

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I Kilpeläinen, P., Leppänen, K., Spetz, P., Kitunen, V., Ilvesniemi, H., Pranovich, A., and Willför, S. (2012): Pressurised hot water extraction of acetylated xylan from birch sawdust, *Nord. Pulp & Pap. Res. J.* 27(4), 680-689.

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Contribution of the author

The author is responsible for the planning, experimental work and the main author of article I. The author is responsible for planning, extractions, part of the sugar analyses, and as main author of article II. The author completed the extractions, co-planned the article and was second writer in the article III. The author was responsible of planning, part of the extractions and analyses, and as main author of article IV. The author planned the article in co-operation with other authors and was main author of article V.

Supporting publications, proceedings, and presentations

Adamczyk, B., Kilpeläinen, P., Kitunen, V., Smolander, A. (2014) Potential activities of enzymes involved in N,C,P and S cycling in boreal forest soil under different tree species, *Pedobiologia – J. Soil Ecol.*, 57, 97–102.

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List of essential abbreviations

ASE	Accelerated solvent extraction
DMAc	*N,N*-Dimethylacetamide
DOE	Department of energy
GC	Gas chromatography
GC-FID	Gas chromatography flame ionization detector
GGM	Galactoglucomannan
GPC	Gel permeation chromatography
HPLC	High performance pressure liquid chromatography
HPLC-RI	High performance liquid chromatography refractive index detector
HPLC-DAD	High performance liquid chromatography diode array detector
HPSEC	High performance size exclusion chromatography
PHW	Pressurized hot water
PHWE	Pressurized hot water extraction
MALLS	Multi angle laser light scattering
Mw	Mass average molar mass
RSD	Relative standard deviation
SAXS	Small angle x-ray scattering
SEW	Sulphur dioxide ethanol water
TDS	Total dissolved solids
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)-oxy
WAXS	Wide angle x-ray scattering
XMT	X-ray microtomography

Abstract

Effective processes to fractionate the main compounds in biomass, such as wood, are a prerequisite for an effective biorefinery. Water is environmentally friendly and widely used in industry, which makes it a potential solvent also for forest biomass. At elevated temperatures over 100 °C, water can readily hydrolyse and dissolve hemicelluloses from biomass. In this work, birch sawdust was extracted using pressurized hot water (PHWE) flow-through systems. The hypothesis of the work was that it is possible to obtain polymeric, water-soluble hemicelluloses from birch sawdust using flow-through PHW extractions at both laboratory and large scale. Different extraction temperatures in the range 140–200 °C were evaluated to see the effect of temperature to the xylan yield. The yields and extracted hemicelluloses were analysed to obtain sugar ratios, the amount of acetyl groups,furfurals and the xylan yields.

Higher extraction temperatures increased the xylan yield, but decreased the molar mass of the dissolved xylan. As the extraction temperature increased, more acetic acid was released from the hemicelluloses, thus further decreasing the pH of the extract. There were only trace amounts of furfurals present after the extractions, indicating that the treatment was mild enough not to degrade the sugars further. The sawdust extraction density was increased by packing more sawdust in the laboratory scale extraction vessel. The aim was to obtain extracts with higher concentration than in typical extraction densities. The extraction times and water flow rates were kept constant during these extractions. The higher sawdust packing degree decreased the water use in the extractions and the extracts had higher hemicellulose concentrations than extractions with lower sawdust degrees of packing. The molar masses of the hemicelluloses were similar in higher packing degrees and in the degrees of packing that were used in typical PHWE flow-through extractions.

The structure of extracted sawdust was investigated using small angle- (SAXS) and wide angle (WAXS) x-ray scattering. The cell wall topography of birch sawdust and extracted sawdust was compared using x-ray tomography. The results showed that the structure of the cell walls of extracted birch sawdust was preserved but the cell walls were thinner after the extractions. Larger pores were opened inside the fibres and cellulose microfibrils were more tightly packed after the extraction.

Acetate buffers were used to control the pH of the extracts during the extractions. The pH control prevented excessive xylan hydrolysis and increased the molar masses of the extracted xylans. The yields of buffered extractions were lower than for plain water extractions at 160–170 °C, but at 180 °C yields were similar to those from plain water and pH buffers. The pH

can thus be controlled during extraction with acetate buffer to obtain xylan with higher molar mass than those obtainable using plain water.

Birch sawdust was extracted both in the laboratory and pilot scale. The performance of the PHWE flow-through system was evaluated in the laboratory and the pilot scale using vessels with the same shape but different volumes, with the same relative water flow through the sawdust bed, and in the same extraction temperature. Pre-steaming improved the extraction efficiency and the water flow through the sawdust bed. The extracted birch sawdust and the extracted xylan were similar in both laboratory and pilot scale. The PHWE system was successfully scaled up by a factor of 6000 from the laboratory to pilot scale and extractions performed equally well in both scales. The results show that a flow-through system can be further scaled up and used to extract water-soluble xylans from birch sawdust. Extracted xylans can be concentrated, purified, and then used in e.g. films and barriers, or as building blocks for novel material applications.

Keywords

Pressurized hot water extraction, Flow-through, Birch, Sawdust, Xylan, Hemicelluloses, Lignin, Biorefinery, Wood biorefinery, *Betula pendula*

Sammanfattning

För en effektiv bioraffinering av biomassa, såsom ved, krävs att man kan fraktionera de huvudsakliga komponenterna. Vatten är miljövänligt och används i hög grad i industrin, vilket gör det till ett potentiellt medium också för fraktionering av skogsbiomassa. Vatten kan lätt hydrolysera och lösa ut hemicellulosor från biomassa vid temperaturer över 100 °C. I detta arbete extraherades sågspån från björk genom att använda trycksatt hetvattenextraktion (eng. pressurized hot water extraction, PHWE) i såkallade genomflödessystem. Arbetets hypotes var att det är möjligt att extrahera polymera och vattenlösliga xylaner från björkvedssågspån genom att använda genomflödes-PHWE i både laboratorieskala och stor skala. Extraktionerna utfördes i både laboratorie- och pilotskala. Hetvattenextraktion i en flödesreaktor användes för att erhålla acetylerade och vattenlösliga xylaner från sågspån av björk. Effekten av temperaturen på xylanutbytet undersöktes genom att variera temperaturen i intervallet 140–200°C. De extraherade hemicellulosorna analyserades för att bestämma mängden acetylgrupper och furfural, förhållanden mellan olika sockerenheter samt xylanutbyte.

Högre extraktionstemperaturer resulterade i ett ökat xylanutbyte men också en minskning i xylanernas molmassa. När extraktionstemperaturen ökade, frigavs mera ättiksyra från hemicellulosorna, vilket sänkte extraktets pH ytterligare. Endast små mängder av furfural detekterades efter extraktionerna, vilket indikerar att behandlingen var tillräckligt mild så att ytterligare sockernedbrytning till mindre komponenter undveks. Packningsgraden av sågspån ökades genom att lägga till mera sågspån i extraktionskärlet i laboratorieskala. Målet var att erhålla extrakt med en högre koncentration än de koncentrationer som erhålls vid typiska extraktioner. Extraktionstiderna och vattenflödet hölls konstant i dessa experiment. Den högre packningsgraden resulterade i minskad vattenanvändning samt en högre koncentration av hemicellulosor jämfört med extraktioner med lägre packningsgrad av sågspån. Hemicellulosornas molmassor var ungefär lika i båda fallen.

Strukturen av det extraherade sågspånet studerades med hjälp av lågvinkel/vidvinkel röntgenspridning (eng. small angle/wide angle x-ray scattering SAXS/WAXS). Cellväggens topografi hos björksågspånet och hos det extraherade sågspånet jämfördes med hjälp av röntgentomografi. Resultaten visade att strukturen hos björksågspånets cellväggar bevarades men att cellväggarna var tunnare efter extraktionen. Efter extraktionen bildades större porer i fibern och cellulosans mikrofibriller var tätare packade.

Acetatbuffert användes för att kontrollera pH under extraktionen. Genom att kontrollera pH förhindrades ytterligare hydrolys av xylanerna vilket ledde till ökad molmassa. Utbytet var lägre i extraktioner vid 160–170°C där buffert använts än jämfört med bara vatten, men vid 180 °C var utbytet ungefär det-

samma i båda fallen. Genom att använda acetatbuffert vid extraktion kan pH alltså kontrolleras och därigenom kan xylaner med en högre molmassa erhållas.

Björksågspån extraherades både i laboratorie- och pilotskala. Flödesreaktorns extraktionskapacitet vid hetvattenextraktion bedömdes i både laboratorie- och pilotskala genom att använda kärl av samma form men olika volymer och med samma relativa genomströmningshastigheter samt samma extraktionstemperaturer. Förbehandling med ånga förbättrade extraktionseffektiviteten och vattenflödet genom sågspånsbädden. De extraherade björksågspånen och de extraherade xylanerna var liknande vid jämförelse mellan laboratorie- och pilotskala. Hetvattenextraktionssystemet kunde skalas upp 6000-faldigt och extraktionerna var likartade i båda storleksklasserna. Resultaten visar att trycksatt hetvattenextraktionsgenomflödessystem kan skalas upp och användas för att extrahera polymera och vattenlösliga xylaner från björkvedssågspån. De extraherade xylanerna kan sedan koncentreras, renas och användas i t.ex. filmer, barriärer eller som byggstenar i nya material.

Yhteenveto

Biomassan, kuten puun, eri komponenttien kokonaisvaltainen fraktiointi on edellytys tehokkaalle biojalostukselle. Vesi on ympäristöystävällinen aine ja laajalti käytössä teollisuudessa, minkä takia vettä voisi myös käyttää liuottimena puubiomassan fraktioinnissa. Lämpötilan ollessa yli 100 °C, vesi voi helposti hydrolysoida ja liuottaa hemiselluloosia biomassasta. Tässä työssä koivun sahanpurua uutettiin läpivirtauslaitteistoilla laboratorio- ja pilottimittakaavassa paineistettua kuumavesiuuttoa käyttäen (eng. pressurized hot water extraction, PHWE). Työn hypoteesin mukaan on mahdollista saada polymeerimuotoisia ja vesiliukoisia hemiselluloosia koivusahanpurusta läpivirtauskuumavesiuuttoa käyttäen sekä laboratorio- että suuremmassa mittakaavassa. Kokeissa käytettiin eri uuttolämpötiloja 140–200 °C välillä lämpötilan vaikutuksen selvittämiseksi ksylaanin saantoon. Uutetut hemiselluloosat analysoitiin, jotta voitiin määrittää asetyyliryhmien ja furfuraalien määrät, eri sokeriyksikköjen suhteet sekä ksylaanisaannon puusta.

Korkeammalla uuttolämpötilalla ksylaanien saanto puusta nousi, samanaikaisesti liuenneen ksylaanin moolimassa laski. Uuttolämpötilan noustessa hemiselluloosista vapautui enemmän etikkahappoa, jonka myötä uutteen pH laski. Uuttojen jälkeen uutteessa oli hyvin pieniä määriä furfuraaleja jäljellä, mikä osoittaa että tarpeeksi hellävaraisella käsittelyllä vältyttiin sokereiden lisäpilkkoutumiselta. Sahanpurun pakkautumistiheyttä kasvatettiin pakkaamalla laboratoriokoon uuttoastiaan enemmän sahanpurua. Sahanpurun lisäyksen tarkoituksena oli kasvattaa uutteen sisältämän ksylaanin konsentraatiota verrattuna konsentraatioon, joka saadaan tyypillisissä uutoissa. Uuttoajat sekä veden virtausnopeudet pidettiin vakioina näissä kokeissa. Koivupurun suurempi pakkautumistiheys vähensi uutoissa käytetyn veden määrää ja lisäksi hemiselluloosien määrä uutteessa kasvoi verrattuna tavalliseen pakkautumistiheyteen. Uutettujen hemiselluloosien moolimassat olivat samankaltaisia molemmissa tapauksissa.

Uutetun sahanpurun rakennetta tutkittiin käyttämällä kapeakulmaista (eng. small angle x-ray scattering, SAXS) ja laajakulmaista (eng. wide angle x-ray scattering, WAXS) röntgensirontaa. Koivun sahanpurun ja uutetun sahanpurun soluseinien topografiaa vertailtiin röntgentomografian avulla. Tulokset osoittivat, että koivun sahanpurun soluseinien rakenne säilyi, mutta soluseinät olivat ohuempia uuton jälkeen. Kuituun muodostui isompia huokosia ja selluloosan mikrofibrillit olivat pakkautuneet tiheämmin uuton jälkeen.

Uuton aikana pH:ta säädettiin asetaattipuskureiden avulla. Puskurin käytöllä estettiin ksylaanin liiallista hydrolyysiä uuton aikana ja kasvatettiin uutetun ksylaanin moolimassaa. Saanto uutoista oli pienempi puskuroiduissa uutoissa jotka tehtiin 160–170 °C:ssa kuin pelkällä vedellä tehdyissä uutoissa.

Lämpötilan noustessa 180 °C:een puskuroidun ja puskuroimattoman saannot uutoista olivat samankaltaiset. Näin ollen pH:ta pystytään säätämään uuttojen aikana asetaattipuskureilla ja sitä kautta saadaan ksylaaneja joilla on korkeampi moolimassa kuin pelkällä vedellä uuttaessa.

Koivun sahanpurua uutettiin sekä laboratorio- että pilottikokoluokassa. Läpivirtauslaitteistojen uuttokykyä vertailtiin laboratorio- ja pilottikokoluokassa käyttämällä samanmuotoisia uuttoastioita eri tilavuuksilla, mutta samanlaista suhteellista läpivirtausta ja lämpötilaa käyttäen. Esihöyrytys paransi uuttotehokkuutta ja veden virtausta sahanpurun läpi. Uutetut koivun sahanpurut ja uutetut ksylaanit olivat samankaltaisia kummassakin kokoluokassa. PHWE-kuumavesiuuttolaitteiston kokoa pystyttiin kasvattamaan 6000-kertaiseksi laboratoriokoosta pilottiskaalaan ja uutot toimivat yhtä hyvin kummassakin kokoluokassa. Tulokset osoittavat että läpivirtauslaitteiston kokoa voidaan kasvattaa ja sitä voidaan käyttää vesiliukoisten ksylaanien uuttamiseen koivun sahanpurusta. Uutetuttuja ksylaaneja voidaan väkevöidä, puhdistaa ja käyttää esimerkiksi kalvoissa tai uusien materiaalien rakennusaineina.

1 Introduction

Fossil based oil and coal are presently the main sources of energy and chemicals. The amount of oil in the world is decreasing and new oil fields are harder to access leading to increased oil prices. Oil is the major feedstock source for platform chemicals that are used by industry. The price of oil is also dependent on the political situation of the world, which can vary quite rapidly. If all the oil that is available were burned, it would increase the amount of carbon dioxide in atmosphere so that the temperature of atmosphere would increase and excess carbon dioxide could acidify seawater. Hence, alternatives to oil need to be developed preferably from renewable resources.

Biomasses are renewable sources of energy and platform chemicals. Currently most of the easily processable biomasses are grown for food use. The use of food-based first generation biofuels can be hard to promote since they are competing against food production feedstock demands. As the population of the world is constantly growing, more food and other resources, such as chemicals are needed. Second generation feedstock such lignocellulose and waste from agriculture or woods are more promising raw material sources. Trees usually grow in areas that could not be used for agriculture and in many parts of the world these can usually be harvested from local sources, which are not dependent on international politics. Forests do not need large amounts of fertilizers or pesticides during their growth. Wood contains less inorganic materials than grasses, so less inorganic material has to be dealt with during processing.

In 2010, the estimated total forest growing stock was about 527 billion m^3 (FAO 2010). Forest areas are increassing in Europe and in Asia, but decreasing in Africa and Southern America. In 2010, the composition of forest growing stock worldwide was 39% coniferous trees (softwood) and 61% broadleaved trees (hardwood). In Europe, 71.4% of the forests are composed of coniferous species.

In Finland, the agricultural growth season is shorter than in middle Europe or Africa. The amount of fertile land is limited. Therefore, the main available lignocellulosic source in Finland is wood. The Finnish forest growing stock was 2332 million m^3 in 2012 (Finnish Statistical Yearbook of Forestry 2013). The annual increment was 104.4 million m^3 of stemwood, of which 99.1 million m^3 was available for wood products. In 2012, 69.8 million m^3 were used, so growing wood stock increased 34.5 million m^3 per year. The dominant species in Finland is pine with 50% proportion of stemwood volume, then spruce with 30% and the remaining 20% of broadleaves. Forest industry used a total of 70.8 million m^3 of wood, of which pulp mills in Finland used 37.4 million m^3 of wood. Pulp mills and the forest industry

have used almost the same amount of wood during the last two years, which is 20% less than in 2006 and 2007. Therefore, in Finland there is a potential sustainable wood source that could be utilized as feedstock for new biorefinery processes.

