Åbo Akademi University

Intensification of hemicellulose hot-water extraction from spruce wood by parameter tuning

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“Gör om, gör rätt”

Preface

The work presented in this thesis has been carried out in the framework of Johan Gadolin Process Chemistry Center at the Laboratory of Wood and Paper Chemistry at Åbo Akademi University under supervision of Docent Andrey Pranovich and Professor Stefan Willför. Financial support was provided by the FuBio 2 Joint research program within the Finish Bioeconomy Cluster (FIBIC), Johan Gadolin Process Chemistry Center at Åbo Akademi University, the Walter Ahlström foundation, European Polysaccharide Network of Excellence (EPNOE), the Graduate School for Biomass Refining (BIOREGS), and NordForsk; all gratefully acknowledged.

First of all, I would like to express my gratitude against my supervisor Professor Stefan Willför for giving me the opportunity to carry out the research needed for this thesis and for all the advices and ideas as well as always finding funding for me so that I could focus on the academical work. I would also like to thank my other supervisor Docent Andrey Pranovich for his supervision and always thoroughly answering all my questions concerning anything in the vast field of wood chemistry. To Docent Anna Sundberg; thank you for helping with me with both academical and more practical questions along the way. Thank you Dr Risto Korpinen, for fighting for my field of work throughout numerous project meetings. And a huge thanks to all my co-workers at 3PK, past and present, Chunlin, Affi, Anders, Hanna, Sylvia, Katja, Daniel, Matti, Janne, Viktor, and all the rest for all the coffee break discussions, parties, trips etc. It wouldn't have been as fun without you. A special thanks to Jalle Hemming for always finding time to solve my problems concerning various analytical problems, to Agneta for always solving my practical problems and Afo for always finding the references I needed.

I would like to thank my family for the encouragement. Especially my mother for my early interest on the natural sciences in general and specifically chemistry. And also for my interest in trying to understand how everything works, it has taken me throughout this work, that and "sittmuskler".

And last, but definitely not least, my huge gratitude to my wife Anna, who stuck by me throughout this work and helped me with it, although maybe not always understanding what I was doing or how she was helping. And to our two beautiful kids, Felix and Molly, just for existing.

Table of contents

Important terms and abbreviations

4-O-MeGlcA	4-O-Methyl Glucuronic acid
ASE	Accelerated solvent extraction
Ara	Arabinose
BSTFA	*N,O*-Bis(trimethylsilyl)trifluoroacetamide
DP	Degree of polymerization
E^0	Standard electrode potential or intercept
Gal	Galactose
GalA	Galacturonic acid
GC	Gas chromatography
GGM	Galactoglucomannan
GlcA	Glucuronic acid
Glc	Glucose
GPC	Gel permeation chromatography
HMF	Hydroxymethylfurfural
HPLC	High-pressure liquid chromatography
HPSEC	High-pressure size exclusion chromatography
ICP-AES	Inductively coupled plasma – atomic-emission spectrometry
LCC	Lignin-carbohydrate complex
L/W	Liquid to wood
MALLS	Multi angle laser light scattering
Man	Mannose
M_n	Number-average molar mass
MTBE	Methyl *tert*-butyl ether
M_w	Weight-average molar mass
MWD	Molecular weight distribution
o.d.w.	of dry wood
P	Primary cell wall
PHWE	Pressurized hot-water extraction
PTFE	Polytetrafluoroethylene
Py-GC-MS	Pyrolysis-gas chromatography-mass spectroscopy
R^2	Coefficient of determination
RI	Refractive index

RSD	Relative standard deviation
S	Calibration slope
S_1, S_2, S_3	Secondary cell wall 1, 2, and 3
SN	Signal noise
TBAH	Tetra-n-butylammonium hydroxide
TDS	Total dissolved solids
THF	Tetrahydrofuran
TMAH	Tetramethylammonium hydroxide
TMP	Thermomechanical pulp
TMCS	Trimethylsilyl chloride
UV-Vis	Ultraviolet-visible
Xyl	Xylose
YS	Yttria-stabilized

Abstract

The growing population on earth along with diminishing fossil deposits and the climate change debate calls out for a better utilization of renewable, bio-based materials. In a biorefinery perspective, the renewable biomass is converted into many different products such as fuels, chemicals, and materials, quite similar to the petroleum refinery industry. Since forests cover about one third of the land surface on earth, ligno-cellulosic biomass is the most abundant renewable resource available.

The natural first step in a biorefinery is separation and isolation of the different compounds the biomass is comprised of. The major components in wood are cellulose, hemicellulose, and lignin, all of which can be made into various end-products. Today, focus normally lies on utilizing only one component, e.g., the cellulose in the Kraft pulping process. It would be highly desirable to utilize all the different compounds, both from an economical and environmental point of view. The separation process should therefore be optimized.

Hemicelluloses can partly be extracted with hot-water prior to pulping. Depending in the severity of the extraction, the hemicelluloses are degraded to various degrees. In order to be able to choose from a variety of different end-products, the hemicelluloses should be as intact as possible after the extraction. The main focus of this work has been on preserving the hemicellulose molar mass throughout the extraction at a high yield by actively controlling the extraction pH at the high temperatures used.

Since it has not been possible to measure pH during an extraction due to the high temperatures, the extraction pH has remained a “black box”. Therefore, a high-temperature in-line pH measuring system was developed, validated, and tested for hot-water wood extractions. One crucial step in the measurements is calibration, therefore extensive efforts was put on developing a reliable calibration procedure. Initial extractions with wood showed that the actual extraction pH was ~0.35 pH units higher than previously believed.

The measuring system was also equipped with a controller connected to a pump. With this addition it was possible to control the extraction to any desired pH set point. When the pH dropped below the set point, the controller started pumping in alkali and by that the desired set point was maintained very accurately. Analyses of the extracted hemicelluloses showed that less hemicelluloses were extracted at higher pH but with a higher molar-mass.

Monomer formation could, at a certain pH level, be completely inhibited. Increasing the temperature, but maintaining a specific pH set point, would speed up the extraction without degrading the molar-mass of the hemicelluloses and thereby intensifying the extraction.

The diffusion of the dissolved hemicelluloses from the wood particle is a major part of the extraction process. Therefore, a particle size study ranging from 0.5 mm wood particles to industrial size wood chips was conducted to investigate the internal mass transfer of the hemicelluloses. Unsurprisingly, it showed that hemicelluloses were extracted faster from smaller wood particles than larger although it did not seem to have a substantial effect on the average molar mass of the extracted hemicelluloses. However, smaller particle sizes require more energy to manufacture and thus increases the economic cost.

Since bark comprises 10 – 15 % of a tree, it is important to also consider it in a biorefinery concept. Spruce inner and outer bark was hot-water extracted separately to investigate the possibility to isolate the bark hemicelluloses. It was showed that the bark hemicelluloses comprised mostly of pectic material and differed considerably from the wood hemicelluloses. The bark hemicelluloses, or pectins, could be extracted at lower temperatures than the wood hemicelluloses. A chemical characterization, done separately on inner and outer bark, showed that inner bark contained over 10 % stilbene glucosides that could be extracted already at 100 °C with aqueous acetone.

Keywords: Biorefinery, spruce hemicelluloses, GGM, hot-water extraction, high-temperature pH, pH measuring, pH calibration, pH control, diffusion, molar-mass, acid-catalyzed hydrolysis, water auto-ionization, spruce bark, extraction kinetics

Svensk sammanfattning

Den ökande världspopulationen, de minskande tillgångarna på råolja och den pågående klimatdebatten kräver bättre utnyttjande av våra förnyelsebara, biobaserade råmaterial. Utifrån ett bioraffinaderiskt synsätt är det biobaserade råmaterialet förädlat till en mängd olika produkter, så som bränsle, baskemikalier och olika material, inte helt olikt ett oljeraffinaderi. I och med att ungefär en tredjedel av landmassorna på jorden är täckt av skog, är biomassa baserad på ligno-cellulosa den vanligaste förnybara råvaran vi har.