Birch is a hardwood that is mainly used in pulp mills and to make plywood and veneer, in construction, and furniture. Birch is sawn in sawmills producing large amounts of birch sawdust. Birch sawdust can be burned to produce energy, but a more value added method would be to use it as a source of raw material in biorefineries. Sawdust has a small particle size so it can be more densely packed and in water extractions the mass transfer of extracted compounds out of sawdust will be faster than from larger birch chips.

Water is an attractive extraction medium, since it is non-toxic and it is found with low salt content in most places in the world. It is cheap and is widely used in industry. Water treatment methods, separation methods and also synthesis in the water phase are known and still developed to further refine extracted compounds, to make value added products.

1.1 Biorefinery

Definitions and products

According to USA's Natural renewable energy laboratory, "A biorefinery is a facility that integrates biomass conversion processes to produce fuels, power and chemicals from biomass" (NREL). Other wider definitions include CO_2 neutrality and an ecosystem-friendly standpoint: "A process for fractionating and/or converting biomass (a CO_2 neutral feedstock) in an eco-friendly way into energy, as well as a variety of chemicals and other biomaterials" (Alén 2011). Integrated production utilizing biomass-derived feedstocks is analogous to oil-based refineries (Cherubini 2010). Biorefineries can produce transport fuels such as bioethanol, biodiesel and other fuels that are of low value but high volume products, similar to oil-based refineries. A biorefinery product portfolio can also include commodity chemicals, specialty chemicals, and other materials, which are low volume but high priced products. The basic principle of a biorefinery is to use all available product streams and leave as low amount of waste as possible. Bioproducts can substitute similar chemical compounds that are produced in oil industry or provide new compounds with unique properties. Products from renewable biomass can replace current products with a similar molecular structure or substitute them with different molecules (Stuart and El-Halwagi 2013) or with new novel products (Werpy and Pedersen 2004). Biorefinery production needs a steady stream of biomass that can be converted to value-added products.

Biomass utilization

Most of the biomass is composed of different polymeric organic molecules such as cellulose, hemicelluloses, starch, chitin, and lignin. Biomass can also include different amounts of proteins, lipids, waxes, organic acids, small organic compounds, and inorganics in variable amounts (Sanders et al. 2012). With a varied feedstock, this poses problems since the feedstock should have a specified quality and purity according to the needs of expected end uses. Some crops can contain a lot of water and others are harvested at high dry weight. The production network should be flexible enough to use very complicated, variable and even low quality feedstock to produce a large diversity of high-quality products (Figure 1). The figure represents theoretical idea for biorefinery. In practise biorefineries can have more complex processes that require larger capital costs still producing value added products.

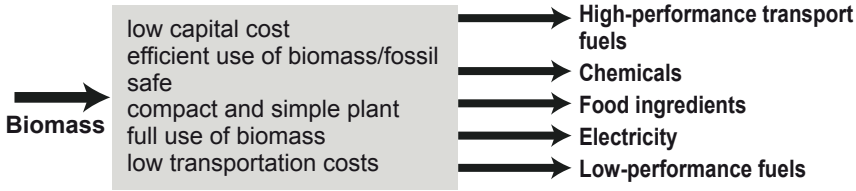

Figure 1. *Requirements for an ideal process utilization of biomass (Sanders et al. 2012).*

Biomass utilization routes

Different strategies to utilize bio-based chemicals have been proposed (e.g. Menon and Rao 2013, Sanders et al. 2013). In the value chain approach, value-added compounds in biomass are identified and isolated. The remaining biomass is then used as a raw material for other chemical products. Chemicals and polymers are isolated from biomass rather than degrading biomass to small molecular building blocks. The same treatments, such as hot-water extraction, can be used to isolate compounds from biomass or degrade them to sugars.

The second strategy, the integrated process chain approach, is similar to that used for chemical production in the oil industry (Menon and Rao 2013). Biomass is first transformed to low molecular weight building blocks and the desired chemicals are then produced from these compounds (Sanders et al. 2013). Typical methods in this group can be pyrolysis, gasification, or more severe acid/alkali treatments that produce sugars. The first thermochemical conversion of biomass to fuels was reported in the eighteenth century (Dusselier et al. 2014).

The third strategy to produce chemicals could be to develop selective reactions to convert single components of biomass to valuable chemicals that could be isolated after the reaction (Sanders et al. 2013).

An ideal biomass pre-treatment method should separate biomass to its constituent parts: extractives, lignin, hemicelluloses and cellulose. In practice, fractions contain some amount of other components such as hemicellulose and lignin. So in a general sense, biomass pretreatments are a mixture of three previously mentioned strategies (Sanders et al. 2013).

In some cases it could be beneficial to retain minerals, soil-improving compounds, and water from biomass at the fields or forest where they were grown. These compounds would be removed using smaller scale fractionation units and then returned to nature to promote further biomass growth. The remaining isolated biomass fractions would be transported to a central processing plant with lower overall transportation cost (Sanders et al. 2013).

When land use, trade, and employment issues are considered in Europe, there is a potential for ca. 100 straw or softwood based biorefineries (Thornley et al. 2014). These biorefineries would produce e.g. ethanol, furfural, bio-oils, lignin and 2,5-furandicarboxylic acid.

Sugar fermentation to ethanol, even at 100% theoretical efficiency, loses one third of the carbon of sugars to carbon dioxide (Dusselier et al. 2014). Therefore, new methods to reuse carbon dioxide produced in industry have been introduced (Perathoner and Centi 2014). Carbon dioxide would be recycled to produce, for example, polypropylene. The carbon dioxide emissions from different biorefinery types and products can have a large variation in the kg CO_2/kg of product (Kajaste 2014). Chemical pulp has a low carbon dioxide emission being 0.4–0.6 kg CO_2/kg product, with ethanol from grain having the largest variation of 0-20.7 CO_2/kg.

Biorefinery products

There have been propositions from the U.S. Department of Energy (DOE) for the TOP 10 biochemicals from carbohydrates (Table 1),(Werpy and Pedersen 2004). Recently this list has been modified with more possible chemicals (Bozell and Petersen 2010). The DOE also issued a report about possible lignin derived chemicals (Holladay et al. 2007) and recently new insights into lignin valorization have been reported (Ragauskas et al. 2014).

Other possible chemicals produced by fermentation could be amino acids, vitamins, biopolymers, enzymes and antibiotics (Kajaste 2014). Some larger polymeric biomaterials could be derived from lignin, hemicelluloses, and cellulose (Menon and Rao 2012). Bio-chemicals could be produced either chemically or enzymatically from sugars obtained from hemicelluloses and cellulose. Cellulose conversion to sugars, to be utilized for fermentation, was first proposed almost 200 years ago (Dusselier et al. 2014).

Table 1. *Some potential platform chemicals from biomass.*

DOE report 2004	DOE report 2007	Bozel and Petersen
Fumaric acid	Methanol	Ethanol
Malic acid	Dimethyl ether	Lactic acid
Succinic acid	Benzene	Succinic acid
Aspartic acid	Toluene	Isoprene
Glucaric acid	Xylene	Biohydrocarbons
Glutamic acid	Phenols	Furfural
Levulinic acid	Vanilin	Hydroxymethylfurfural
Sorbitol	Vanilic acid	Levulinic acid
Xylitol/arabinitol	Carbon fibers	Sorbitol
Glycerol/derivatives	Polyelectrolytes	Xylitol
Itaconic acid	Polymer alloys	Glycerol/derivatives
3-Hydroxyburyrolactone	Polymer extenders/fillers	3-Hydroxypropionic acid
3-Hydroxypropionic acid	Composites	2,5-Furan dicarboxylic acid
2,5-Furan dicarboxylic acid	Adhesives and binders	

In industrial use, hardwood hemicellulose xylan is hydrolysed to xylose and is then hydrogenated to xylitol (Sjöström 1993). Furfural is another compound that is industrially produced from xylose (Zeitch 2000). Ethanol, sorbitol, vanillin are other examples of fuels and chemicals that are currently produced in industrial scale (Rødsrud et al. 2012, Sjöström 1993).

Polymeric and oligomeric products from biomass and wood

Cellulose, hemicelluloses and lignin are natural polymeric compounds. Polysaccharides are hard to synthesize, so it is preferable to isolate them from biomass. Wood cellulose has been used for a long time to make paper and packaging materials. The cellulosic product value chains are well established and pulp mill operations to remove lignin are highly optimized. Nowadays, cellulose fibres are the main product of pulp mills. Cellulose is mainly used to make paper, packaging materials, regenerated cellulose and some specialty products such as cellulose acetates, -ethers etc.

Starch can be isolated from biomass and it has unique properties. It is used to make food products and first generation biofuels. Usually starch originates from food sources and as more food in world is currently needed, it would be preferable not to use it as raw material for chemicals or fuels. Woody materials have a small amount of starch and starch molecules are quite easily degraded during different processes.

Hemicelluloses and lignin do not have as wide use as cellulose. Lignin and hemicellulose have usually been used as energy in pulp mills. Lignin has

been used in industry to produce lignosulphonates (Rødsrud et al. 2012). New industrial methods have recently surfaced such as the LignoBoost process (Tomani 2010), to isolate sulphur-containing lignin from Kraft pulp mill black liquor.

New ideas and approaches to use hemicelluloses have emerged. Isolated hemicelluloses should preferable be water-soluble so that they can be modified in water phase or at least pumped or transported without using organic solvents, or strong acids or bases. Hemicelluloses isolated from biomass have unique properties, or properties that enable their use in various end products. They can be used to make oxygen barriers films for food packaging (Mikkonen and Tenkanen 2012) in the food industry. Aerogels is another interesting field of application (Mikkonen and Tenkanen 2013). Aerogels are used to insulate products since they have low thermal conductivity. Other proposed uses for xylans have been biomedical applications (Ebringerová and Hromádková 1999) and composite films with cellulose (Saxena et al. 2011). Xylo-oligosaccharides are potential source for food additives and nutraceuticals (Moure et al. 2006). In the medical field, xylan microparticles could be used as drug carriers in the pharmaceutical industry (Silva et al. 2012). Xylan can also be functionalized to cationic xylans (Schwikal et al. 2005) that can be attached on cellulose to modify surface properties. In some applications, it would be beneficial to have xylans with larger molar masses. One way to increase the size of the xylan molecules is reducing end amination (Daus et al. 2010).

Galactoglucomannans (GGM) from spruce have been modified by TEMPO oxidation and amidation (Leppänen et al. 2013) to attach modified GGM to cellulose. GGM could be also enzymatically modified and allylated (Leppänen et al. 2010, 2012) to form monoallyl alcohols. Attached allyl units would be a reactive linker where new active moieties could be attached to GGM. Nanofibrilated cellulose has been modified using amphiphilic block-structured GGM (Lozhechnikova et al. 2014). Anionic polysaccharides from GGM can be attached on cellulose (Parikka et al. 2012). A new approach is to mix GGM with polyaniline to from conducting biocomposites (Leppänen et al. 2013). Cationic polysaccharides can be synthesized from GGM (Kisonen et al. 2014) for cellulose surface modifications or perhaps water treatment applications. GGM can also be hydrophobized to form barriers (Kisonen et al. 2012). Cationic hydrogels of GGM have been synthesized to remove arsenic and chromium from aqueous solutions (Dax et al. 2014).

1.2 Wood

Trees belong to seed bearing plants (*Spermatophytes*). Coniferous trees or softwoods belong to the gymnosperm ("naked seeds") group and hardwoods

to the angiosperm group, with flowering plants having fruits. In Europe, there are 10 softwood and 51 different hardwood species that exist naturally. The dominant species in Finland are softwoods with 80% of stem volume and the remaining 20% are hardwoods (Metla 2013). Softwoods have needle or scale like leaves, while hardwoods usually have broad, flat, and thin leaves.

Hardwoods and softwoods have different kinds of cellular structures. The cellular structure of softwood is simpler than for hardwoods (Sixta 2006). Softwoods are mainly composed of tracheids (90–95% of total volume) and ray cells (5–10%) (Sjöström 1993). Hardwoods contain several cell types that are specialized for different functions. For example vessels (25% of birch stemwood area) form long tubes for water transportation through wood (Figure 2).

Cell wall layers have different structures and chemical compositions (Sjöström 1993). The middle lamella binds cells together and is mainly lignin (Figure 3). Thin primary wall (0.1-0.2 µm) has randomly oriented microfibrils on the outside and more transverse orientated microfibrils towards the cell axis on the inner surface (Sixta 2006). Three secondary layers are formed when the cell matures. The S2 layer is thickest (1–5 µm) and around it are the outer S1 (0.1–0.3 µ) and inner layer S3 (0.1 µm). The microfibrils of the inner and outer layers are oriented perpendicular towards the middle S2 layer. The thick S2 layer contains most of the cell wall components, 90% of cellulose and 70–80% of hemicelluloses. The warty layer W can be found

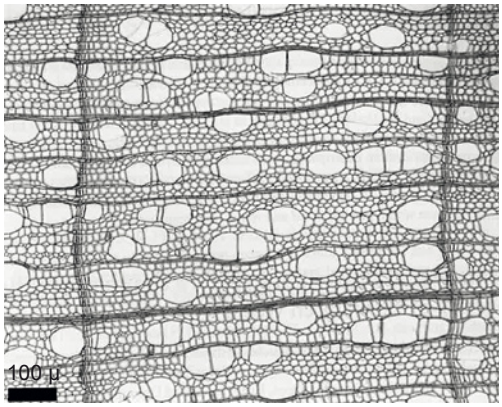

Figure 2. *Transverse section of 33 year old silver birch wood grown in southern Finland (Piispanen 2004).*

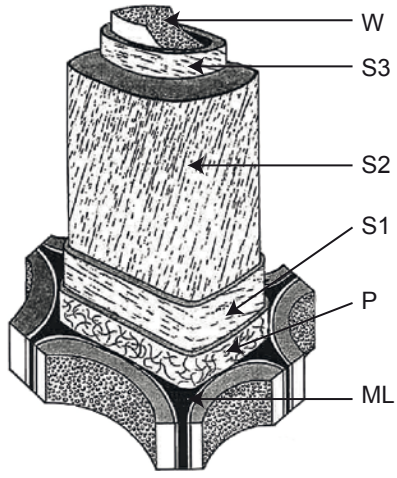

Figure 3. *Cell wall model of wood: primary cell wall (P), secondary cell wall (S1-S3), middle lamella (ML) and warty layer (Modified from Côte et al. 1964).*

in phylogenetically more primitive cells, such as softwood (gymnosperm) tracheids, but more specialized cell types have lost this layer (Parham and Baird 1974). The warty layer is comprised of granules and amorphous structures. The one proposed function of the warty layer is that it restricts the access of rumen micro-organisms (Himmel 2008).

1.2.1 Hemicelluloses

Hemicelluloses function as supporting material in the cell walls with cellulose (Sjöström 1993). Unlike cellulose, hemicelluloses are heteropolysaccharides with different sugar moieties (Sixta 2006). Most hemicelluloses have a degree of polymerisation (DP) under 200 (Sjöström 1993) and from 50 to 200 (Sixta 2006). Hemicelluloses are quite easily hydrolysed under acidic conditions to monosaccharides (Sjöström 1993). Softwood and hardwood hemicelluloses differ significantly. They have different sugars, such as hexoses and pentoses in the main chain and different amounts of side moieties.

Hardwood

The main type of hemicellulose in birch is xylan (Sjöström 1993). Birch xylan is formed from a xylose backbone appended with 4-*O*-Methylglucuronic units (Figure 4). Approximately 60% of the xylose units of hardwoods can also carry an acetyl group attached either to the C-2 or C-3 position (Teleman et al. 2000).

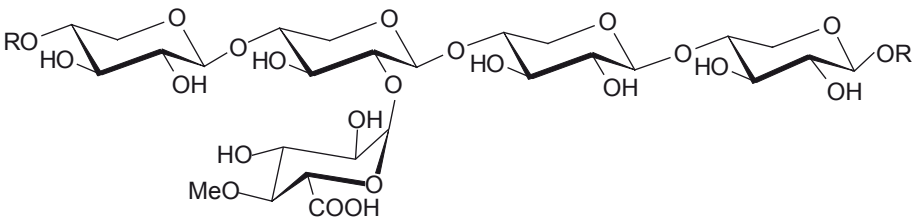

Figure 4. *Hardwood xylan.*

Softwood

Galactoglucmannans are the main hemicelluloses in softwoods comprising 20% of the wood (Sjöström 1993). They have linear chains of β-1→4 linked monosaccharides consisting of β-D-glucopyranose and β-D-mannopyranose units, linked at C6 to α-D-galactose as one unit side group(Figure 5). The hydroxyl groups at C-2 and C-3 in the chain are substituted by O-acetyl groups.

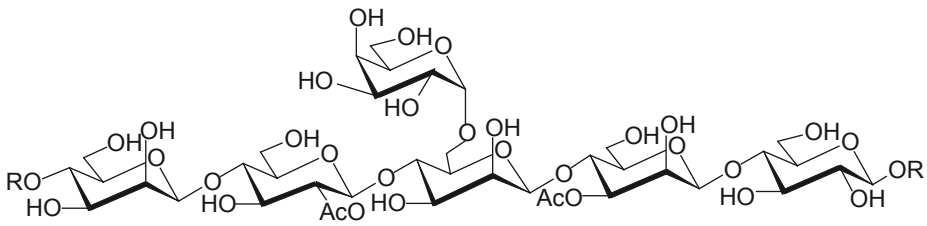

Figure 5. *The structure of softwood galactoglucomannan.*

Pectins

Pectic polysaccharides mostly consist of D-galactouronic acid with D-galactose, L-arabinose, and L-rhamnose residues (Sjöström 1993). Pectic substances are not commonly classified as hemicelluloses even though they have a polymeric structure. Pectins can be found in primary cell wall and middle lamella. Minor amounts of pectins and galactans can be found in normal and tension wood but even up to 10% can be found in tension wood. Pectin is more abundant in bark than in wood, where it plays a part in early stages of cell development (Sixta 2006).

Starch

Trees use starch as their main reserve polysaccharide (Sixta 2006). Starch is composed of two components, linear amylose and branched amylopectin. They both have very high molecular mass. Amylopectin has higher molecular mass than cellulose (Sjöström 1993).

1.2.2 Lignin

Lignins are abundant and complex plant biopolymers that account for approximately 30% of the organic carbon in the biosphere (Ralph et al. 2004, 2007, Heitner et al. 2010, Ragauskas et al. 2014) Lignification of plants during evolution enabled plants to form large upright vascular plant forms (Himmel 2008). These upright forms enabled some plants to collect more photons in competition with other photosynthesising plants and also spread more spores over wider areas. Lignins are crucial for structural integrity of the cell and give stiffness and strength to stem and roots (Ralph et al. 2007, Heitner et al. 2010). Lignin polymers are aromatic and therefore very hydrophobic (Himmel 2008). Lignin hydrophobicity gave plants a way to control water uptake, use, and storage, which allowed them to diversify onto most landscapes of earth (Himmel 2008). Lignins have also a role in protecting plants against pathogens (Ralph et al. 2007).

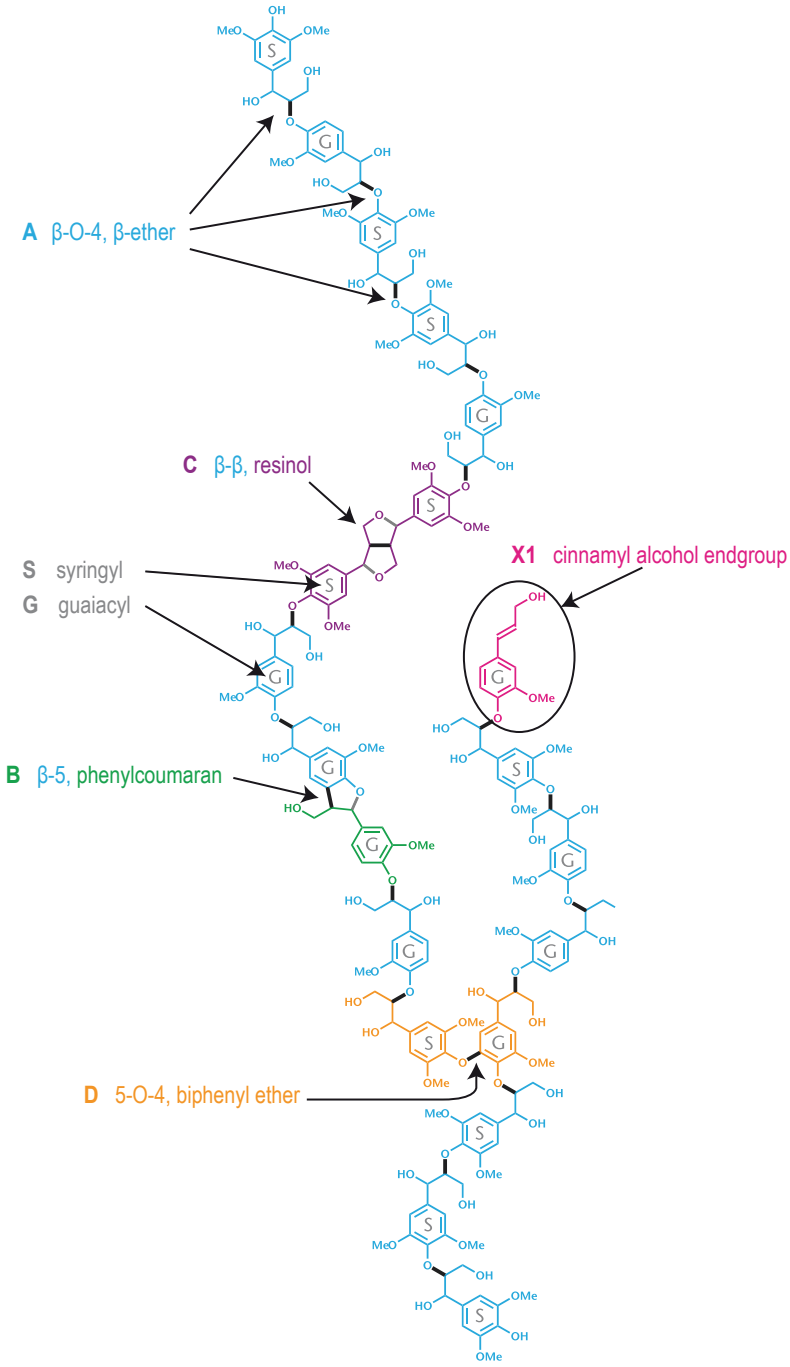

Figure 6. *A lignin polymer model for a hardwood (poplar) lignin with 20 units.*

Lignins are formed from 4-hydroxyphenylpropanoids, which are oxidatively coupled (Ralph 2004). The primary monomers for lignification are three p-hydroxycinnamyl alcohols: p-coumaryl, coniferyl and sinapyl alcohols. These lignols are incorporated in lignin as p-hydroxyphenyl (H), guaiacyl (G) and Syringyl (S) forms, respectively. Lignin polymerisation forms three-dimensional amorphous polymers (Sjöström 1993). Lignin monomers are linked through ether or carbon-carbon bonds. The lignin model for hardwood is shown in figure 6 (Modified from Ralph et al. 2007).

Bold black bonds indicate the bonds formed by radical coupling (Figure 6). Lighter (grey) bonds result from post coupling internal rearomatization reactions. The α-OH groups, resulting from nucleophilic addition of water assume the colours of parent structure. The branch point (D, 4-O-5-units in orange) is differentiated by unique colour, even though unit can also be β-ether. Each of these structures represents one of billion isomers (Ralph et al. 2004).

The chemical nature of the carbohydrate matrix and the orientation of cellulose microfibrils influences lignin deposition (Ralph et al. 2004). In the middle lamella and the primary wall, lignin forms spherical structures. In the secondary wall, lignin forms lamellae, which follow the orientation of microfibrils. During deposition, lignin could form chemicals bonds with hemicellulose components in the cell walls as it gradually eliminates water, forming a hydrophobic environment (Ralph et al. 2007).

1.2.3 Cellulose

The wood mainly consists of cellulose, which is most abundant in the secondary cell wall (Figure 7). It consists of 40–45% of the dry substance of most wood species (Sjöström 1993). Cellulose is a homopolymer consisting of β-1,4-glycosidic linked D-glucopyranose units (Sjöström 1993, Sixta 2006). Cellulose molecules in crystalline regions are strictly linear and form intra- and intermolecular hydrogen bonds.

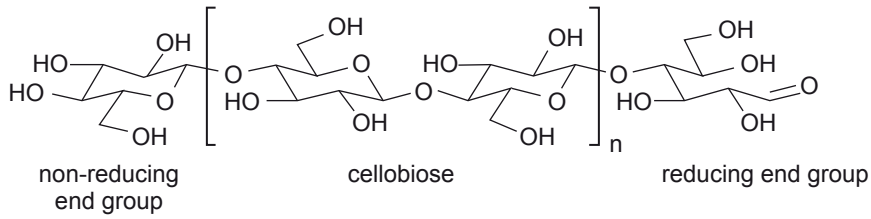

Figure 7. *Cellulose structure.*

Intramolecular H-bonds are formed between adjacent monomers in one cellulose chain and intermolecular H-bonds between adjacent cellulose chains, in crystalline cellulose. Due to the repeating H-bonds and fibrous structure, native cellulose has a high tensile strength and is resistant to most solvents. The supramolecular structure of cellulose makes it a complex substance (Sixta 2006). Highly ordered domains are called crystalline and less ordered domains are called amorphous. Cellulose microfibrils with diameter of 3.5 nm, build fibrils with larger diameter of 3–35 nm, and fibrils form larger cellulose fibers with hemicelluloses with even larger 10–30 nm diameter (Sjöström 1993, Sixta 2006). The microfibrills form so-called macrofibrils (Sixta 2006), also termed fibrillar bundles, with hemicellulose and lignin.

1.3 Biomass pre-treatment and conversion

Usually biomasses have to be pre-treated before they can be utilized. Biomasses are mostly recalcitrant, meaning that they are resistant to microbe and enzyme deconstruction (Himmel 2008). Pre-treatments aim to separate hemicellulose from cellulose, disrupt and remove the lignin sheath, decrease crystallinity of cellulose, increase the accessible surface area of cellulose and to increase the cell wall pore size of facilitate the penetration of hydrolysing catalysts (Alén 2011). Thermal and chemical pre-treatments must be severe enough to access the cellulose core, but not too severe as sugars could be modified to non-fermentable or toxic compounds (Himmel 2008).

Typical conversion or pre-treatment methods for biomasses could be pyrolysis, gasification, direct combustion, acid, alkali, enzymatic hydrolysis or chemical fractionation, for example, with organic solvents (Figure 8). These methods can produce a wide range of products from energy, pyrolysis oil, synthesis gas, or sugar monosaccharides. Synthesis gas can be converted to fuels and sugars can be further chemically or enzymatically modified to produce fuels (hydrocarbons) or chemicals (Alén 2011, Stuart and El-Hawagi 2013). One recent fractionation method to produce fuels and chemicals from wood lignocellulosics is sulphur dioxide ethanol water (SEW) fractionation (Yamato et al. 2011, 2014 and Iakolev and van Heiningen 2012).

Recently emerging methods for biomass conversions to fuels and chemicals use ionic liquids. Ionic liquids are molten salts that are liquid below 100 °C (Brandt et al. 2013). They typically have very low vapour pressure at room temperature (Mäki-Arvela et al. 2010). Ionic liquids can dissolve hemicelluloses, lignin, and cellulose. They can also be used to dissolve sawdust and chips with varying yields to obtain cellulose (Brandt et al. 2013). Nevertheless, the cost of ionic liquids is quite high, the biomass loadings in processes need to be increased, the recyclability of ionic liquids needs to be improved and health or environmental impacts need to be looked into (Brandt et al. 2013).

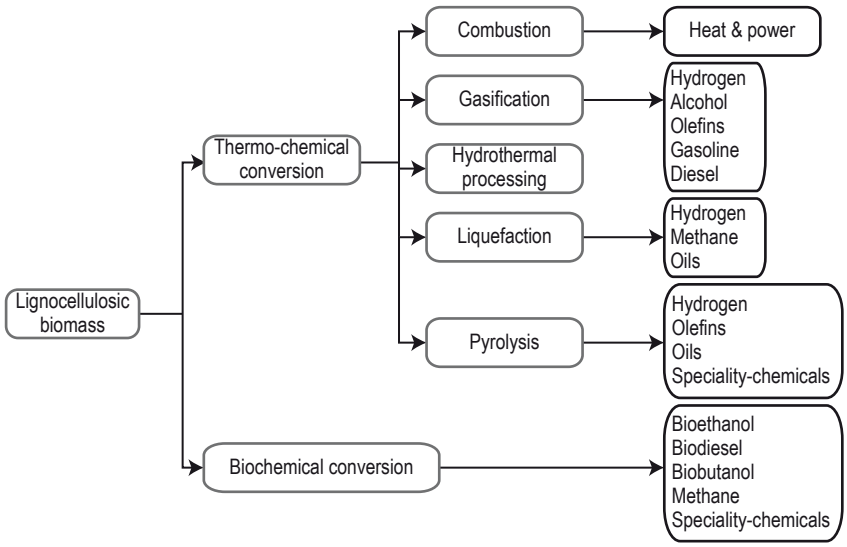

Figure 8. *Examples of lignocellulosic pretreatments (Menon and Rao 2012).*

1.3.1 Water extractions

Water is an environmentally friendly extraction media (Garrote et al. 1999) and it is extensively used in industry. Water extractions usually do not need catalysts or chemicals when used. From an atom economy viewpoint, hydrolytic reactions preserve the carbon contained in the biomass carbohydrates for further processes (Dusselier et al. 2014). Pressurized hot water extraction (PHWE) at subcritical temperatures can be used to modify the physical properties of water (Carr et al. 2011, Kronholm et al. 2007). The dielectric constant (relative permittivity) of water decreases at subcritical temperatures (Teo et al. 2010) and therefore water can dissolve more semi-polar compounds. Water at 200 °C has a similar dielectric constant to dimethylsulfoxide (DMSO). The pressure has a negligible effect on the dielectric constant under 1000 bar. The temperature also enhances the penetration capabilities of water into the sample matrix by lowering the viscosity and surface tension of water. The increased diffusivity improves the mass transfer of dissolved compounds, thus improving the extraction efficiency (Mustafa and Turner 2011). Temperature and pressure have an effect on the surface of the particles (Ong et al. 2006) by disrupting the strong van der Waals and H-bonding solute-matrix interactions, as well as dipole attraction of the solute molecules and active sites of the matrix. The desorption process of extracted material is increased with higher thermal energy on the surfaces of particles. The flow of fresh fluid in a flow-through

extraction vessel increases the concentration gradient between the extraction fluid and surface of the sample matrix, which increases the mass transfer from the matrix, i.e. increases extraction efficiency. The ionic-product of water increases by rising temperature, providing more H+ and OH- ions in water, allowing for an increase in the catalysis of hydrolytic reactions (Akiya and Savage 2002). Dissociation of weak organic acids, such as acetic acid, is slightly decreased in higher temperatures reducing the amounts H+ ions that catalyse hydrolytic reactions. (Shock 1995).

Many synonyms have been used in the literature to define different how water or steam treatments, with different effects, on lignocellulosic materials: Autohydrolysis, hydrothermolysis, aqueous liquerification or extraction, aquasolv, water prehydrolysis, hydrothermal pre-treatment, hydrothermal treatment, steam pre-treatment and steam treatment have been used in literature (Garrote et al. 1999). Hardwoods are more favourable than softwoods for hydrothermal treatments because they have more acetyl groups present in the hemicelluloses.Accordingly, more acetic acid is released from hardwoods than from softwoods during pressurized hot-water treatments, which catalyses further hydrothermolytic biomass degradation (Garrote 1999).

1.3.2 Batch extractions

Batch extractions are performed using a reactor, where the biomass is placed. Reactors are heated and water is kept under constant pressure. To remove hemicelluloses, temperatures are usually under 200 °C, since water heating consumes energy and furfurals start to form from monosaccharides released at higher temperatures. Hardwood such as birch sawdust (Borrega et al. 2011a, 2011b) and chips (Testova et al. 2011, Borrega et al. 2013) have been treated with hot water. Aspen, birch and maple chips were hydrolyzed with hot water (Li et al. 2010) and hardwood chips with a mixed continuous batch reactor (Chen et al. 2010). Eucalyptus (Vázquez et al. 2005), sugar maple (Mittal et al. 2009a), aspen, and sugar maple (Mittal et al. 2009b) have been extracted with hot water.