Det första steget i ett bioraffinaderi är att separera och isolera de olika komponenterna i biomassan. De mest förekommande komponenterna i ved är cellulosa, hemicellulosor och lignin, som alla kan processeras till en mängd olika slutprodukter. Idag utnyttjas oftast bara en av komponenterna, cellulosan, i den kemiska massapappersframställningen. Att även kunna utnyttja de övriga komponenterna skulle gynna pappersindustrin både ekonomiskt och miljömässigt. Därför bör separeringsprocessen utvecklas och optimeras.

Hemicellulosan kan partiellt extraheras ur veden genom hetvattensextraktion vid 170-180 °C innan massatillverkningen. Beroende på extraktionens längd och temperatur är de extraherade hemicellulosorna degraderade i varierande omfattning. För att kunna bestämma slutprodukterna ur ett brett spektrum, borde hemicellulosorna vara i så nativ och intakt form som möjligt efter extraktionen. Fokusen i detta arbete har legat i att, med fortsatt högt utbyte, bevara hemicellulosornas nativa molmassa med hjälp av en aktiv pH kontroll under hela extraktionen vid dessa höga temperaturer.

I och med att det inte har varit möjligt att mäta pH under en hetvattenextraktion just på grund av de höga temperaturerna, har pH under extraktionens gång varit en ”svart låda”. Därför utvecklades, utvärderades, och testades ett in-line högtemperatur pH mätsystem för att användas under hetvattenextraktioner av ved. En mycket viktig del för korrekta mätningar är kalibrering och därför sattes mycket tid på att utveckla ett trovärdigt kalibreringsutförande. Inledande hetvattenextraktioner av ved med pH-mätning visade att pH vid 170 °C är 0.35 enheter högre än vad man tidigare trott.

Hela systemet bestyckades ytterligare med en regulator och pump som gjorde det möjligt att även kontrollera pH till valfri nivå under extraktionen. När pH sjönk under ett valt börvärde

aktiverade regulatorn pumpen att pumpa in alkali vilket gjorde att det valda pH börvärdet bibehölls. Analys av de extraherade hemicellulosorna visade att hemicellulosautbytet minskade vid högre extraktions-pH men de extraherade hemicellulosornas molmassa var högre än vid lägre extraktions-pH. Vid ett visst pH kunde sockermonomerformationen helt undvikas. Genom att höja temperaturen för extraktionen och samtidigt bibehålla pH på en vald nivå kunde extraktionshastigheten ökas utan att sänka molmassan på de extraherade hemicellulosorna och på så sätt intensifierades extraktionen.

Diffusionen av de lösa hemicellulosorna från vedpartikeln ut i vattnet är en viktig del av extraktionsprocessen. Därför undersöktes den inre massöverföringen av hemicellulosorna med hjälp av att extrahera olika stora vedpartiklar mellan 0.5 mm och flisbitar med samma storlek som används inom industrin. Föga förvånande visade undersökningen att hemicellulosorna extraherades snabbare ur mindre vedpartiklar än från större vedpartiklar. Molmassan av de utextraherade hemicellulosorna verkade emellertid inte påverkas av vedpartiklarnas olika storlekar. Man bör dock minnas att det krävs mer energi för att producera mindre vedpartiklar, vilket skulle höja kostnaderna

I och med att 10-15 % av trädet består av bark är det viktigt att också inkludera barken i ett bioraffinaderikoncept. Gran inner- och ytterbark extraherades separat med hetvatten för att undersöka möjligheter att extrahera och isolera barkens hemicellulosor. Studien visade att barkens hemicellulosor är mycket olika vedens hemicellulosor och består till största del av pektiner eller pektinliknande sockerarter. Dessa hemicellulosor, eller pektiner, extraherades ut vid betydligt lägre temperaturer än hemicellulosorna i veden. Kemisk karakterisering, separat gjord på innerbarken och ytterbarken, visade att innerbarken innehåller över 10 % stilben glukosider som kunde extraheras redan vid 100 °C med en aceton-vattenblandning.

Nyckelord: Bioraffinaderi, gran hemicellulosor, GGM, hetvattenextraktion, högtemperatur pH, pH mätning, pH kalibrering, diffusion, molmassa, sur hydrolys, vattnets autojonisering, granbark, extraktionskinetik

List of publications

This thesis is a summary of the following original publications:

I Krogell, J., Korotkova, E., Eränen, K., Pranovich, A., Salmi, T., Murzin, D., Willför, S., (2013), **Intensification of hemicellulose hot-water extraction from spruce wood in a batch extractor – Effects of wood particle size**, *Bioresource Technology* 143, 212-220

II Krogell, J., Eränen, K., Granholm, K., Pranovich, A., Willför, S., (2014), **High-temperature pH measuring during hot-water extraction of hemicelluloses from wood**, *Industrial Crops and Products* 61, 9-15

III Krogell, J., Eränen, K., Pranovich, A., Willför, S., (2015), **In-line high-temperature pH control during hot-water extraction of wood**, *Industrial Crops and Products* 67, 114-120

IV Krogell, J., Eränen, K., Pranovich, A., Willför, S., (2015), **Utilizing active pH control for enhanced hot-water extraction of wood**, submitted April 20 to *Nordic Pulp and Paper Research Journal*

V Krogell, J., Holmbom, B., Pranovich, A., Hemming, J., Willför, S., (2012), **Extraction and chemical characterization of Norway spruce inner and outer bark**, *Nordic Pulp and Paper Research Journal* 27(1), 6-17

The Roman numerals I-V are used when referring to the original papers. The original publications are reproduced with the kind permission of the respective copyright holders.

Contribution of the author

Papers I-IV: The author planned the experimental design along with the co-authors, did all the experimental and analytical work, interpreted the results, wrote the manuscript, and finalized them with the co-authors.

Paper V: The author planned the experimental design along with the co-authors, did all the experimental work, interpreted the results with the co-authors, wrote the experimental, results, and conclusion part of the manuscript, and finalized it with the co-authors. The ICP-AES

analysis was made by the Natural Research Institute Finland (Luke), formerly Finnish Forest Research Institute (METLA).

Supporting publications

Grénman, H., Eränen, K., Krogell, J., Willför, S., Salmi, T., Murzin, D. Yi., (2011), **Kinetics of aqueous extraction of hemicelluloses from spruce in an intensified reactor system** *Industrial & Engineering Chemistry Research* 50, 3818–3828

Aho, A., DeMartini, N., Pranovich, A., Krogell, J., Kumar, N., Eränen, K., Holmbom, B., Salmi, T., Hupa, M., Murzin, D.Yu., (2013), **Pyrolysis of pine and gasification of pine chars – Influence of organically bound metals**, *Bioresource Technology* 128, 22–29

Krogell, J., Eränen, K., Pranovich, Willför, S., (2013), **High-temperature pH measuring during hot-water extraction of hemicelluloses from wood – equipment setup, calibration and validation**, 3rd EPNOE conference, Nice, France, October 21-24 (oral presentation)

Krogell, J., Korotkova, E., Eränen, K., Pranovich, A., Salmi, T., Murzin, D., Willför, S., (2012), **Intensification of hot-water extraction of hemicelluloses from Spruce wood by different particle size and stirring**, EU BC&E Conference June 18-22, Milano (oral presentation)

Krogell, J., Korotkova, E., Eränen, K., Pranovich, A., Salmi, T., Murzin, D., Willför, S., (2011), **Intensification of hemicellulose extraction from spruce wood with hot-water and stirring**, 2nd EPNOE conference, Wageningen, The Netherlands, August 29 - September 2 (oral presentation)

Le Normand, M., Krogell, J., Willför, S., Holmbom, B., Ek, M., (2010) **Hot-water extraction and characterization of hemicelluloses and pectins from bark of Norway spruce (*Picea abies*)**, 11th EWLP Conference, Hamburg, Germany, August 16-19 (poster presentation)

1. Introduction

1.1 General approach

Since only about half of the wood (mainly cellulose) is used in the papermaking process and the rest (hemicelluloses and lignin) is burned for energy, it would be a great improvement for the forest industries if the "non-used" compounds in the trees were utilized more efficiently for more refined high-value end-products. This way of thinking goes hand in hand with the biorefinery concept where the whole renewable biomass, e.g., the whole tree should be used.