Spruce galactoglucomannans have been extracted with hot water in a batch system (Song et al. 2008), using sodium bicarbonate to adjust pH during extraction (Song et al. 2011) and phthalate buffer (Song et al. 2011). The effect of particle size on the extraction yields of has been studied (Song et al. 2012, Krogell et al. 2013). GGM has been extracted and then isolated with membranes and ethanol precipitation (Song et al. 2013). The degradation of GGM in water has also been modelled (Visuri et al. 2012).Importantly, pH during extractions has been measured using a special high-temperature pH probe to monitor the actual pH inside a reactor (Krogell et al. 2014).

1.3.3 Flow-through extractions

In 1964, Bobleter and Pape patented a method using flow-through extraction for biomass. Since then this hydrothermal extraction method has been used to treat several different types of biomasses. Birch shavings (Hörnmeyr et al. 1988), aspen (Bonn et al. 1983), cellulose, and straw (Bobleter et al. 1976) have been treated with this method. A comprehensive review of these hydrothermolytic treatments can be found in a review paper (Bobleter 1994).

More recent studies have been completed using flow-through extractions as pretreatment to produce ethanol from corn stover (Liu and Wyman 2003, 2004). Authors compared batch and flow-through methods and found that flow-through extraction resulted in higher xylose yield, greater lignin removal and less extensive hydrolysis or general breakdown of hemicelluloses (Liu and Wyman 2004). Flow-through with a partial flow was used to reduce the usage of water with corn cob extraction (Liu and Wyman 2005). Flow-through extraction has been compared with other techniques such as aqueous ammonia recycling, ammonia freeze explosion, batch, controlled pH, dilute acid hydrolysis, partial flow-through, and lime pretreatments using corn stover (Liu and Wyman 2004, Wyman et al. 2005, Mosier et al. 2005). Flow-through pre-treatment increased the accessible surface, removed hemicelluloses and lignin, and modified remaining solids. Remaining solids could be digested with enzymes to produce ethanol. Pressurized hot water (PHWE) flow-through extraction has been used to extract galactoglucomannan from spruce (Leppänen et al. 2011). The extraction kinetics of spruce hemicelluloses was studied with an experimental cascade reactor (Grénman et al. 2011). Extraction kinetics and mass transfer of spruce wood with different sized chips have been studied with a cascade reactor and a model was built based on these results (Rissanen et al. 2014a). Similar research was done with the same cascading reactor system to find conditions to obtain hemicelluloses with desired molar mass (Rissanen et al. 2014b).

2 Hypothesis and objectives

The hypothesis of the work was that it is possible to obtain polymeric, water-soluble hemicelluloses from birch sawdust using flow-through PHW extractions at both laboratory and large scale.

The main objectives of this study were thus to obtain naturally acetylated hemicelluloses from birch sawdust using PHW extractions performed mainly under 200 °C and to isolate polymeric, water-soluble hemicelluloses of high molar mass. The aims were to characterize extracts and extractions residues and to make mass balances based on results of different extraction temperatures.

The extraction yield and water usage are also important parameters, so sawdust was packed to higher density to obtain extracts with higher concentration than typical extractions. The information about the properties of the extracted sawdust is important allow for further processing in biorefineries and was to be included in the study. Finally these experiments were to be scaled up from the laboratory scale experiments to pilot scale, to see if there would be any differences in mass balances or extraction kinetics between two different sized extraction systems.

3 Materials and methods

3.1 Birch sawdust

Birch sawdust was obtained from Ruotsinkylä research station in southern Finland and used as a raw material or the experiments reported in the four papers (I-IV). For the scale up experiments (V), birch sawdust was obtained from Koskisen Oy sawmill (Hirvensalmi). After samples were collected, they were transferred to Vantaa and stored in a freezer at -20 °C in closed plastic bags. The particle size distribution of sawdust was determined with three different sized sieves in papers I-IV and with 8 sieves in paper V. Birch sawdust particle size used in papers I -IV is given in Table 2.

The particle size fraction that had the largest amount of sawdust was between 0.2-0.63 mm. The most of the sawdust was larger than 0.2 mm. The above-mentioned birch sawdust that was used in papers I-IV had 40% of cellulose, 27% of lignin and 25% of hemicelluloses. The rest of the compounds were acetyl groups and extractives. The particle size distribution of the sawdust that was used in paper V, obtained from Koskisen Oy sawmill, is presented in Table 3.

The distribution percentage shows how much each sieve retained. The most of the birch sawdust particles were larger than 0.5 mm, with more than 50% in the size range of 1-2 mm. Cellulose, hemicelluloses, lignin, and extractives contents of birch sawdust are shown in Table 4.

Table 2. *Birch sawdust particle size distribution (Papers I-IV).*

Sieve	Sawdust, %
> 2.0 mm	1
2.0–0.63 mm	28
0.63–0.2 mm	53
0.2–0.063 mm	18
< 0.063 mm	0

Table 3. *Particle size distribution of the birch sawdust from Koskisen Oy sawmill (Paper V).*

Sieve	Sawdust, %
Pan	<0.1
0.05 mm	1.0
0.1 mm	1.7
0.2 mm	9.2
0.5 mm	22.6
1 mm	51.0
2 mm	8.2
4 mm	4.3
8 mm	2.0

Table 4. *Chemical composition of birch sawdust from Koskisen Oy Sawmill (Paper V).*

Compound	Wt %
Cellulose	39
Hemicelluloses	30
Lignin	21
Extractives	3
Other compounds	7
Sum	100

The sawdust from the Koskisen sawmill contained more hemicelluloses and less lignin than the sawdust obtained from Ruotsinkylä research station. Other compounds include acetyl groups, ash, and proteins and were calculated by subtracting the sum of known compounds from the total.

3.2 Analytical methods

Sawdust, extracts, and extracted sawdust were characterized with various analytical methods. The amounts of hemicellulose, lignin and cellulose were determined in papers I, II, IV and V. Extracts were characterized from the liquid phase. Sawdust and extracted sawdust were characterized with similar methods. The main aim of work was to characterize the amount of the hemicelluloses so the main analysis method used in these studies was acid methanolysis (Willför et al. 2009).

Solid sample analyses

Sawdust and extracted sawdust was freeze-dried before analyses. Extractives from sawdust were removed with accelerated solvent extraction (ASE) using a mixture of ethanol/toluene 1:2 (v/v) and were determined gravimetrically (Paper I). Extractives were determined gravimetrically from sawdust in paper V by extracting them with ASE using acetone-water (95/5, v/v). Samples were ground with a mill using 0.5 mm sieve before analysis.

For mass balance calculations, the extracted sawdust was removed from the extraction vessel, freeze-dried to a constant weight, and the amount of extraction residue was weighted. This weight was used in the comparison of cellulose, lignin, and hemicelluloses in original and extracted sawdust.

Hemicelluloses were analysed with acid methanolysis. Depending on the sample, 10-20 mg of sawdust or extracted sawdust was treated (Willför et al. 2009). Acid methanolysis cleaves hemicelluloses and forms methylglycosides, which are analysed with GC-FID. Methanolysis gives the amounts of sugars

in hemicelluloses, oligosaccharides, and monosaccharides in the sample. Methanolysis cannot cleave highly ordered (crystalline) celluloses, so the amount of glucose in hemicelluloses can be determined (Willför et al 2009.).

The amount of cellulose in samples was determined with acid hydrolysis according to Sundberg et al. (2003). Samples were hydrolysed and the amount of glucose was determined with GC-FID. To calculate the amount of cellulosic glucose, the amount of glucose in hemicelluloses obtained with acid methanolysis was subtracted from the amount of glucose obtained with acid hydrolysis.

Lignin in solid samples was determined using two different Klason lignin methods. In paper I lignin was hydrolysed using maceration of samples. In papers II, IV and V lignin was determined according to the so-called KCL method using an ultrasonic bath to promote hydrolysis (Raiskila et al. 2007). The material remaining after hydrolysis was weighted to get the amount of Klason lignin. Soluble lignin was measured with an UV-spectrometer from the liquid phase at 203 nm.

The samples were delignified using the Wise-method followed by treatment in ethylenediamine (EDA) prior to the analysis of molar mass distribution by gel permeation chromatograph (GPC) (Wise et al. 1946; Yamamoto et al. 2011). The solutions formed were diluted with pure DMAc (0.5 ml of cellulose solution 4.5 mL of DMAc) to a concentration of 1 mg/mL and were filtered using 0.2 mL syringe filters prior to the GPC analysis.

The GPC system consisted of one PLgel Mixed-A (7.5 x 50 mm) column , four PLgel Mixed-A (7.5 x 300 mm) columns and a Shodex RI-detector (RI-101). The analysis was carried out at room temperature using 9 g/L LiCl/DMAc as eluent. Duplicate analyses were performed with each sample. Pullulan standards were used to calibrate the system. The molar masses of pullulan standards were corrected to those of cellulose by using a previously published correction factor (Berggren et al. 2003). The molar mass distributions were calculated using a MATLAB script written at Aalto University.

X-ray scattering measurements under perpendicular transmission geometry were executed with a setup consisting of a Seifert ID 3003 X-ray generator (voltage 36 kV, current 25 mA) equipped with a Cu tube (wavelength 1.54 A°), a Montel multilayer monochromator and either a Bruker AXS HI-STAR two-dimensional wire detector (SAXS measurements, sample-to-detector distance 59 cm) or a MAR345 image plate detector (WAXS measurements, sample-to-detector distance 7 cm).

WAXS intensities of the samples with smallest particle size (0.063–0.2 mm) were also measured using a four-circle diffractometer in symmetrical reflection mode.

In the X-ray microtomography (XMT) experiments, two individual grains of both freeze-dried original sawdust and extracted sawdust (Extracted sawdust 0.2 kg/l) were attached to a carbon fibre rod using a cyanoacrylate adhesive, and scanned with Nanotom 180NF XMT equipment (PhoenixjX-ray systems and services GmbH, Germany). Each scan consisted of 1,200 X-ray transmission images, with the imaging geometry resulting in an effective pixel size of 0.5 x 0.5 μm^2. The X-ray tube voltage was set to 60 kV. The three-dimensional XMT volumes were reconstructed with 0.5 x 0.5 x 0.5 μm^3 voxel size, using datosjx-reconstruction software supplied by the equipment manufacturer. They were then visualized and binarized with using the Avizo Fire software (VSG, France). From the binarized data, volume-weighted thickness distributions for the cell walls were calculated using the Local Thickness plugin to ImageJ software (Dougherty and Kunzelmann 2007).

Extract analyses

Extracts were collected in volumetric flasks during the extraction. The pH of the sample was measured straight from the extracts at room temperature. After the pH measurements the extracts were diluted to a constant volume. The extracts were either analysed straight away or stored in freezer at -20 °C to wait for later analyses.

To obtain real time information during the extraction, the refractive index of the sample was measured using the °Brix scale. The refractive index gives the amount of hemicellulose and other sugars in the samples. A calibration curve for hemicelluloses was made using the results from acid methanolysis. Calibration curves were made for galactoglucomannans (GGM) of spruce and xylans from birch. By using these calibration curves, it was possible to estimate the amount of hemicelluloses in the extracts during the extractions.

Total dissolved solids (TDS) gave the amount of the material dissolved from sawdust during the extractions. The different methods were used to determine the amount of TDS. In the papers I and II, TDS was determined with a Kern mlb 50-3 moisture analyser. At least duplicate samples were dried at 138 °C. The second method was freeze-drying after the sample was frozen. Freeze-dried samples were placed in desiccator after drying and then weighted. The third method was traditional oven drying at 105 °C. After drying, samples were cooled down in desiccator and weighted. The TDS result includes all solid and suspended material, but volatile compounds in extracts such as acetic acid evaporates during drying.

The amount of hemicellulose in the extracts was determined using acid methanolysis (Willför et al. 2009). The samples were frozen and then freeze dried to a constant weight. The acid methanolysis forms methylglycosides from hemicelluloses, oligosaccharides and monosaccharides. The amount of

these compounds in the extract can be calculated after silylation and GC-FID analyses.

Monosaccharides were analysed straight from the freeze-dried samples by silylating and analysing with GC-FID. The ratio of hemicelluloses and oligosaccharides from acid methanolysis can be compared against the amount of monosaccharides present in extract. Since hydrolysis cleaves hemicelluloses to oligosaccharides and then to monosaccharides, the amount of monosaccharides gives indication of how strong hydrolysis was during the extraction.

Furfurals were analysed from samples with high performance liquid chromatography with a diode array detector (HPLC-DAD). The amount of furfural and hydroxymethyl furfural were quantified against external standards at wavelengths 277 and 284 nm, respectively.

Acetic acid in the extracts and acetyl groups present in hemicelluloses were determined using HPLC with either ultraviolet (UV) or refractive index (RI) detectors. The amount of acetic acid was determined straight from the extract using external standards. The amount of acetyl groups in hemicelluloses were determined by saponifying the acetyl groups at high pH. The acetyoxy groups were then determined by HPLC after lowering the pH to form acetic acid. The amount of acetyl groups in xylans were calculated by subtracting the amount of acetic acid in the extracts from the amount of acetic acid in the extracts after saponification.

Molar masses of the extracted hemicelluloses were determined using size exclusion chromatography multi angle laser light scattering (SEC-MALLS). Extract was diluted and filtered before injection to the SEC-MALLS. The light scattering in MALLS detector gives the hydrodynamic radius distribution of the hemicelluloses. With known literature values of different sized hemicelluloses, it is possible to calculate the molar mass distributions of xylan.

3.3 Pressurized hot water flow-through extraction systems

Laboratory scale extraction system

Most of the experiments were performed in laboratory scale (I-II, IV-V). Pressurized hot water extraction (PHWE) was performed using a constant flow of water through the extraction vessel during the whole extraction time. The scheme of the laboratory scale extraction system is shown in Figure 9.

The extraction vessel was filled with typically 10 g of sawdust. A HPLC pump was used to pump the ion exchanged water into a pre-heating capillary where it was heated. Usually a 4 mL/min flow was used but the flow could be adjusted from 0.1–10 mL/min. The heated water the flowed through the sawdust bed in the extraction vessel (Figure 10).

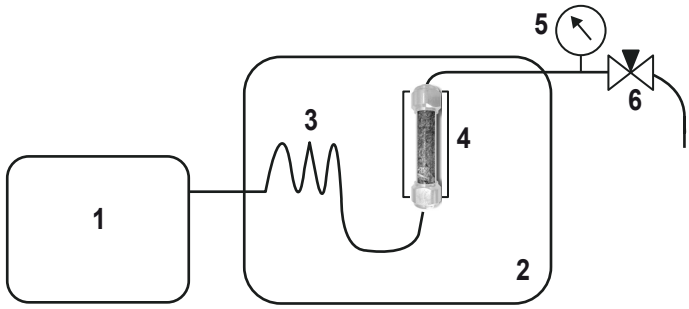

Figure 9. *Scheme of the laboratory scale extraction system used in papers II, III-IV. 1. HPLC pump; 2. GC-oven; 3. Heating capillary; 4. Extraction vessel; 5. Manometer; 6. Valve (Paper I).*

Figure 10. *A 50 mL flow-through extraction vessel (Paper V).*

The pressure inside extraction vessel was controlled using a manometer and a valve. The pressure was kept at 50–70 bars during the extraction to keep water in liquid form. Usually 10 minutes of successive fractions were collected. To get more detailed information about the extraction kinetics 5 minute fractions were collected in some cases. The overall extraction times were from 30 minutes to 60 minutes. Longer extraction times were not used in order to reduce the amount of water used in the extractions.

Pilot scale extraction system

In the scale up experiments, a laboratory pilot scale extraction vessel was used (V). The height and width of the vessel was chosen to be the same ratio as the laboratory scale extraction vessel.

The volume of the extraction chamber was 300 L. Pressures between 0-25 bar could be utilized and extraction temperatures could be adjusted between 10–225 °C with a flow rate of 6–100 kg/min. Approximately 50-60 kg of dry spruce sawdust and 65-75 kg of dry birch sawdust could be placed inside the extraction chamber. The sawdust bed could be heated by hot water flowing through the sawdust bed. The other method was to allow the hot water to evaporate and let the hot steam to pre-warm the sawdust bed. The scheme of the pilot scale system is shown in Figure 11.