One convenient and environmentally friendly way of separating hemicelluloses from the wood is with subcritical hot water. Most hemicelluloses are extractable with only water at temperatures below 200 °C (**I**, Kilpeläinen et al., 2012; Lundqvist et al., 2002; Song et al., 2008). Depending on the temperature and severity of the extraction, hemicelluloses are degraded to a various degree during the extraction. The extracted hemicelluloses can then be used as feedstock for a variation of different products such as bioethanol, biopolymers, animal feed, and even possible health applications. The end uses are much dependent on what kinds of hemicelluloses are extracted and with what molar-mass.

Lately, much focus has been on producing bio-based fuels from renewable resources (Ragauskas et al., 2006, 2005). Wood falls well into this category since it is possible to make bioethanol from wood carbohydrates, i.e., cellulose and the hemicelluloses. In this process, the carbohydrate chains are degraded into monomers and further fermented into ethanol. Although this is an appealing process, great opportunities are lost for other, end-products of higher value when the carbohydrate chains are destroyed. If the molar mass were preserved during the extraction, more options regarding the end-products would be available. Extracting high-molar-mass hemicelluloses is challenging due to a substantial drop in pH during the extraction. This pH drop is the result of a combination of auto-ionization of the water at high temperatures and release of acetic acid, originated from the cleavage of acetyl groups from the hemicellulose chains. The lower pH will in turn hydrolyze the hemicellulose chains with lower molar mass as result. It is possible to extract high-molar-mass hemicelluloses, but the yield will then be very low.

In order to be able to extract high-molar-mass hemicelluloses with high yield, the pH should stay at a satisfying level during the extraction. Problems occur when trying to control the extraction pH since there have been no methods to measure pH during extraction. Conventional pH electrodes cannot withstand temperatures higher than 80-100 °C, since the ion sensitive membrane starts to decompose at these temperatures. Therefore, the pH during a high-temperature extraction is a "black box". It is possible to measure pH before and after the extraction, but this will not give an accurate picture of what happens with the pH during the extraction when the pH sensitive reactions takes place. Besides, when trying to control the pH by cooling down samples taken during extraction, not only does a considerable delay occur between measuring and adjustment actions but the actions are also based on the room temperature pH, which is not necessary the pH where the crucial reactions takes place.

With the possibility to measure pH in-line during a hot-water extraction it would open up the possibility to be able to control the extraction and perhaps maintain a high molar-mass for the extracted hemicelluloses. It would offer the possibility to either utilize the high-molar-mass hemicelluloses or selectively degrade the hemicelluloses afterwards. With uncontrolled degradation during the extraction, this option becomes unavailable. Another positive consequence is "opening the black box" to acquire more knowledge about the high-temperature pH and the extraction process in general.

1.2 Hypothesis and objectives of the work

The main aim of this work was to improve the hot-water extraction of spruce wood to obtain high yield of high-molar-mass hemicelluloses. By understanding, monitoring and controlling several important extraction parameters, such as temperature, time, and pH, the extraction process could be tailored to get the desired end-products. A specific objective was to develop a high-temperature pH measuring and -controlling system that would enable in-line control of the pH during a hot-water extraction. The hypothesis was that by controlling the pH during extraction the chain degradation could be inhibited, or at least suppressed, and high-molar-mass hemicelluloses could be extracted at high yield. Secondly, spruce bark was thoroughly investigated for better characterization and furthermore utilization in accordance with the biorefinery concept of using as much of the biomass as possible.

2. Background

2.1 Biorefinery

The growing interest in environmental issues, the finite amount of fossil raw materials and fuels, global climate change, and the increasing world population have boosted the interest of developing alternative production chains beside the petrochemical industry. The biorefinery is a promising alternative that uses renewable and carbon neutral biomass as raw material (Ragauskas et al., 2006). The biorefinery raw material can be provided from several different sectors such as agriculture (preferably non-food agricultural waste), forestry, process side streams and residues from existing industries, municipal waste, or aquaculture (mainly algae) (Cherubini, 2010). The biorefinery concept is to use the components in the biomass (mainly cellulose, hemicelluloses, and lignin) as base for a vast array of different end-products. Besides the main components in the wood, the extractives can also be used for producing end-products such as turpentine, tall oil, and biodiesel.

In a sense, biorefineries have already existed for centuries in the pulp and paper industry. The paper mill uses cellulose from wood for paper and board production. The pulping process has been refined throughout the years (Sixta, 2006) so that today the cellulosic part of the wood is utilized very efficiently, while the rest is burned to obtain energy and heat. For the pulp and paper mill to be a fully developed biorefinery (a sound and new alternative to paper production in the Nordic countries), processes for the non-cellulosic components in the wood need to be developed alongside the pulping process. New high-value end-products (or large volume bulk chemicals) should be produced from the other wood components without compromising the main end-product in quality and volume. It is also preferable that the new processes are integrated into the existing processes so few new investments, for example, on infrastructure, and logistics are needed.

Separation of the different biomass components is usually a crucial first step in a biorefinery process. Depending on the desired end-products, different separation methods with different severity can be used (more in section 2.3). Overall, the separation process should strive to be as mild and structure-preserving as possible for more options regarding production of high-value end-products. Synthesizing complex polymers similar to those found in wood is extremely demanding, if not impossible, and definitely not economically feasible when

considering industrial-scale production. A well-adapted biorefinery should have the flexibility of producing a variety of high-value end-products and a possibility to change production according to market demands.

2.2 Wood and bark chemistry

Wood is a heterogeneous natural material that consists mainly of structural components and some non-structural components. The structural components are cellulose, hemicelluloses and lignin and constitutes typically 95-99 % of the wood from temperate zones (Fengel and Wegener, 1984a). The quantity of the different structural components varies from hardwood and softwood and specie to specie, but hardwood generally constitutes of 35-50 % cellulose, 20-35 % hemicelluloses, and 20-30 % lignin and softwood 35-45 % cellulose, 20-30 % hemicelluloses, and 30-40 % lignin (Sjöström, 1993). Bark is much more heterogeneous than wood. Besides the structural components, bark normally contains more extractives and also other unique components not present in wood such as condensed tannins and suberin. The chemical composition of bark differs much depending on species, even more than the chemical composition of wood. Therefore, end products from bark utilization present an interesting opportunity in fields other than wood (Le Normand et al., 2014).