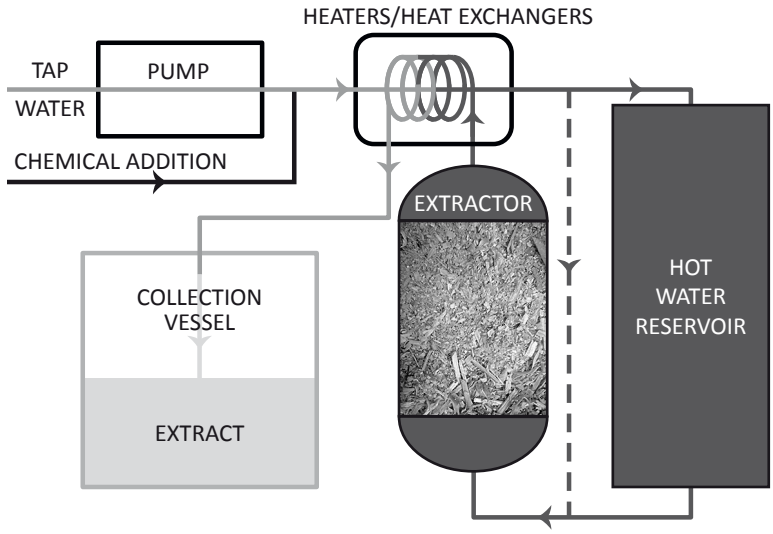

Figure 11. *Scheme of the pilot scale PHWE flow-through system (Paper V).*

Typical pilot scale extraction started when the tap water was pumped to heat exchangers. Heat exchangers heated the hot water and it was pumped into the hot water reservoir. The reservoir was filled until there was enough water for the whole extraction. Hot water was then pumped from the reservoir into the extractor. The temperature inside the extractor was monitored in three different heights and depths in the sawdust bed. The extraction started when the first drop of extract fell to the collection vessel. Usually the extraction time was 60 minutes with a 20 kg/min flow rate.

The flow rates of water and extract inside the extraction system were controlled by 5 valves and 3 manometers. The extraction temperature was monitored inside extraction vessel with 9 thermocouples at three different heights. There were three thermocouples at each height inside hollow metal rods, which were placed into extraction vessel. The extract was collected in

container with volume of one cubic meter. The container was placed on a scale to monitor the weight of the extract during and after extraction.

3.3.1 Residence time

The residence time is the time over which the hot water was inside the extraction vessel. The residence time in a batch reactor is the time that extracted material is inside the extraction vessel. In case of a flow-through vessel, water is constantly flowning through the extraction vessel, so the residence time of the water inside the vessel varies according to the extraction flow rate. It can be calculated using equation 1,

$$t = \frac{V\,vessel - V\,sawdust}{q} \tag{1}$$

where t = residence time, min, V_{vessel} = volume of the extraction vessel, mL, $V_{sawdust}$ = volume of sawdust solids, mL, q = flow through the extraction vessel, mL/min (Papers II, V).

The sawdust cell walls take some volume when they are in extraction vessel. The volume of sawdust solids can be calculated using their density, which is 1.530 g/mL (Sixta 2006).

4 Results and discussion

4.1 Pressurized hot water extraction of acetylated xylan from birch sawdust (I)

The first experiments with pressurized hot water were performed to find suitable initial extraction conditions, to obtain acetylated xylans from birch. Temperatures were chosen from the results of the earlier PHWE flow-through extractions with spruce (Leppänen et al. 2011). Extraction times were short, 30 minutes, and one important aim was to use less water than in previous experiments. The whole 30 minutes extract was collected in one volumetric flask and was then analysed.

Total dissolved solids (TDS) and mass balance

Total dissolved solids give an overview of how much material is extracted from the sawdust. The first extraction series performed over the range of 140–200 °C gave information about how much material is possible to extract out of the birch sawdust.

The highest yield of obtained at 190°C closely followed by 180°C. There was a large increase in TDS between 160°C and 170°C compared to the yields at lower temperatures, which were under 5% of the birch sawdust. Two TDS determination were performed at 140°C, 150°C and 200°C. At temperatures between 160 and 190°C, results are an average of two extraction series with four determinations.

The second extraction series was performed over the range where yields were highest, i.e. between 160 and 200 °C (Table 5). The large variation at 200 °C is a result of the formation of a black precipitate during the extraction. The black precipitate fouled the lines and glassware. Similar variation in yields was observed from the mass balances of the extractions.

Table 5. *Mass balances of the second extraction series at temperatures from 160–200 °C.*

Temperature, °C	Dry residual weight, %	TDS,%	Sum, %	Lost material, %
160	87.1	5.2	92.3	7.7
170	75.4	17.9	93.3	6.7
180	67.6	27.8	95.4	4.6
190	57.9	33.7	91.6	8.4
200	55.1	29.4	84.5	15.5
Sawdust	100.0	0.0	100.0	0.0

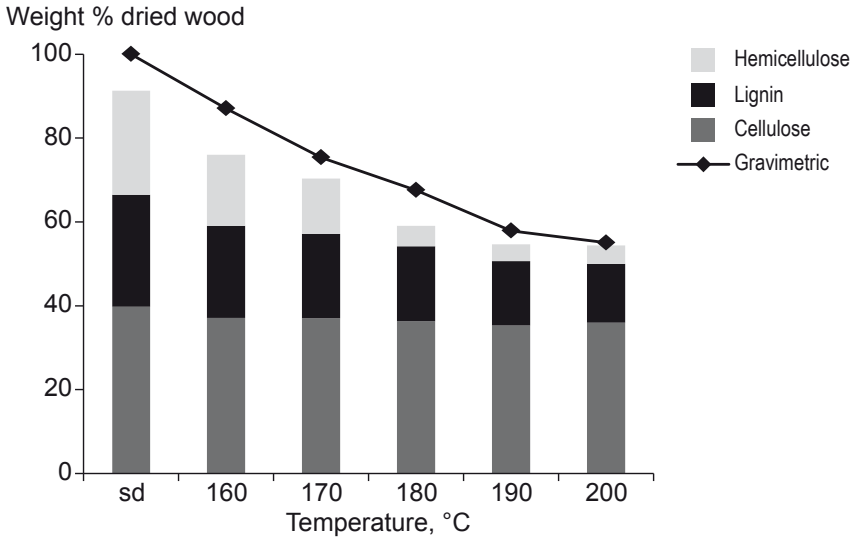

Figure 12. *Comparison of gravimetric and analytical data of the extraction residues.*

The mass loss in the sum balance could be explained due to the loss of volatile compounds, such as acetic acid and furfural. They are lost when the extract is evaporated during the TDS determination. Uronic acids could also be de-methylated and de-carboxylated at high temperatures.

To see the effect of the extraction on the extracted sawdust, the amounts of hemicellulose, lignin and cellulose in the extracted sawdust were analysed. The extracted sawdust was collected, freeze-dried, weighted, and then analysed (Figure 12).

Gravimetric analysis shows the mass percentage of wood left after extraction, compared to the original amount of the dry wood. Hemicelluloses, lignin, and cellulose values are from analytical determinations of extracted residue (Paper I).

At 200 °C almost half of the wood was solubilized during the 30 minute extraction. The amount of cellulose was almost constant at all extraction temperatures. Only a small 5–10% difference was observed at temperatures between 160 and 200 °C. The amount of lignin remaining in sawdust decreased from 82% of original lignin left at 160 °C to 52% left at 200 °C. Hemicelluloses were solubilized rapidly during the extractions. Most of the hemicelluloses, 70%, were left after 160 °C extraction. But at 200 °C, only 18% of the hemicelluloses were remaining in the wood material.

Extracted hemicelluloses and hemicelluloses in the extraction residue

The extracted residual wood still contained hemicelluloses after 30 minutes PHW extraction at 160–200 °C (Table 6).

Table 6. *Carbohydrate composition of extracts and extraction residues (Paper I).*

Extract (weight % of dried wood)										
T, °C	**Man**	**Glc**	**Gal**	**Xyl**	**Ara**	**Rha**	**GlcA**	**GalA**	**MeGA**	**total**
160	0.3	0.7	0.1	1.0	0.2	0.1	0.0	0.2	0.0	2.5
170	0.5	0.9	0.3	5.6	0.2	0.2	0.0	0.4	0.1	8.1
180	0.8	1.1	0.4	9.5	0.2	0.2	0.0	0.5	0.2	12.9
190	1.0	1.2	0.4	12.1	0.3	0.3	0.0	0.5	0.3	16.0
200	0.9	1.3	0.4	9.8	0.3	0.2	0.0	0.3	0.2	13.5
Extraction residue and starting birch sawdust (weight % of dried wood)										
T,°C	**Man**	**Glc**	**Gal**	**Xyl**	**Ara**	**Rha**	**GlcA**	**GalA**	**MeGA**	**total**
160	0.1	0.9	0.6	13.7	0.0	0.2	0.0	0.6	0.8	16.9
170	0.0	0.8	0.5	10.6	0.0	0.2	0.0	0.5	0.6	13.2
180	0.0	1.4	0.2	3.2	0.0	0.0	0.0	0.0	0.0	4.9
190	0.0	1.6	0.1	2.3	0.0	0.0	0.0	0.0	0.0	4.0
200	0.0	1.6	0.1	2.6	0.1	0.0	0.0	0.0	0.0	4.4
sd	1.1	2.1	0.8	16.6	0.3	0.3	0.1	1.6	2.0	24.9

Man = mannose, Glc= glucose, Gal = Galactose, Xyl = Xylose, Ara = Arabinose, Rha = Rhamnose, GlcA = Glucuronic acid, GalA=Galacturonic acid, MeGA = 4-*O*-methylglucuronic acid and sd=sawdust. Carbohydrates were determined by methanolysis. Results are represented as the weight percent of original dry sawdust and calculated as anhydrosugars.

The amount of carbohydrates in the extraction residue and in the extract was smaller than the amount in the starting birch sawdust. The lost sugars were mostly xylose and 4-*O*-Mmethylglucuronic acid. The amount of pectins (galactouronic acid and rhamnose) in the extraction residue decreased at higher temperatures. The glucose amount probably increased due to the hydrolytic cleavage of cellulose at under acidic conditions and at high temperatures. The amount of xylose in the extract at 200 °C was substantially lower than in the extract at 190 °C. This could be due to the formation of furfurals from xylose and the formation of black sticky precipitates (Borrega et al. 2011).

Molar mass of extracted xylans

The average molar mass (Mw) of extracted xylans was determined with SEC-MALLS. Xylans and lignin can form aggregates, which can have a strong effect on the molar mass measurements. HPSEC chromatograms determined with a refractive index detector are shown in Figure 13.

Larger molecules elute earlier than smaller molecules in the HPSEC system. There is a wide peak at 37 min at temperatures from 140 °C to 170 °C. This could be from non-cellulosic carbohydrates such as starch, since starch

is solubilized at lower temperatures than xylans. The average molar mass of extracted xylans decreased as the extraction temperature increased (Table 7).

At higher temperatures than 180 °C the largest peak is near 40 minutes (Figure 13), showing that the amount of polymeric xylans increases. The peak area at approximately 42–43 minutes is significantly larger at 190 °C and 200 °C. There could be more monosaccharides and oligosaccharides derived from hemicelluloses than at lower extraction temperatures. The molar masses at 180–200 °C were lower than the molar masses at lower extraction temperatures.

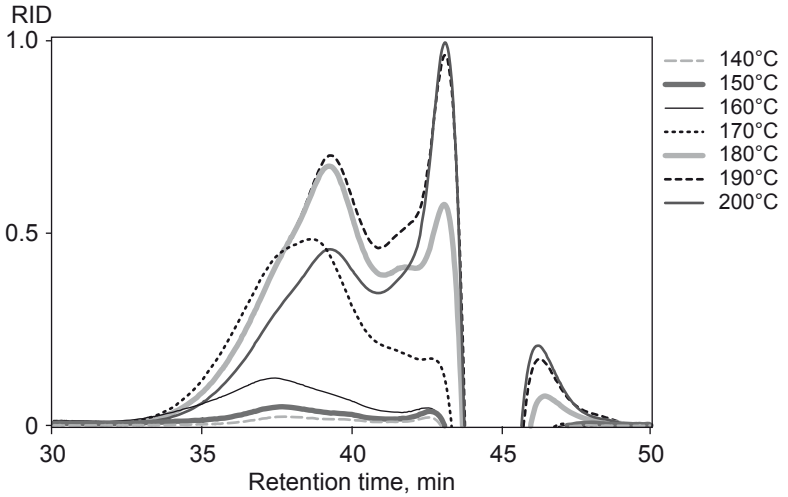

Figure 13. *SEC-RID chromatogram of carbohydrates in extracts obtained at different extraction temperatures. Chromatograms in the range 140–150 °C are from the first extraction series, while the others from the second extraction series at 160–200 °C (Paper I).*

Table 7. *Average molar mass of xylans (Paper I).*

Extraction temperature, °C	Mw, kDa
140	a
150	a
160	33
170	5.6
180	2.5
190	2.6
200	2.3

a = reliable molar mass of xylans could not be determined due to the presence of particles/aggregates

The proportion of monosaccharaides compared to oligo- and polysaccharides increased at higher extraction temperatures. The largest amount of monosaccharides compared to oligo- and polysaccharides were obtained at 200 °C, where 26% of carbohydrates were present as monosaccharides. At 180 °C and 190 °C the proportion of monosaccharides was 16% and 10%, respectively. On the other hand, the amount of total carbohydrates in the extracts dropped dramatically when the extraction temperature was raised from 190 to 200 °C. The amount of xylan and pectins decreased (Table 6) and this degradation of pentoses and uronic acids can lead to the formation of furfurals. Furfurals in these conditions could then form condensates and resins.

Acetic acid and acetyl groups in xylans

During PHWE, some acetyl groups of the native xylan are cleaved to give acetic acid. The amount of released acetic acid increased with increasing extraction temperature (Figure 14).

Some free acetic acid was probably lost during the extraction at 190 °C, because of problems recovering volatile compounds with this extraction set-up. The release of acetic acid from xylans also decreased the pH of the extracts. The pH was highest in the extract obtained from 140 °C, where it was near 5. As the temperature increased to 180–200 °C, pH was near 3.2.

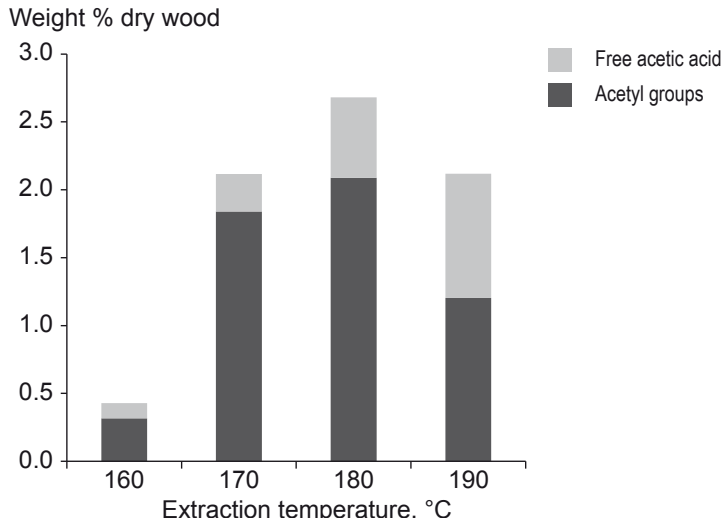

Figure 14. *The amount of free acetic acid and acetyl groups in hemicelluloses in extracts (Paper I).*

Furfurals
Only trace amounts of furfurals could be found in the extracts. The amount of furfural increased to79 μg/L as the temperature increased to 190 °C and pH decreased. Furfural can be formed form pentoses at these extraction temperatures (Zeitch 2000). Formation of furfural condensation products (resins) mixed with extracted lignin related substances could explain the black sticky precipitate, which was formed at the 200 °C extraction. The amount of hydroxymethyl furfural, which is formed from hexoses, was under 6 μg/L in all extraction temperatures between 140–190 °C.

Precipitate
When the extracts cooled down, some precipitate was formed. At 160 °C, 0.9% of the sawdust formed precipitate. The largest amount of precipitate was found at 190 °C, where the amount of precipitate was 5.4% of the original sawdust. The precipitate was mostly comprised of lignin, but there were 6–12% of carbohydrates, which were mainly glucose and xylose. The presence of lignin and carbohydrates could be due the lignin-carbohydrate complexes (LCC) that have been found in hardwoords (Henriksson et al. 2007) and after water extraction (Tunc et al. 2010).

Concluding remarks
Pressurized hot water flow-through extraction can extract acetylated and water-soluble polymeric xylans. About 70% of the total xylan can be extracted from birch sawdust and the most of the extracted xylan is still polymeric and oligomeric. The amounts and rates of the extracted poly-, oligo- and monosaccharides can be controlled, by using different extraction temperatures and flow rates. Based on these results, if a high yield of xylans and low yield of lignin is wanted, extraction should be performed at 180 °C. Xylan could be further concentrated and purified using ultrafiltration and precipitation with ethanol.

4.2 The effect of sawdust density and sawdust size (II-III)

The objective of the packing experiments was to increase the concentration of xylans in the PHW extracts. In a typical water extraction, sawdust was lightly pressed inside the extraction vessel and then the vessel was closed. The sawdust density in the extraction vessel was increased by packing the sawdust more tightly into the vessel. The volume of the extraction vessel (50 mL) and the amount of water (280 mL) was constant during these experiments. Therefore it was possible to use less water, compared to the amount of sawdust, with higher packing degrees.

The different sized sawdust was also packed in three different densities to evaluate the effect of the density and particle size on the extraction process.

Extraction parameters

Two different extraction series were performed for birch sawdust. The extraction temperature was 180 °C for 60 minutes with 4 mL/min flow through the extraction vessel. The first extraction series was performed with unfractionated sawdust with six different sawdust densities (Table 8). The second extraction series was performed with three different sized sawdust and three different densities.

Table 8. *The degree of packing, sawdust sizes, residence times, and liquid to wood ratios of the two extraction series. The two extraction series with unfractionated and fractionated sawdust are separated with the dotted line (Paper II).*

Degree of packing, kg/L	Fraction, mm	Residence time, min	Liquid to wood ratio
0.2	Unfractionated	10.9	28
0.3	Unfractionated	10.2	20
0.4	Unfractionated	9.4	15
0.5	Unfractionated	8.6	12
0.6	Unfractionated	7.8	10
0.7	Unfractionated	7.0	8
0.2	0.063–0.2	10.9	29
0.5	0.063–0.2	8.6	12
0.5	0.063–0.2	8.1	10
0.2	0.2–0.63	10.9	28
0.5	0.2–0.63	8.5	12
0.6	0.2–0.63	7.7	10
0.2	0.63–2.0	10.9	29
0.5	0.63–2.0	8.5	11
0.6	0.63–2.0	7.6	9

Total dissolved solids

Typically 0.2 kg/L (10 g) of sawdust have been used in previous flow-through extraction experiments (Paper I). In these experiments the density of sawdust was increased by packing it in the extraction vessel.

In the first extraction series with unfractionated sawdust, 58–59% of the birch sawdust was left with 0.2–0.5 kg/L density. With higher densities the amount of residue increased and the amount of dissolved solids decreased.

At the lowest density of fractionated sawdust, the largest particle size had the smallest amount of dissolved solids and largest amount of residue left. The smaller sawdust sizes had the higher TDS yield.

On the other hand, at packing degrees of 0.5 kg/L and 0.6 kg/L of fractionated sawdust, extraction residues and TDS were quite similar over all particle sizes. The density of the sawdust could reduce the void space between sawdust and the flow could go more evenly through even the largest particles. Some material was missing which could be volatile compounds such as acetic acid and furfural formed in the extraction. These compounds likely have evaporated during the drying of the extract.

The chemical composition and structure of the extraction residues

The amount of cellulose in the extraction residue did not change significantly after the extraction. The molar mass (Mw) of the cellulose of the extraction residues decreased during the extraction and was lower than for typical paper grade pulps (Figure 15).

The peaks were fitted to give for hemicelluloses of around 30 kg/mol for delignified sawdust, and 20 kg/mol for the extracted samples (First large peak at the left and small peak under it in left in Figure 15). The bimodal distribution of the higher molar masses corresponded to cellulose. Delignified

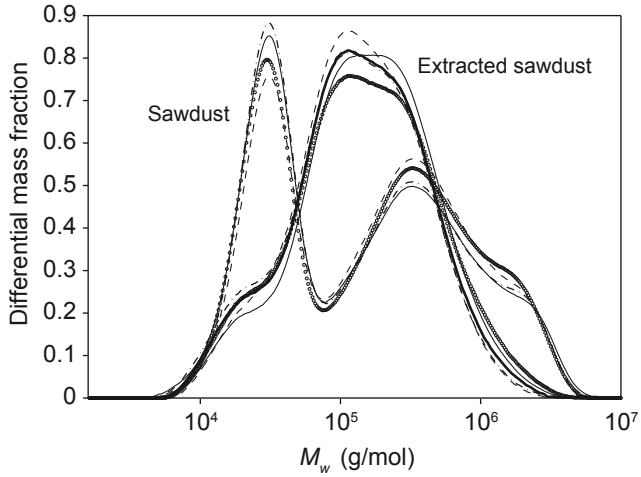

Figure 15. *Molar mass distributions of the birch sawdust samples: Unfractionated (thin line), sawdust 0.063–0.2 mm (dashed line), sawdust 0.2–0.63 mm (circles), sawdust 0.63–2.0 mm (dash-dot line), extracted 0.2 kg/L (thin line), extracted 0.5 kg/L (dashed line), extracted sawdust 0.063–0.2 mm (circles), extracted sawdust 0.2–0.63 mm (dash-dot line), extracted sawdust 0.63–2.0 mm (thick line) (Paper III).*

sawdust had a maxima around 340 kg/mol and 2000 kg/mol. The delignified extracted samples had a maxima around 75 kg/mol and 200 kg/l range. The molar mass averages and hemicellulose contents varied marginally between the samples having different particle sizes but no consistent trend was discovered. The data suggest, however, that the 0.5 kg/L density led to more severe depolymerisation than for the case of the 0.2 kg/L loading. Still, it would be enough to produce dissolving grade pulps, if the rest of the hemicellulose were removed and the extraction residue would be further delignified.

The cellular structure of the wood was mostly preserved during the PHW extraction (Figure 16). The cell walls of the sawdust were thinner after extraction and pores with rough surfaces were opened between microfibrillar aggregates. Microfibrils in the cell wall fibres became more closely packed after the hemicellulose removal.

The original sawdust contained xylans as the main hemicelluloses. Birch sawdust also contains minor amounts of glucomannans, some uronic acid and galactose-containing hemicelluloses. Ca. 18–24% of the hemicelluloses were still left in the extraction residue. The main sugars in the extraction residue

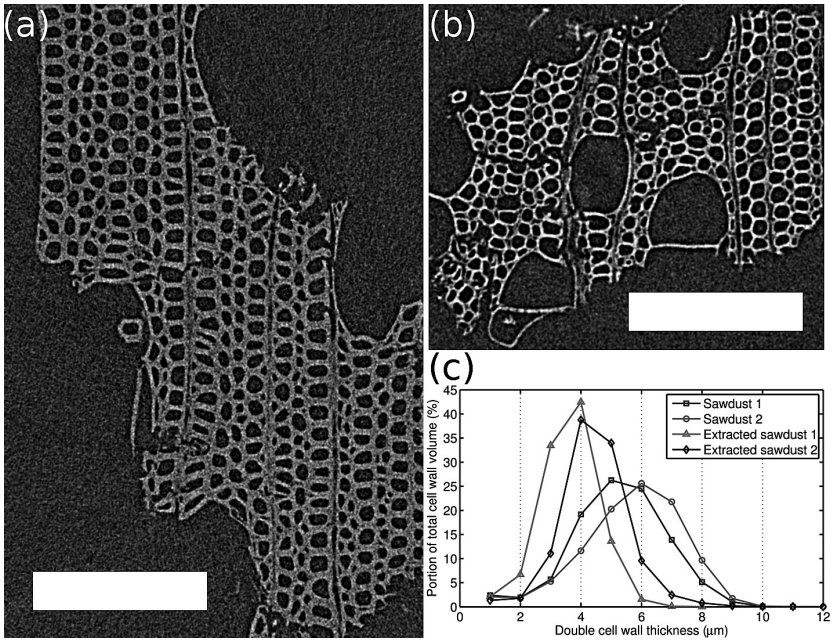

Figure 16. *Example slices from X-ray microtomography reconstructions (scale bar 150 µm): a) Sawdust b) Extracted sawdust 0.2 kg/L c) Double cell wall thickness distributions calculated from the three-dimensional reconstructions (Paper III).*

were xylose and glucose. Cellulose could form amorphous regions during the PHW extraction, which could then be hydrolysed during acid methanolysis and observed as glucose in the analyses. The higher sawdust packing degrees, with 0.6 kg/L and 0.7 kg/L, had somewhat more hemicelluloses left compared to the lower sawdust packing degrees. The major part of the xylan was extracted from the wood. There was 13–18% of xylose left after extraction and almost no 4-*O*-methylglucuronic acid was present in the extraction residues. The xylan in the extraction residues has been described as 'slow reacting xylan', since more severe extraction conditions are needed to remove it (Borrega et al. 2011, Mittal et al. 2009a and Mittal et al. 2009b).

Similar results were obtained from extractions with particle size fractioned sawdust. After the extractions 15–18% of the hemicelluloses were left in the extraction residue, for all degrees of packing and particle sizes.

Hemicelluloses and monosaccharides in extracts

The highest concentrations achieved with all degrees of packing were at 20–30 minutes of extraction (Figure 17).

The concentration profile was similar to that obtained with a continuous mixed flow reactor (Chen et al. 2010). The highest concentration of xylans in the extract was ca. 100 g/L, with a 0.5 kg/L packing degree. With densities

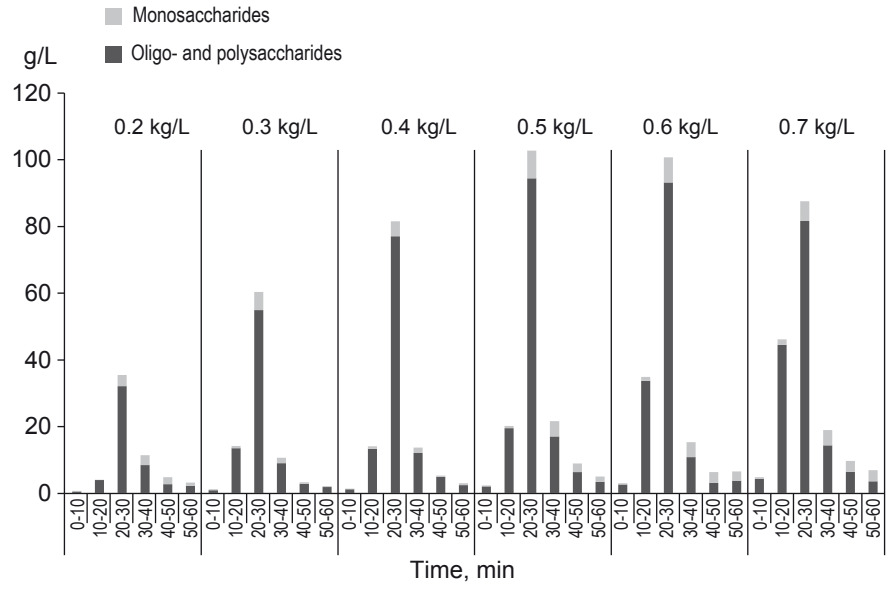

Figure 17. *Concentrations of oligo-, poly- and monosaccharides obtained with different unfractionated sawdust packing degrees, using a 50 mL extraction vessel at 180 °C for 60 minutes (Paper II).*

higher than 0,5 kg/L of sawdust, the concentration started to decrease at 20–30 minutes. Still, with 0.6 kg/L and 0.7 kg/L packing densities, more xylans were extracted earlier at 10–20 minutes. This is probably due to a larger amount of organic acids, such as uronic and acetic acid, in the extracts, which decrease the pH of the extracts and catalyse more hydrolysis at an earlier stage of the extraction.

If all of the 10 minute extract fractions from the 60 minute extraction would be combined, the lowest concentration of hemicelluloses in the combined extracts would be ca. 10 g/L for a 0.2 kg/L packing degree. The concentration increased linearly against the packing density, to the 30 g/L concentration with the 0.7 kg/L packing degree.

The highest hemicellulose concentration was achieved using fractionated sawdust at different packing degrees and was found at 20–25 minutes (not shown). The pH had not yet reached the lowest value, which was at 25–30 minutes. In the first extraction series with unfractionated sawdust, the pH was at its lowest point, giving the highest yield at 20–30 minutes. The amount of monosaccharides compared to oligo- and polysaccharides increased after 15–20 minutes.

With 0.2 kg/L of sawdust the largest particle size released the hemicelluloses later during the extraction compared to the smaller particle sizes. The concentration maximum of glucose was earlier than other sugars, in the 15-20 minutes, for all fractions. There could be some starch in the sawdust, which could dissolve earlier than hemicelluloses. Part of the glucose could also originate from glucomannan. With the highest packing degree of 0.6 kg/L of sawdust, relatively more hemicelluloses were extracted earlier during the extraction compared to packing degrees of fractioned sawdust. Similarly, more hemicelluloses were extracted earlier during the extraction, with a 0.6 kg/L degree of packing, compared to when 0.2 kg/L and 0.5 kg/L packing were used with different sized sawdust fractions.

Acetyl groups in xylans, acetic acid, and pH in the extracts

During PHW extraction, acetyl groups are de-esterified mainly from xylans and released as acetic acid (Borrega et al. 2011, Kilpeläinen et al. 2012 and Testova et al. 2011). However, a smaller amount of acetyl groups are present in glucomannans (Teleman et al. 2003). Thus, the acetic acid formed together with other released acids decrease the pH of the extract.

The highest concentration of acetic acid was found at 20–30 minutes of extraction, where the concentration maximum of hemicelluloses was present. At higher packing degrees more acetylated hemicelluloses were released earlier, already at 10–20 minutes extraction time. The most of the acetyl groups were still bound to the extracted xylans after extraction. The lowest amount of acetyl groups in the extracted xylans was found at the end

of the extraction, at the 40–50 and 50–60 minute extraction times. When the packing degree of the birch sawdust was increased, the residence time of water inside the extraction vessel decreased. Therefore, with higher packing degrees the released acetic acid would be removed from the extraction vessel faster than with lower degrees of packing. The increased amount of acetic acid and acetyl groups can be seen at 10–20 minutes, with 0.6 kg/L and 0.7 kg/L densities. The amount of extracted xylans and acetyl groups in xylans are compared in Figure 18.

The amount of extracted xylans and the amount of acetyl groups correlated linearly (Figure 18). The average degree of acetylation of xylans was 0.52. With different particle sizes, the largest particle size, 0.63–2.0 mm, had a lower amount of acetic acid and acetyl groups, compared to the other particle sizes (not shown). The degree of acetylation of xylan was highest with 0.2 kg/L of unfractionated sawdusts, ranging from 0.4 to 1.1. The amounts of extracted xylan and acetyl groups were linearly correlated. The average degree of acetylation of xylans, extracted from different sawdust sizes and degrees of packing, was 0.53. The most of the acetyl groups were still bound to xylans as in the case of different packing densities.

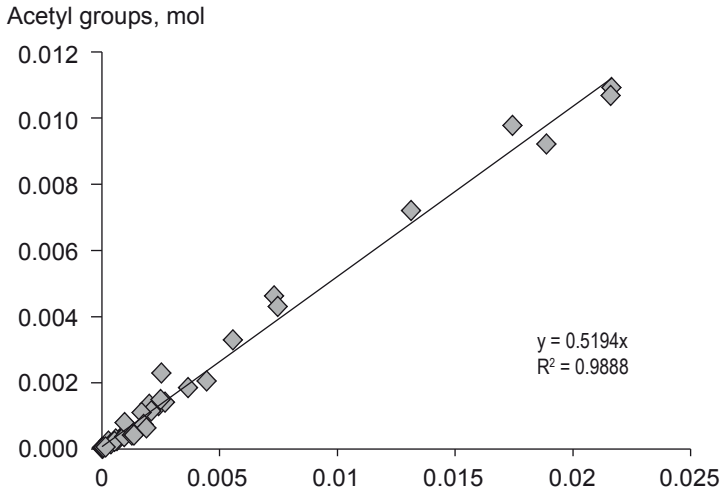

Figure 18. *The amount of extracted xylans and the amount of acetyl groups in the extracted xylans. Xylans were extracted from unfractionated birch sawdust (Paper II).*

pH of the extracts

The pH of the extracts decreased during the extraction time, as acidic groups from sawdust were released. The pH was above 3 in the first 20 minutes of extraction (Figure 19).

The lowest pH was found for the extracts collected in the interval between 20–30 minutes. After the lowest value, pH started to increase again. Since fresh water is constantly flowing in and through the extraction vessel, acids released from the sawdust are removed with the extract, out of extraction vessel. Thus, after 30 minutes when the major part of organic acids had been released from the hemicelluloses, the pH of the extracts increased slowly remaining under 4, at the end of the extraction. The pH was lower at 20–30 minutes than compared to a similar batch extraction (Borrega et al. 2011a, 2011b) but then increased above 3. With the 0.2 kg/L packing degree, pH was somewhat higher at 10–30 minutes of extraction but after this, the pH was similar to the other packing degrees of sawdust.

In case of the different sized sawdusts and packing degrees, the pH values are shown in Figures 20 a-c.