Cellulose is the main polysaccharide in wood and consists of long chains of glucose units linked together with β-(1→4)-glycosidic bonds (Alén, 2000). The degree of polymerization (DP) of the cellulose chains are believed to be in the range of 10 000 in native wood with a rather low polydispersity (< 2). Due to the nature of the β-bond, the glucose polymer forms a straight chain and the many hydroxyl groups in the cellulose chains form both intra- and intermolecular hydrogen bonds to each other. This will result in a bundle of highly aligned cellulose chains closely attached together, which is called a microfibril. The cellulose chains in the microfibrils are mostly highly ordered (crystalline) regions but also some less ordered (amorphous) regions occur. The high crystallinity of the cellulose makes it more resistant to chemical treatments than the other structural components, which has been utilized in the chemical pulping process.

Hemicellulose is a collective name for a variety of heteropolysaccharides consisting of various different sugar units, heavily depending on the tree species. The most common sugar units are mannose (Man), xylose (Xyl), arabinose (Ara), galactose (Gal), and glucose (Glc) as

well as some uronic acids such as galacturonic acid (GalA), glucuronic acid (GlcA) and 4-*O*-methyl glucuronic acid (4-*O*-MeGlcA). The hemicelluloses found in the most abundant Nordic hardwood species, i.e., birch, are mostly comprised of xylose and thus are often called xylans. In the common Nordic softwoods (spruce and pine) the hemicelluloses mainly consist of mannose and glucose and are therefore often called mannans or glucomannans (Fengel and Wegener, 1984b; Willför et al., 2005). The hemicelluloses have a lower DP than the cellulose, up to only 200 repeating sugar monomer units (Sjöström, 1993). In comparison to cellulose, hemicelluloses can be branched and sometimes have other than hydroxyl functional groups attached, i.e., carboxyl or acetyl groups. Depending on the sugar composition, level of branching and the different functional groups, hemicelluloses can have very different properties compared both to cellulose and also other hemicelluloses. Due to the lower molar mass and branching, the hemicelluloses are generally more amorphous than cellulose which makes hydrogen bonding between molecules more difficult. Therefore, hemicelluloses are normally easier to dissolve in water and more easily hydrolyzed by acid than cellulose.

Galactoglucomannan, or GGM, are the dominant hemicellulose in spruce wood (Willför et al., 2005) comprising approximately 60 % of the total hemicelluloses and about 15 % of the total wood (Song et al., 2008). The GGM backbone consists of β-(1→4)-linked mannose and glucose units with α-(1→6)-linked galactose units attached to the mannose units (Fig. 2.1) (Timell, 1967; Hannuksela and Hervé du Penhoat, 2004).

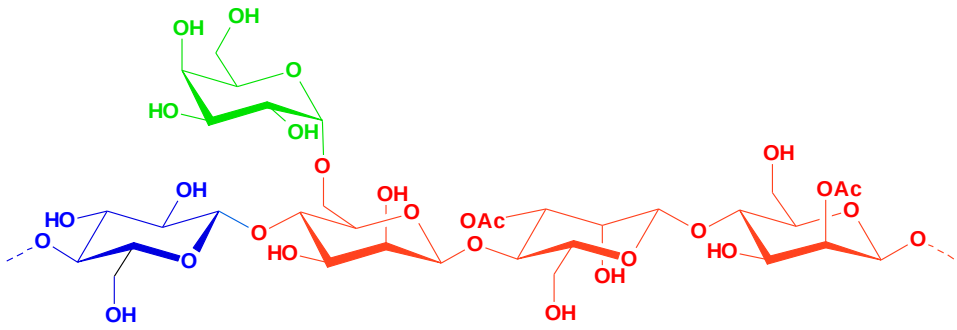

Figure 2.1 Chemical structure of *O*-acetyl galactoglucomannan with mannose sugar units in red, glucose sugar units in blue, and galactose sugar units in green.

The mannose units in the GGM are acetylated at positions C-2 or C-3 with a degree of acetylation of 0.3-0.5 giving GGM a sugar-acetyl ratio of 3.5-4.5:1:0.5-1.1:0.3-0.5 (Man:Glc:Gal:Ac) (Capek et al., 2000; Song et al., 2008; Willför et al., 2008 and references therein). It is important to remember that any separation and isolation method will affect the native hemicellulose so different isolation methods probably also give hemicelluloses with different sugar unit ratios and degree of acetylation. Therefore, the GGM composition discussed here is an estimated average. The average molar mass of the water-extracted GGM is suggested to be 20 – 50 kDa, corresponding to a degree of polymerization of 100 – 400 (Timell, 1967; Sjöström, 1993; Willför et al., 2003) but the isolation method will also strongly affect the molar mass of the GGM. Besides GGM, spruce wood also contains smaller amounts of other non-cellulosic carbohydrates. These are mainly arabinoglucuronoxylans, arabinogalactans, and pectins (Sjöström, 1993; Willför et al., 2002).

Lignin is a three-dimensional heteropolymer consisting of three different phenylpropane units; trans-*p*-coumaryl, trans-coniferyl, and trans-sinapyl alcohol (Fengel and Wegener, 1984c) of which coniferyl alcohol is the main unit in spruce lignin. These units are linked to each other with different carbon-to-carbon and ether bonds (Fig. 2.2).

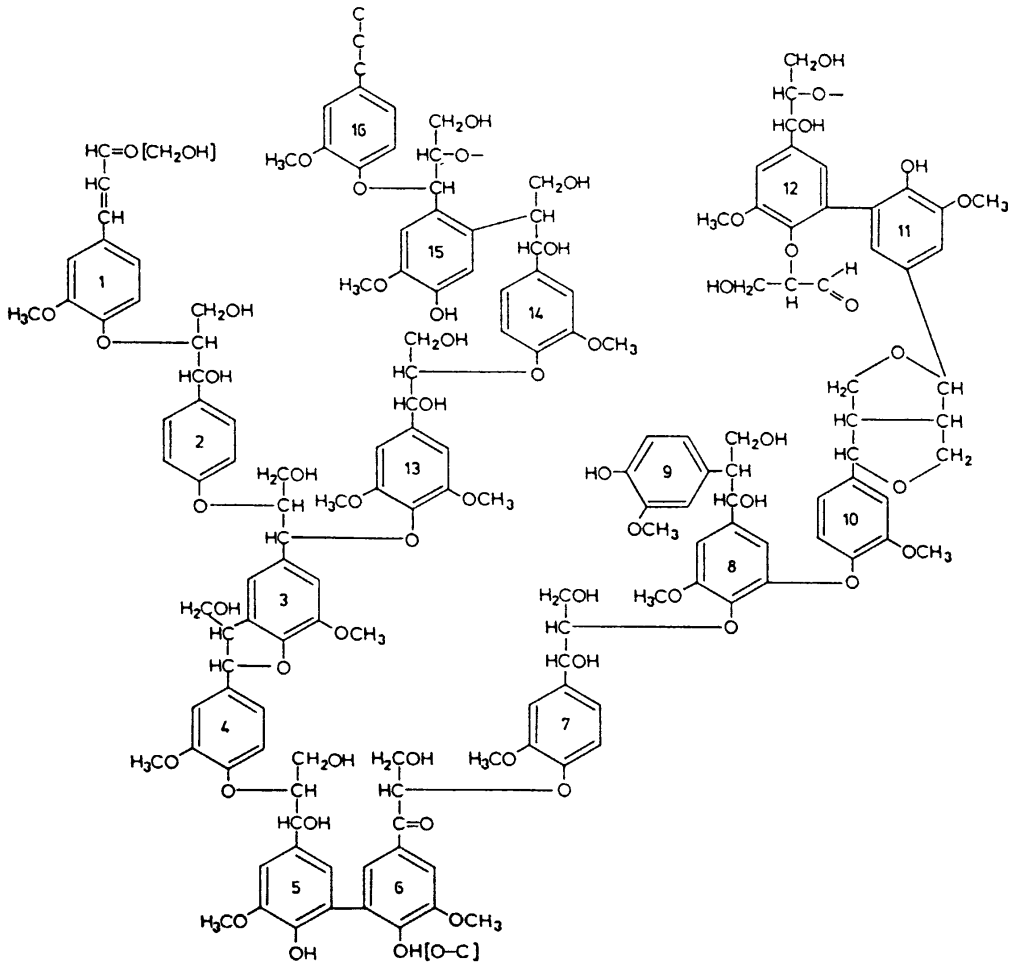

Figure 2.2 A structural segment of softwood lignin suggested by Adler (1977)

The main functions of lignin are believed to act as glue binding the cells together, as well as strengthening the cell walls. It is the amorphous and sticky lignin combined with the hemicelluloses and the strong and rigid cellulose that gives the tree its unique mechanical strength properties that enables it to grow to astonishing heights and withstand heavy abuse (e.g., wind).