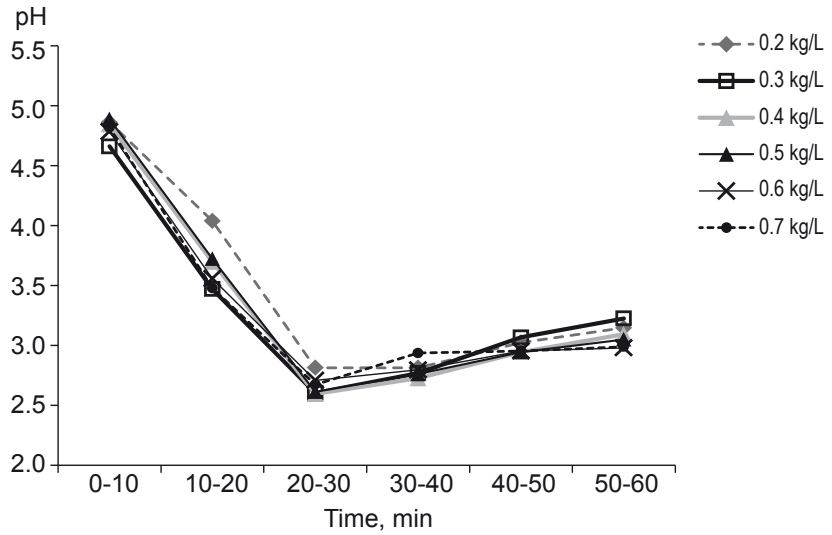

Figure 19. *The end pH of the birch PHW extracts. Unfractionated birch sawdust was packed inside the 50 mL extraction vessel and extraction was performed at 180 °C for 60 minutes (Paper II).*

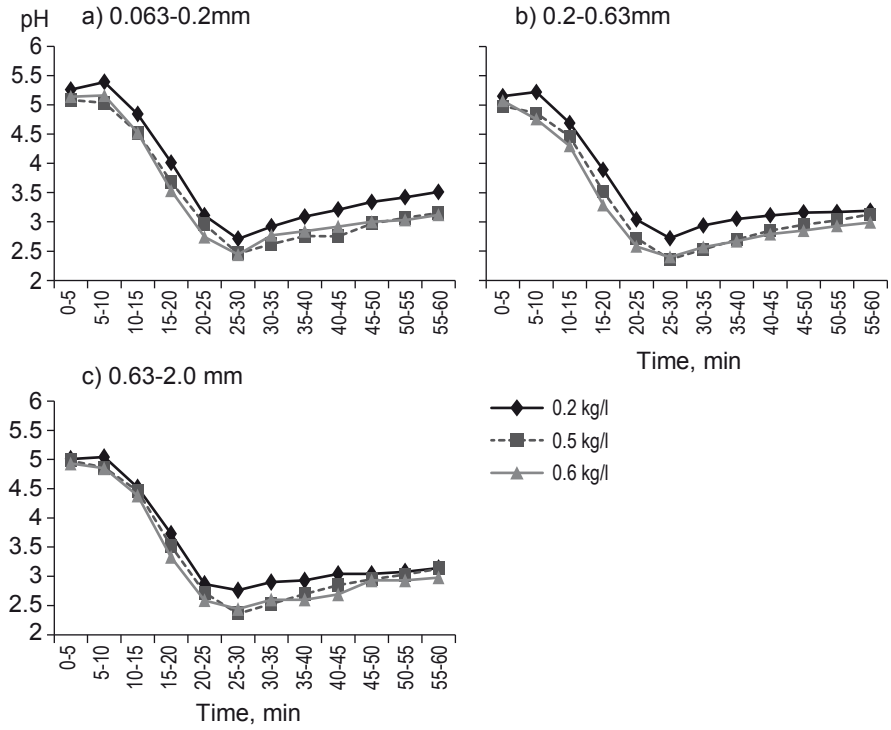

Figure 20*. The pH of the extracts using fractionated sawdust. a) 0.063-0.2 mm; b) 0.2-0.63mmc) 0.63-2.0 (Paper II).*

The lowest pH was found for all sawdust particle sizes and packing degrees in the extracts sampled between 25–30 minutes. The pH with the 0.2 kg/L sawdust packing degree decreased to 2.8 with all particle size fractions. In the case of the higher packing degrees, the pH of the extract decreased to a level of 2.4–2.5 at 25–30 minutes. The pH for extracts obtained after the minimum pH at 25–30 minutes increased to 3.5, mostly with the smallest 0.063–0.2 mm sawdust fraction (Figure 20 a). This effect was not observed with the unfractionated sawdust (Figures 19 and 20a). The smallest sized sawdust could release acetic and other organic acids earlier the during extraction, compared to larger sized sawdust, due to faster diffusion. Therefore, there are lower amounts of acids present in the extract during the last phase of the extraction at 40–60 minutes.

Molar masses of extracted xylans

The molar masses of the xylans decreased as the extraction time increased. The highest molar masses (Mw) were obtained with unfractionated sawdust during the beginning of the extraction at 0–20 minutes. Some extracts

had some aggregates with larger molar mass than hemicelluloses. HPSEC chromatograms of the extracts, obtained from 0.2 kg/L and 0.5 kg/L of sawdust, showed similar molar mass distributions. The first 0–20 minutes extract contained a small amount of polysaccharides with high molar mass, possibly attributable to starch or glucomannans. A large variation in scattering, especially with the 30–60 minute extracts, allowed for only an estimation of the molar masses.

The molar masses of hemicelluloses extracted at 30–60 minutes were at ca. 1 kDa. This molar mass corresponds to similar average degree of polymerization (DP) of 7 as Chen et al. (2011) and Borrega et al. (2011) obtained at the end of their extractions, at 160 °C and 180 °C respectively. At 20–30 minutes of extraction, molar masses varied between 2–8 kDa. The average DP is in the range of the highest DP obtained by Chen et al. (2011) and higher than Borrega et al. (2011), obtained using a batch reactor. This could be due to the lower residence times of hemicelluloses in a flow-through vessel compared to a batch reactor.

Concluding remarks

The hemicellulose concentration at the maximum point could be increased from about 40 g/L to 100 g/L, with a 0.5 g/L packing degree. The highest 30 g/L average concentration of hemicelluloses in the combined extracts could be achieved with the highest 0.7 kg/L packing degree. In addition, with the highest packing degree it was possible to extract more hemicelluloses earlier than with the typical packing densities.

The same amount of water was used in all extractions, resulting in a decreased use of water when the packing degree was increased. Therefore, sawdust packing also improved the L/W ratio of the extraction. The smallest particle sizes gave the highest yields of xylans at typical packing densities. However, with higher degree of packing the yields of hemicelluloses were similar, irrespective of sawdust particle size.

Based on these results, the larger scale flow-through extraction system using sawdust could benefit from higher degrees of sawdust packing. For continuous processes, similar extractions could be performed using continuous systems. Higher packing degrees would improve the L/W ratio and increase the hemicellulose concentration of the extracts.

4.3 pH control with acetate buffer (IV)

In a typical PHW extraction, only time and extraction temperature are controlled. This usually leads to a low pH during the extraction. The low pH catalyses hydrolysis of the hemicelluloses and the molar masses of the extracted hemicelluloses can therefore be low. On the other hand, some

hydrolysis is needed to cleave hemicellulose chains and release them from the wood structure.

The aim of the use of pH control was to find an appropriate value where hemicelluloses can be extracted from sawdust, but their molar masses would be preserved for polymer applications,e.g. for film formation. This can be achieved using a buffer.

Pressurized hot water extractions were performed at 160–180 °C. The flow rate was 4 mL / min and 10 g of birch sawdust was placed in the 50 mL extraction vessel. Sodium acetate/acetic acid buffer (0.05 M and 0.1 M) was used to control the pH. The pH was adjusted to 4.0, 4.2, or 4.6. The extraction time was 30 minutes and 10 minutes fractions of extract were collected. The buffer strength was chosen to be quite low, since as excessive amount of buffer would also increase the amount of sodium in the extract.

Extract pH

Birch sawdust was PHW extracted with ion-exchanged water, or with acetate buffers. Water extraction decreased the pH quite steeply (Figure 21 a,b,c) in all extraction temperatures. At all extraction temperatures, the initial pH of the buffered extractions was lower than with ion-exchanged water. At 160 °C the final pH was near the buffer pH but at 170 °C and 180 °C the pH was lower than the buffer pH in the final 20–30 minute fractions.

The results are similar to those obtained by Chen et al. (2010) using a mixed batch reactor with hardwood chips and acetic acid, at pH 3.3 and 3.8. The pH decreased similarly with hot water, but kept constant when acetic acid was used. The pH was relatively stable even at 180 °C, although the pH decreased more steeply than at 160 °C or 170 °C. Similar buffered extraction have been performed with Norway spruce, using 0.1 M $NaHCO_3$ (Song et al. 2011a) and phthalate (Song et al. 2011b) buffers. The pH decreased from 8 when $NaHCO_3$ was used and with phthalate from 3.8, 4.0, 4.2, and 4.4. The pH in case of $NaHCO_3$ decreased to 3.1 at 180 °C. The change of pH was lower with phthalates, the pH dropped most at 180 °C with 0.8 pH units.

Total dissolved solids (TDS)

The amount of total dissolved solids (TDS) increased at higher extraction temperatures. Both plain water and buffer extractions had a similar amount of TDS, except at 170 °C. At 170 °C the buffered solutions extracted lower amounts of solids, than with extractions using ion- xchanged water. When extractions and TDS were compared against extraction time, the buffered extractions had lower TDS, at with the 20–30 minute fractions compared to plain water extractions. The pH buffer could slow down hydrolysis of xylans and therefore most of the material would be extracted after 20–30 minute fraction with buffered extractions. At 180 °C, the combination of temperature

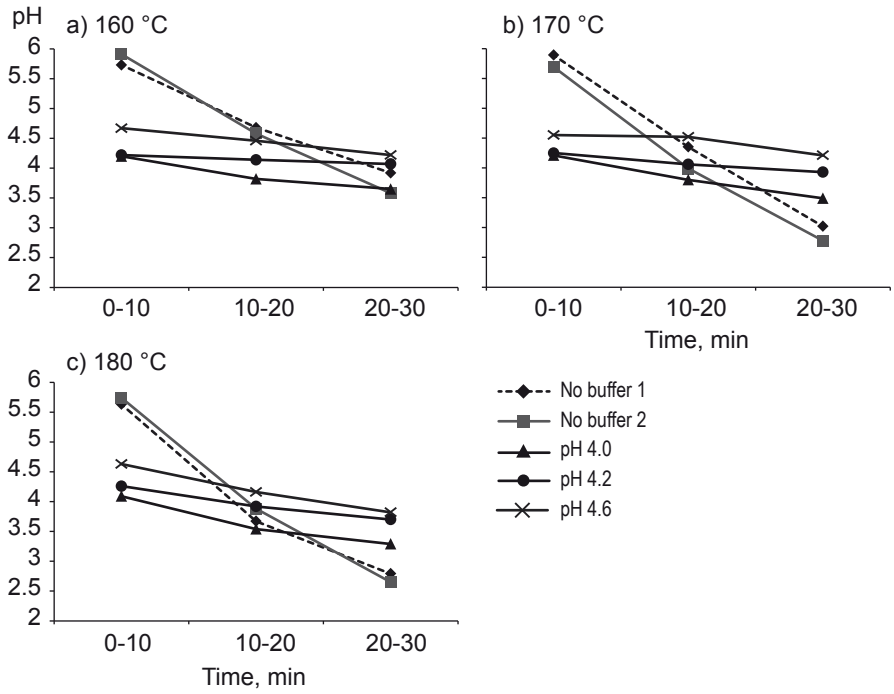

Figure 21. *The end pH of 10 minute successive fractions after a) 160°C, b) 170°C, and c) 180°C PHW extractions (Paper II).*

and pH was enough to release the same amount of material as with compared to plain water.

Extracted carbohydrates

The amount of extracted xylans from birch sawdust was similar as the TDS results. The highest yield of carbohydrates was obtained at 180 °C. There were some differences between carbohydrate yields when plain water or buffered solutions were used (Figure 22).

The yield of plain water extraction was higher at 170 °C than that of buffered solutions, which was similar outcome as to the TDS results shown earlier. The addition of buffer probably decreased the extraction yield by inhibiting hydrolysis, as was with Norway spruce extractions performed with the batch system (Song et al. 2011 a,b) at 170 °C with sodium bicarbonate and phthalate. The pH of the extraction is an important parameter during the extraction, but the temperature of the extract must be high enough to release xylans from birch sawdust

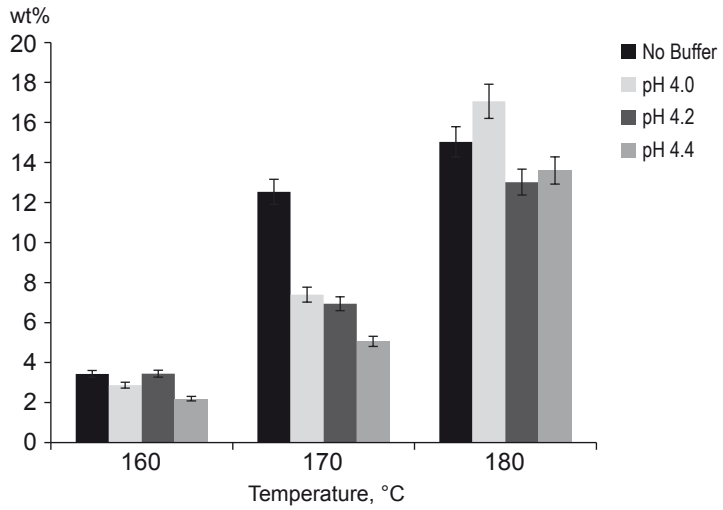

Figure 22. *PHW-extracted non-cellulosic carbohydrates at different extraction temperatures. Error bars represent a 5% relative standard deviation (Paper IV).*

The sugar compositions of the extracts were similar for all buffers. Yields and compositions of the different carbohydrates in oligo- and polysaccharides were similar for both plain water and the pH buffered extraction series. The only difference was the yield of hemicelluloses at 170 °C for the 20–30 min fraction.

The amount of hemicelluloses and monosaccharides

During extractions, part of the xylans were hydrolysed from polysaccharides to oligosaccharides and finally to monosaccharides. The ratio of monosaccharides vs. extracted oligo- and polysaccharides gives an indication of the severity of the hydrolysis of xylans. Xylose and arabinose from xylans can further react to form furfurals (Borrega et al. 2011, Sixta 2006). Furfurals can further react with xylose intermediates and polymerize to condensates and resins (Zeitch 2000). Some monosaccharides were released at the beginning of the extraction between 0 to 10 minutes. Most of the early extracted monosaccharides were easily solvated sugars such as glucose and fructose, which could originate from starch and saccharose.

Molar masses of the extracted xylans

The addition of sodium acetate buffer had an influence on the molar mass of the extracted hemicelluloses. When the plain water was used and the extraction temperature was increased from 160 °C to 180 °C, the average molar mass (Mw) of extracted carbohydrates between 20 to 30 minutes decreased from ca. 19 kDa to ca. 1 kDa (Figure 23).

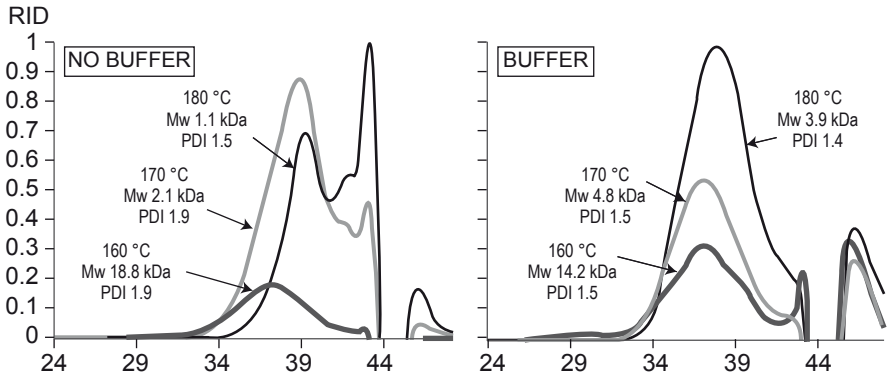

Figure 23. *HPSEC-MALLS chromatograms of extracts from birch wood, collected during the 20-30 min extraction time at different temperatures with and without the addition of pH 4.0 buffer (Paper IV).*

The addition of buffer, however, prevented hydrolytic cleavage of the extracted polysaccharides. Even after extraction at 180 °C, their average molar mass was ca. 4 kDa. On the SEC chromatogram of the extracts obtained with only water at 180 °C, the most pronounced peak was related to low molar mass hydrolysis products. When the buffer was used, formation of these low molar mass compounds was suppressed. The Mw of the extracts obtained at 160 °C with buffer addition was lower than that without buffer. Buffer gave a lower starting pH compared to plain water i.e. more substantial hydrolytic cleavage of the polysaccharide backbone was observed with lower pH values.