The non-structural components, or extractives, consist of a vast variety of mostly low-molar-mass compounds that can be classified as lipophilics (soluble in nonpolar solvents) and

hydrophilics (soluble in polar solvents). Common lipophilic extractives are fatty and resin acids, triglycerides, and steryl esters and common hydrophilic extractives are sugars, stilbenes, stilbene glucosides, and flavonoids (Sjöström, 1993). Their portion in the wood is only a few percentages, although the amount varies between species and even between individual specimens. The concentrations varies much in the different parts of the tree, e.g., heartwood and sapwood, bark (Fengel and Wegener, 1984d), roots, needles, and leafs (Backlund et al., 2014). One task of the extractives is to protect the tree from microbial and insect attacks if the tree is damaged. Therefore, it is not uncommon that some of the extractives are bioactive and has a variety of antibacterial properties (Välimaa et al., 2007). Besides protection, some extractives also act as an energy source for biological activities in the wood cells (Alén, 2000).

The xylem in softwoods is composed of different cells: the main ones are longitudinal tracheids (90-95 %) and transversal ray cells. Tracheids, or fibers, are long cells (2-4 mm) with the main function of providing the tree mechanical strength, but also to transport water and nutrients (Sjöström, 1993). The cell wall in the tracheids consists of several different cell wall layers. The first layer that is formed at cell division is called the primary cell wall (P) and is a thin layer that protects the cell core. A thicker layer is then formed inside the first primary wall. This is called the secondary cell wall and after it is completely developed it has three different layers called S_1, S_2, and S_3 (Fig. 2.3).

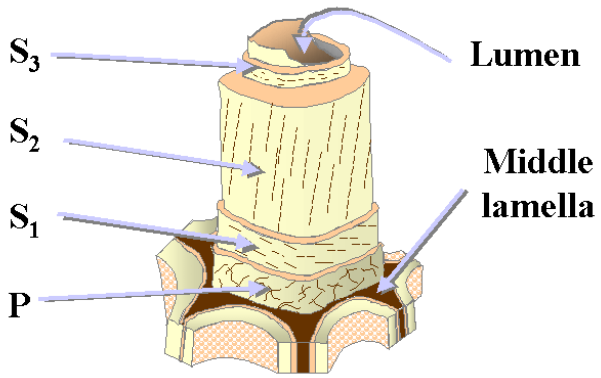

Figure 2.3 General description of cell wall layers in a wood cell (Hubbe, 2015)

These layers differ from each other both in thickness and fibril orientation. The outermost layer of the secondary wall, S_1, is 0.1-0.3 µm thick and has a fibril orientation angle of 50-70°. The middle layer, the S_2, is the thickest and forms the main part of the cell wall. Its thickness varies between earlywood and latewood from 1 µm to 8 µm. The fibril orientation angle is 5-30°, also varying between earlywood and latewood. The innermost layer, S_3, is less than 0.1 µm with a corresponding fibril angle of 60-90°. The hollow center of the cell or fiber is called the lumen (Alén, 2000).The area between cells is called middle lamella and it is rich in lignin and pectins and binds the adjacent cells to each other.

Although the chemical composition of the cell wall is well known, the matrix of the cellulose-hemicellulose-lignin and other smaller substances in the cell wall is very complex and not fully understood to this day. One well supported suggestion is that the highly ordered cellulose microfibrils are aligned with each other and surrounded by less ordered hemicelluloses and lignin (Fengel and Wegener, 1984e) (Fig. 2.4).

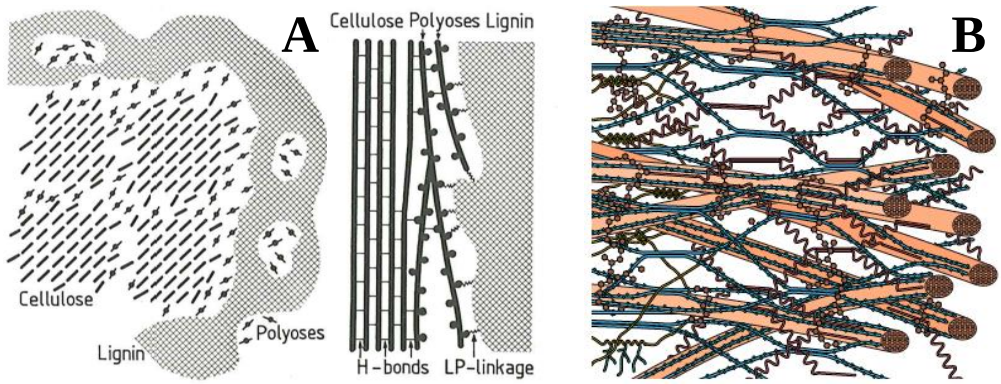

Figure 2.4 Models of cellulose-hemicellulose-lignin matrix in the cell wall. **A**: Transversal and longitudinal cross section (Fengel and Wegener, 1984e). **B**: Suggested wood matrix by Carpita and McCann (2000).

It is believed that some hemicelluloses are linked to the cellulose microfibrils by hydrogen bonding adding stability, as well as flexibility to the framework (Carpita and McCann, 2000). It has also been suggested that the two dominating hemicelluloses in spruce, mannans and

xylans, are somewhat selectively associated to cellulose and lignin separately (Salmén and Olsson, 1998); mannans to cellulose and xylans to lignin. The lignin is connected to the carbohydrates by covalent bonds, forming so called lignin-carbohydrate complexes (LCCs) (Lawoko and Henriksson, 2005; Lawoko et al., 2006). It is important to point out that investigating the wood matrix is extremely difficult because the available solid-state analytical methods are inadequate and the traditional analytical methods for wood used today require separation, dissolution, and often degradation of the components. By destroying the wood matrix, valuable structural information is lost. Furthermore, the required treatment of the wood may alter the interaction of the components, perhaps even producing new matrix formations.

2.3 Hot-water extraction

Using plain water extraction to first separate the hemicelluloses from the other structural components is a promising method. Water is a very good solvent for any industry since it is non-toxic, causes less equipment corrosion, easy to handle, and probably less expensive compared to other solvents. Furthermore, it can definitely be considered a green chemical or solvent which will improve the public opinion for any process using it. For good extraction yield, temperatures above the boiling point are required. Pressurized hot-water extraction (PHWE), sub-critical water extraction or hot-water extraction are a few names that are used for water extraction of hemicelluloses from various biomasses. Normally the temperatures used vary between 150 °C and 240 °C, highly depending on the biomass used and extraction products desired. Different hot-water extraction methods are discussed in more detail in section 2.3.1 Several water properties will change at higher temperatures; the dielectric constant decrease, the viscosity decreases, and the surface tension decreases (Yang et al., 1998). These changes will affect the dissolution properties of water, which might be one explanation why hot-water extraction is such a good method for hemicellulose extraction.

The mechanisms comprising the dissolution and extraction of hemicelluloses from wood are, due to the complex wood matrix and variety of hemicelluloses in different tree species, very difficult to define and still today not understood in detail. One way of trying to explain the extraction process in simple terms could be to divide it into three different steps (Fig. 2.5). The first step is the solvent (in this case the water) that penetrates into the wood and more precise the secondary cell wall, to reach the main location of the hemicelluloses. The

following step is the detachment or dissolution of the hemicelluloses from the wood matrix into the water phase. This is probably the most crucial step and unfortunately the part of the process that is known least about. The third step involves the now dissolved hemicelluloses to diffuse out from the wood and into the water outside in order to be isolated and utilized further. The diffusion out from the wood is also a crucial step since the dissolved hemicelluloses will encounter both mechanical obstacles such as penetrating the wood matrix and chemical obstacles such as reprecipitation, hydrolysis of the hemicellulose chains, or reactions with other wood components that will complicate the diffusion. The most prominent limitation factor affecting the diffusion is probably the molecular size of the hemicelluloses although, as stated earlier, it is a complex situation and very difficult to describe.

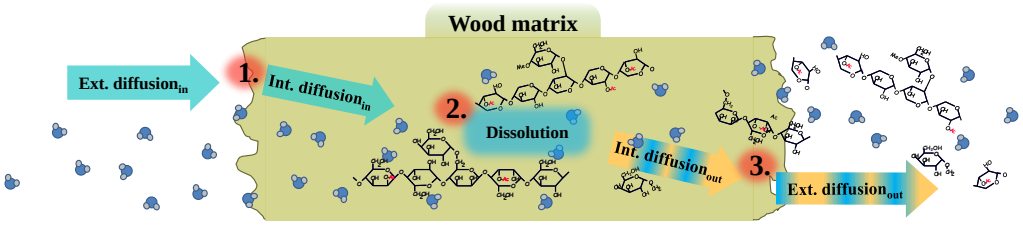

Figure 2.5 Simplified scheme on the extraction process of hemicelluloses from wood with the different extraction steps. 1: diffusion of the water into the biomass reaching the hemicellulose molecules, 2: dissolution of the hemicelluloses from the wood matrix into the water (i.e., solvation), and 3: diffusion of the dissolved hemicelluloses out from the wood into the water phase.

Depending on the final products, the extraction process can be adjusted in order to obtain the hemicelluloses (or monosaccharides) in a desired form for further processing. This could be platform chemicals such as low-molar-mass organic acids or furfural (Holm et al., 2010; Maassen et al., 2014), monosaccharides for fermentation and ethanol production (Gírio et al., 2010), or high-molar-mass hemicelluloses for production of bio-based barrier films, hydrogels, emulsion stabilizers, and possible health-promoting agents (Willför et al., 2008; Mikkonen et al., 2010; Xu et al., 2010; Kisonen et al., 2012; Pan et al., 2012; Maleki et al., 2014; Stevanic et al., 2014;).

Following the extraction, the next step in the process is to isolate and purify the dissolved hemicelluloses from the extract. Although water is an environmentally friendly and safe

solvent to use, it is highly energy demanding (i.e., expensive) to remove due to its high boiling point. Methods used for separating the hemicelluloses from water are ethanol precipitation (Song et al., 2013), filtration (Palm and Zacchi, 2004; Krawczyk and Jönsson, 2011; Al Manasrah et al., 2012), film evaporation (Walton et al., 2010) and spray-drying (Gabrielii et al., 2000; Jacquemin et al., 2012). Ethanol precipitation will produce a quite clean hemicellulose fraction with only minor impurities (Zasadowski et al., 2014). By varying the volume percentage of ethanol to the water extract, it is possible to tailor the molar mass of the precipitating hemicelluloses. However, ethanol precipitation can be rather expensive in industrial scale due to the needed ethanol recovery. Filtration is at one hand a cheaper and a continuous process, but at the other hand the filtrated hemicellulose fraction will contain more impurities such as lignin fragments and degradation products. Furthermore, the filters may foul because of the heterogeneity of the biomass and needs to be cleaned on a regular basis, which might cause production stops. This illustrates the challenges with hemicellulose recovery from the extracts in industrial scale. A combination of pre-concentration with filtration before ethanol precipitation could be a promising combination to finding an economical sustainable solution when upscaling.

2.3.1 Different water extraction techniques

Low temperatures (<100 °C) has been used during extraction to avoid intense hydrolysis and boiling as well as mimic the thermomechanical pulping (TMP) process. Willför and Holmbom (2004) investigated the water extractable polysaccharides from spruce, pine, and larch at room temperature. Only 0.5 % (o.d.w.) polysaccharides were released from spruce and 0.8 % from pine whereas as much as 15 % from larch, all with relatively high molar mass (20-30 kDa). Due to the mild conditions and high yield from larch (much arabinogalactans free in the middle lamella) one can conclude that the small amount of extracted polysaccharides from spruce and pine in their study probably also originated from the middle lamella. Stronger conditions (in this case higher temperatures) are needed to dissolve the hemicelluloses in the cell wall.

Microwave assisted hot-water extraction has been used for extraction of hemicelluloses from spruce chips (Lundqvist et al., 2003, 2002). The microwave treatment strongly shortened the required extraction time since heating the water to the desired temperatures is extremely fast. Lundqvist et al. (2002) reported a mannan yield of 71.4 % with water pre-impregnated wood

chips and extraction temperature and time of 200 °C and 5 minutes, respectively. Their following study (Lundqvist et al., 2003) showed that both shorter and longer extraction times than 5 minutes yielded substantially less hemicelluloses with this technique. A major drawback with the microwave-assisted extraction is that it is difficult to up-scale, not to mention expensive, and therefore might be best suited for academic work on laboratory scale.

Mild alkali addition to the extraction was applied (Capek et al., 2000) to investigate the structure of the extracted galactoglucomannan from spruce sawdust. A detailed and mild extraction scheme was used to minimize the degradation of the extracted hemicellulose chains. A thorough investigation with different analysis techniques could then be conducted on the polymer chain. Another study compared alkaline extraction to water extraction as a pretreatment before anthraquinone pulping and reported in favor of the alkali extraction due to higher pulping yields (Al-Dajani and Tschirner, 2010). Mentionable is that the study was focused on the pulping part and not optimizing the hemicellulose extraction. Addition of acid to the extraction process has been used as catalyst for the hydrolysis of the hemicellulose chains (González-Muñoz et al., 2011; Söderström et al., 2003) in order to extract monosaccharides for further ethanol production.

Varieties in plain hot-water extraction also exist, mainly by different extraction setups. The extractions has been made in batch mode with different reactor setups (Song et al., 2008; Grénman et al., 2011; **I**), in flow-through mode (Leppänen et al., 2010; Kilpeläinen et al., 2012, 2014) or by applying steam explosion (Martin-Sampedro et al., 2014). All these different techniques have both pros and cons regarding the end products and the economics of the whole process. In batch mode, it is more difficult to control the hydrolysis due to the long residence time of the already extracted hemicelluloses. And per definition, batch extraction is not a continuous process which is favorable in industrial production. Despite these drawbacks, batch extraction may be to be preferred due to the advantage of less water volumes, which keeps the subsequent extract concentration costs low. An unfortunate but natural property of flow-through extraction is the vast volumes of water it produces which will greatly increase cost when concentrating the extract by evaporation.