Concluding remarks

Pressurized hot water flow-through extraction with acetic acid/sodium acetate buffer is a convenient way to extract oligomeric and polymeric xylan from birch sawdust. The molar masses of the extracted carbohydrates were higher with buffers and yields at 180 °C were comparable to typical PHW extraction. Buffers could be used to reduce the hydrolysis of xylans. Buffers could be added at the point when the pH of the plain water extraction is at the initial pH of the buffer. The pH buffers have a more pronounced effect at higher extraction temperatures, inhibiting cleavage of xylan.

4.4 Scale up from the laboratory to the pilot scale (V)

The aim of these experiments was to scale up the PHW flow-through extraction, from the laboratory scale to the pilot scale. Previous laboratory scale experiments gave fundamental information about the PHWE flow-

through systems. Scale-up to larger scale is an important step to evaluate processes, prior to industrial scale assesment. Pilot scale extractions were also performed with spruce sawdust but they are omitted from this thesis since the main objective was to develop the extraction with of birch sawdust.

Extraction set-up

PHW flow-through extraction were conducted using similar relative vessel dimensions, sample amount proportional to vessel volumes, and similar extraction condition both in the laboratory and the pilot scale systems (Table 9).

Three identical extractions were conducted for birch sawdust for both laboratory and pilot scale. Extractions were performed at 160 °C. Birch sawdust was extracted at lower temperature to extract mainly xylans, while keeping the amount of extracted lignin as low as possible. The residence time was kept constant, which had an effect on the wood to water ratios, which were 1:16 (w/w).

Table 9. *The extraction and extraction vessel parameters for laboratory and pilot scale systems (Paper V).*

	Laboratory scale	**Pilot scale**
	(0.05 L)	**(300 L)**
Temperature	160 °C	160 °C
Loading (dry sawdust)	11.9 g	71 kg
Wood to water -ratio	1:16	1:16
Flow rate	3.3 mL/min	20 L/min
Length/diameter	60/34 mm	1040/590 mm
Length to diameter -ratio	1.8:1	
Residence time	12 min	
Extraction time	60 min	

Temperature and pH profile of PHW extractions

Temperature is the most important single factor which affects the hemicelluloses yield, with plain water. The average temperature profile for birch extractions are presented in Figure 24.

The extraction vessel heats up faster in the laboratory scale than in the pilot scale. In the laboratory scale the extraction vessel is placed inside the oven, which heats both the extraction vessel and the water flow. Pre-steaming further enhances the heating. The pilot scale system does not have any similar external heating system as the laboratory scale system and is heated only

by the in-flow of the preheated water. Figure 24 b shows the effects of the temperature on the pH of the extract. The faster the temperature rose, the more the pH decreased during extraction.

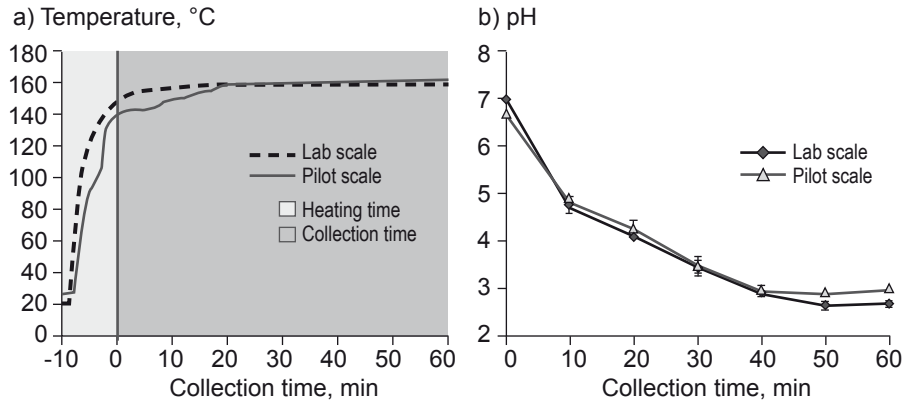

Figure 24. *Birch extraction a) temperature and b) pH profiles for both laboratory and pilot scale extractions. Pilot scale temperature is an average of nine measuring points (Paper V).*

Hemicelluloses

The main product obtained from PHW extraction of sawdust is a hemicellulose-rich fraction. The amount of the extracted hemicelluloses in the laboratory and the pilot scale are shown in Figure 25 for birch sawdust. The amounts and extraction rates of the hemicelluloses extracted from birch sawdust were similar for both laboratory and pilot scale. The total yield of birch hemicelluloses obtained during extraction was 50% with a relative standard deviation (RSD) of 3.2% for laboratory and 4.2% for pilot scale extraction.

The yield of extracted hemicelluloses increased after 20 min and the highest concentration was obtained at 40 min of extraction time. The average molar mass (Mw) of hemicelluloses decreased during the extraction, due to hydrolysis

The decrease in the hemicellulose molar mass (Mw) depends on the extraction temperature, pH, and the residence time inside the extraction vessel. With these extraction parameters, the molar mass of the hemicelluloses obtained was 16–19 kDa at the beginning of the extraction and 4–5 kDa for the peak of the hemicellulose yield. The decrease in the molar mass is due to the hydrolysis of hemicelluloses. The longer the time the hemicelluloses stay at high temperature or lower pH, the more hemicelluloses are cleaved into smaller fragments.

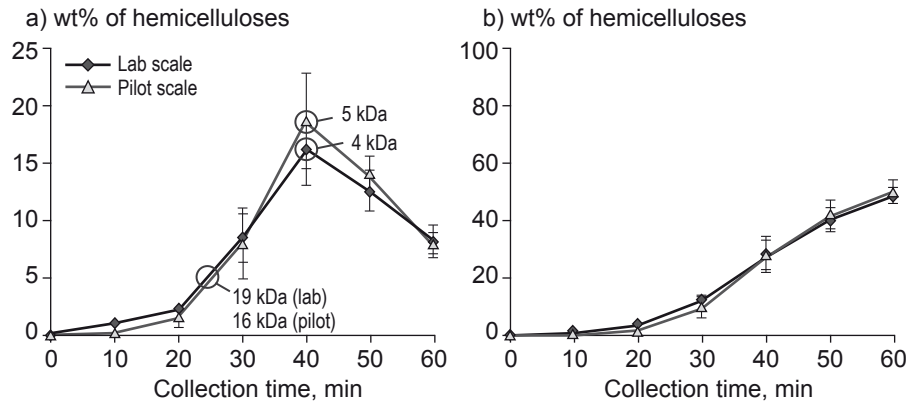

Figure 25. *a) Birch hemicellulose yields, and molar masses b) cumulative yields from the laboratory and pilot scale PHW extractions. Error bars are the relative standard deviations of three parallel extractions. The amounts of hemicelluloses were measured with the °Brix scale (Paper V).*

Mass balances

During the extractions more than 20% of the birch sawdust was dissolved into the extract. During the cooling down phase in pilot scale, some of the hemicelluloses and lignin were still extracted out from the sawdust. The extract collected during the cooling down phase was taken into account in the mass balances (Figure 26).

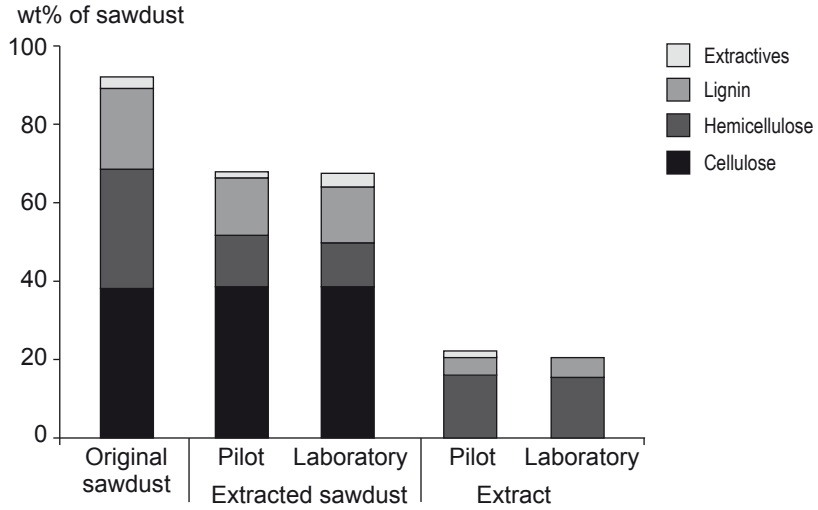

Figure 26. *The mass balances of birch extractions (Paper V).*

There were no significant differences in mass balances between the pilot and laboratory scale extractions. Hardly any of the cellulose was dissolved in the laboratory and pilot scale PHW extractions. The temperatures used in the experiments were not high enough for cellulose degradation to occur (Borrega et al. 2011, Paper I). The extraction yields of the hemicelluloses can be controlled by varying the extraction temperature, time and flow rate. In these experiments about 50% of birch hemicelluloses were dissolved.

In case of wood extractives, about half of the extractives were found in the extract and half was retained in the sawdust. Some part of the lignin was also extracted, but the lignin mainly remained in the sawdust. Some lignin was extracted from the birch sawdust (24%) even at 160 °C.

Figure 27 shows the distribution of the wood compounds, when 1000 kg of sawdust would be extracted. During the extraction, acetic, uronic, and other organic acids were released. Some furfurals and other compounds were also formed. In the previous experiments (Paper I), the amount of furfurals in the extracts was very low. Acetic acid and furfural can be evaporated when samples are dried. These are not included in the calculations. With birch, this kind of weight loss can be large due to the large amount of acetic acid released from xylans. In addition to extractives, lignin, hemicelluloses and cellulose, sawdust and extracts contained some ash and proteins, which were not analysed. These missing compounds a.k.a. acetic acid, ash, and proteins are included as 'others' in Figure 27.

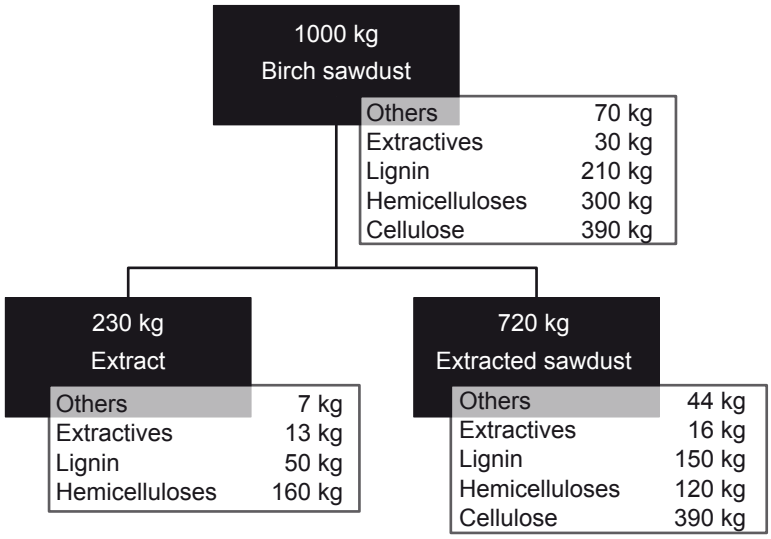

Figure 27. *The distribution of the wood compounds between pilot scale extract and extracted sawdust for birch (Paper V).*

Performance of the pilot scale system

The information of the performance on the pilot scale system is important in further upscaling (Pronyk and Mazza 2009). Heating and cooling rates for the laboratory and pilot extraction vessels were not similar, as the pilot scale flow-through extraction system had larger heat capacity and mass and the pilot scale system was heated only with hot water. The channelling of water through the sawdust bed can mostly be avoided in a smaller laboratory scale system, but in the pilot scale the channelling effect was still present. Channelling can occur if sawdust packing and movement create voids in the sawdust bed. Water moves more rapidly in these voids than in other parts of sawdust bed.

Channelling was monitored with nine thermocouples inside the extraction vessel, which were in three different heights. When the extraction vessel was first filled with sawdust and hot water was pumped into the vessel, the temperature of the thermocouples near the side of the vessel heated up first. In addition, heating was not similar for every part of the sawdust bed, indicating channelling of water. The channelling could be avoided using pre-steaming before extraction.

Black precipitate has been reported to be formed in batch extractions at temperatures near 200 °C (Borrega et al. 2011a, Borrega et al. 2011b). In the pilot scale experiments, lignin-derived compounds and other extracted compounds started to precipitate when the temperature of the extract decreased. Some of the formed precipitate stuck on the surfaces of the heat exchangers and pipelines. Some part of these precipitates could have been so-called pseudo-lignin from carbohydrates (Kumar et al. 2012). There was also some precipitation in the containers where the extracts were stored, which was mainly lignin.

Concluding remarks

The pilot scale extractions were repeatable and scalable. The PHWE system was scaled by a factor of 6000, from 50 mL to 300 L. The laboratory scale extractions gave valuable information about the different extraction parameters, such as extraction temperature and time. The overall extraction yield was similar in laboratory and pilot scale. In the PHWE flow-through extraction system, 50% of birch hemicelluloses were extracted at 160 °C. There were no significant differences in hemicellulose composition and mass balances, between laboratory and pilot scale. Pilot scale extractions gave important information about overall performance and channelling, which could not be revealed from laboratory scale extractions.

5 Conclusions

This work confirmed the hypothesis that it is possible to obtain polymeric, water-soluble hemicelluloses from birch sawdust, using flow-through PHW extractions in both laboratory and large scale. At 180 °C, about 70% of xylans were extracted from the sawdust. The xylans were acetylated and water-soluble after the extraction. When the temperature increased from 160 °C to 190 °C, more acetic acid was released from xylan chains. Only trace amounts of furfurals were found after extractions at 160–190 °C. The amounts of monosaccharides increased as the extraction temperature rose, indicating more severe hydrolysis. Similarly, the molar masses of the hemicelluloses decreased as the temperature increased from 140 to 200 °C. Some dark precipitate formed at 200 °C, which clogged the lines of the extraction system.

The sawdust packing enabled an increase in the concentration of xylans in the extracts and it also improved the liquid to wood ratio of the extraction, by decreasing the water usage during the extraction. The liquid to wood ratio decreased from 28/1, which was used for typical extractions, to 8/1 at the highest sawdust density. The degree of packing did not change the xylan yield as the same amount of xylan was extracted in all packing densities. The highest xylan concentration during the extraction was 100 g/L, with 10 minute fractions. The highest total yield of extraction was 30 g/L. The pressurized hot water extraction removed 80% of the hemicelluloses and 40–50% of the lignin from the sawdust.

The X-ray tomography experiments showed that the cellular structure of birch sawdust was preserved during the extraction. The cell walls were thinner after the extractions and larger pores were opened inside the sawdust. As hemicellulose and lignin were removed, cellulose microfibrils were more closely packed after extraction. Cellulose was preserved during the extractions although some amorphous cellulose was formed.

The pH buffer slowed down xylan hydrolysis and the molar mass of the extracted xylans increased. The pH of the extracts decreased more slowly when pH buffer were used. At lower extraction temperatures, at 160 °C and 170 °C, the acetate pH buffer decreased the xylan yield while the molar mass of the xylan increased. The yield of the extraction with pH buffer was similar compared to plain water extraction at 180 °C. The molar mass increase was more pronounced at 180 °C than at 160–170 °C.

The hemicellulose extraction was successfully scaled up from laboratory to pilot scale. Extractions in these two different scales had the same extraction yields and the molar masses of the extracted xylans were similar. The chemical composition of extracted sawdust and extracts were similar in both the laboratory and the pilot scale.

Further studies could include the study of the effect of density in continuous co-current or counter current extraction systems. The higher sawdust density would improve the liquid to wood ratio of the extractions and it could give a higher concentration of hemicelluloses in the extract.

The pressurized hot water pilot system has been shown to work, so this system could be integrated as a part of a current pulp mill. Integration could make the PHWE flow-through extraction an economically viable system. Lignin fractions obtained from PHWE could be utilized as energy, or the sulphur free lignin could be used to make new products.

Cellulose from sawdust PHW extraction could be further utilized as regenerated cellulose after the bleaching step or one possible use of the cellulose fraction could be for conversion to nanocellulose. Cellulose could be in some cases hydrolysed and fermented to ethanol or other more valuable platform chemicals.

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