Biomass extraction assisted by ultrasound may be a promising addition to the process. The ultrasonic waves create micro cavities, or microbubbles, inside the biomass(García et al.,

2011). These bubbles will grow until they become unstable and collapse. A result of the treatment is an improved diffusion in the biomass. García et al. (2011) applied ultrasound to extraction of olive tree pruning residue and found that the treatment enhances the fractionation selectivity of different compounds depending on the extraction media. The ultrasound was less effective when alkaline or acidic media was used, so they concluded as a previous study (Romdhane and Gourdon, 2002) that the character of the media highly affects the effect of the ultrasound. However, to harsh ultrasound treatment will increase the amount of free hydroxyl radicals that might react with the hemicelluloses with depolymerization as a result (Kardos et al., 2001). Ultrasound can be a valuable addition to the extraction process but its effect will depend on the extraction media, as well as the biomass itself.

2.3.2 Extraction parameters

Temperature is probably one of the most important extraction parameter during a hot-water extraction. It is the temperature that applies the energy that initiates all the chemical reactions involved in the process. Naturally, with increased temperature more energy is provided and more chemical reactions can occur. A certain amount of energy (i.e., a certain temperature) is required for the reactions involving the hemicellulose dissolution from the wood matrix to start. But if the temperature is too high, unwanted reactions such as depolymerization, degradation, and condensation will likely occur. The extraction temperature is therefore crucial for the whole extraction.

The extraction time is almost as equally important as temperature, since the time will affect the amount of total energy added to the overall extraction process. Besides, some extraction reactions may require longer reaction times so only applying high temperature (high energy) during a short period may not be enough for certain reactions. Furthermore, the dissolved hemicelluloses also require a certain amount of time to diffuse out from the wood into the water phase.

The temperature and time can be combined to a severity factor, also called prehydrolysis factor, or P-factor (Sixta et al., 2006). With the P-factor, the extraction severity can be expressed as a single variable, which can be used as a tool for estimating and comparing extractions with different temperatures and time. Important to notice is that the extraction of hemicelluloses from a wood matrix is controlled by many factors, not only temperature and

time, which the authors also pointed out. The P-factor was originally developed for comparing severity during different delignification treatments (Brasch and Free, 1965). Pedersen and Meyer (2010) present the development of the original P-factor equation into a more fitted model for hemicellulose dissolution and degradation with pH included. Although improvement has been made in the attempts to explain the pretreatment by a severity factor, also Pedersen and Meyer (2010) concluded that a one-dimensional severity factor calculation cannot reliably explain the complexity of biomass pretreatment.

Following temperature and time, pH is another important extraction parameter. The pH plays a vital role in many chemical reactions and specifically hydrolysis of hemicelluloses in biomass hot-water extraction. During hot-water extraction of wood with plain water the pH in the extract normally decreases. This decrease or drop in pH is a result of a combination of the enhanced auto-ionization of water at higher temperatures, which increases the H_3O^+ ion concentration (Zumdahl and Zumdahl, 2007) and an induced formation of acetic acid from cleaved acetyl groups from the hemicellulose chains (Liu, 2010). It is a well-known fact that acidic media (low pH) will catalyze the hydrolysis of the (1→4)-glycosidic bonds between the sugar units in the hemicellulose chains with chain degradation and reducing molar mass as result (Lai, 2001; Kamerling and Gerwig, 2007). The depolymerization reaction of the carbohydrate chain is described in Figure 2.6.

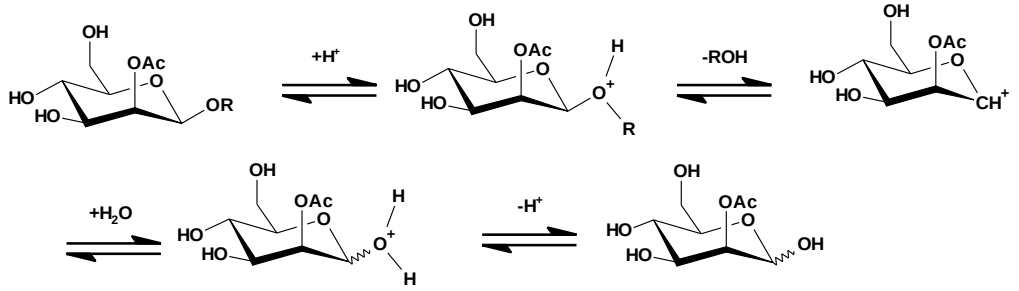

Figure 2.6 Simplified reaction mechanism of acid-catalyzed hydrolysis of hemicellulose. Adapted from Kamerling and Gerwig (2007).

This depolymerization is an unwanted process if high-molar-mass hemicelluloses are the target but can be an advantage if sugar monomers are the desired extraction product. A positive aspect of the chain degradation due to pH is that smaller molecules (low-molar-mass

hemicelluloses) will more easily diffuse out from the matrix than larger molecules will, resulting in faster diffusion and increased yield. These examples show the effect of pH during a hot-water extraction of wood and why it is a relevant extraction parameter. For an efficient and controlled extraction process, correct in-line pH measuring and eventually in-line pH control during extraction of hemicelluloses from wood is highly desirable. More of pH and pH measuring during hot-water extraction is described in sections 2.4 and 3.2.2.

Surface active agents, or surfactants, could be an interesting addition to the hot-water extraction in order to facilitate the diffusion of the hemicelluloses out from the wood matrix. Surfactants has been used as additives in enzymatic hydrolysis processes, both to enhance the hemicellulose extraction (Wei et al., 2011), to prevent unspecific adsorption of cellulase enzymes to lignin (Kristensen et al., 2007), and to improve the solubility and removal of the lignin from the wood matrix, making the cellulose more accessible to the enzymes (Kurakake et al., 1994). All these methods are aiming at the enzymatic degradation of cellulose and hemicelluloses in order to produce bioethanol, biofuels, and other value-added co-products. Because of the specific aims of the above-mentioned studies it is difficult to predict if surfactants would have a positive effect on high-molar-mass hemicellulose extraction.

2.4 High-temperature pH measuring

2.4.1 Theory

Normally when measuring pH, a conventional laboratory pH meter, the glass electrode, is used. The pH meter measures the difference in potential caused by hydrogen ion activity in a solution between a hydrogen ion sensitive electrode and a reference electrode (usually an Ag/AgCl electrode) (Galster, 1991). Normally both electrodes are embedded in the pH probe along with a thermometer. In fact, the measured potential consists of several intermediate potentials within the pH meter. Such are the potential difference between the sample solution and the gel layers on the glass membrane (inside, as well as outside), the potential difference between the reference electrode and the reference electrolyte solution and the potential difference between the reference electrolyte and the sample solution through a junction. These form a potential chain where all but the potential between the sample solution and the glass membrane are constant. With the measured potential and the temperature the pH meter uses Nernst equation (1) (Christian, 1994)

$$E = E^0 - 2.303\,{}^{RT}/_{F} * (-\lg(\alpha_H)) \quad (1)$$

or rearranged (2) to calculate pH from the potential

$$pH = \frac{E^0 - E}{S} \quad (2)$$

where E is the measured potential, E^0 the standard electrode potential, R the gas constant, T the temperature in Kelvin, F Faradays constant, a the activity of the measured species (in this case the hydrogen ion), and S 2.303*RT/F. S is also known as the calibration slope or Nernst potential (U_N). The standard electrode potential, E^0, is a constant that includes the different internal potentials, as well as an asymmetry potential. The asymmetry potential exists across the glass membrane and is a result from physical defects of the membrane such as non-uniform composition, mechanical and chemical attacks, and the degree of hydration. This will slowly change with time and therefore, it is very important to calibrate the pH meter from time to time (Christian, 1994).

2.4.2 Measuring

In hot-water extraction of hemicelluloses from wood it is highly desirable to be able to monitor the extraction pH due to reasons explained in chapter 2.3. The conventional glass pH electrodes are not suitable for measurements of pH at elevated temperatures because the hydrogen ion sensitive glass membrane will degrade and loose its ion sensitivity (Galster, 1991; Morf, 1995). The upper working temperature for commercial laboratory pH electrodes are 80 - 100 °C, depending on if the inner reference electrolyte is in gel or liquid form (SI Analytics GmbH, 2012). Further on, calibration of pH meters is also difficult at higher temperatures. The pH measurement is highly temperature dependent, especially at elevated temperatures (>100 °C), very little information on measured pH values for buffers at these temperatures are to be found. Some literature mention measured pH values above 100 °C but they are few and originates from the 1960s (Le Peintre, 1960; Krykov et al., 1966).

Over the last decades, beginning in the early 1980s, high-temperature and high-pressure compatible pH electrodes have been developed (Niedrach, 1980a, 1984). These electrodes are

mainly ceramic solid metal/metal oxide electrodes and the yttria-stabilized zirconium oxide (YS Zr/ZrO_2) electrode is probably the most common (Niedrach, 1980a; Niedrach and Stoddard, 1985; Lvov et al., 2000, 2003). The YS Zr/ZrO_2 ceramic membrane is in fact an oxygen-ion conducting membrane, but because of the close relation between oxygen and hydrogen ions in the potential determining reaction

$$O_2 + 4\ H^+ + 4\ e^- = 2\ H_2O$$

the electrode also functions well as a hydrogen sensitive (pH) electrode (Niedrach, 1980a, 1980b). Since the Zr/ZrO_2 electrode also is very stable regarding corrosion and redox potential at high temperatures it has been used as an excellent reference electrode when measuring corrosion and redox potentials in high temperature aqueous solutions (Niedrach, 1982; Niedrach and Stoddard, 1985).

3. Experimental

3.1 Materials and chemicals

3.1.1 Spruce wood and bark

Wood

Knot-free sapwood from a healthy 38-year-old Norway spruce (*Picea Abies*), felled in southern Finland, was ground with a Fritsch Universal cutting mill "pulvirisette 19" (Idar-Oberstein, Germany). The roughly grounded wood was sieved into five different particle sizes; 0.5-0.7 mm, 1.25-2 mm, 2-4 mm, 4-8 mm, and 8-12.5 mm with a Retsch Vibratory Sieve Shaker AS 200 basic (Haan, Germany). Wood chips were handmade from the knot-free sapwood with dimensions of 25 × 20 × 4 mm and a cutting angle of ~45°.

Bark

The spruce bark was manually removed from a healthy tree felled in south-western Finland in autumn 2008. Inner and outer bark samples were carefully separated with scalpel, then freeze-dried, and milled with a Model MF 10 Microfine Grinding Mill (IKA Works, Germany) through a 1 mm sieve.

3.1.2 Reactor setup

The extractions were carried out in a batch extractor setup (Figure 3.1). The setup consisted of a 500 mL autoclave (**I**) or a 1000 mL autoclave (**II - IV**), (Autoclave Engineers, PA) equipped with a Dispersimax^{TM} turbine stirrer and a heating mantle. A pre-heating vessel (300 mL) equipped with heating elements was connected to the autoclave adding the possibility to pre-heat the water before extraction. The solvent pre-heating option was used in paper **I**. A sampling valve made it possible to take aliquots of samples during the extraction. Argon gas was coupled to the system for purging the inside of the autoclave, as well as the tubing. The extraction temperature was controlled by an Eurotherm 2416 temperature controller (Eurotherm, VA, USA) and logged to a computer with PicoLog TC08 datalogger and PicoLog software.

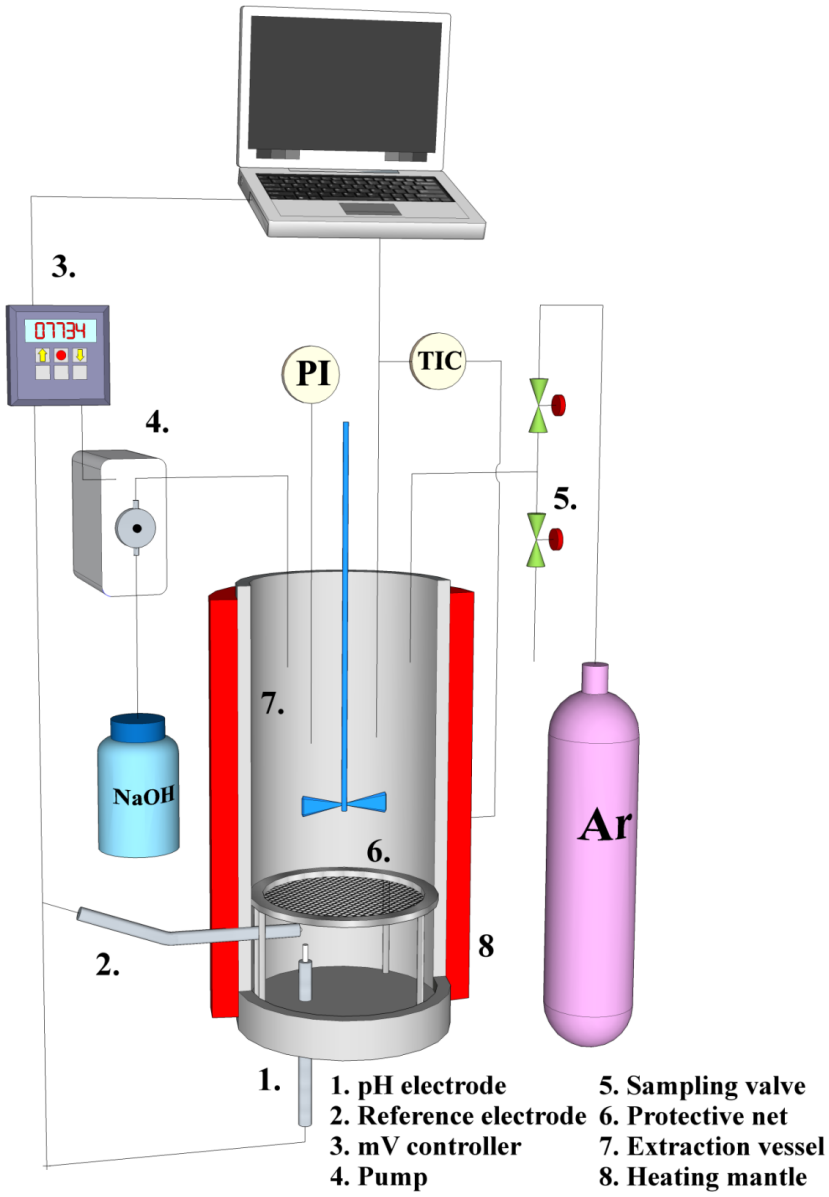

Figure 3.1 Batch extraction setup with pH measuring electrodes and automated alkali addition setup. PI, pressure indicator; TIC, temperature indicator and controller.

The 1000 mL autoclave was further equipped with a solid ZrO_2-based high-temperature and high-pressure pH electrode together with a high-temperature and high-pressure Ag/AgCl

reference electrode (Corr Instruments, TX, USA). The pH electrode was an yttria-stabilized Zr/ZrO_2 membrane electrode with a Queon™ seal and a pressure range of 0-136 bars and temperature range of 90-305 °C. The reference electrode was a 0.1 N KCl filled internal pressure-balanced Ag/AgCl electrode with a ceramic frit as a junction partly based on ZrO_2, Queon™ seal and a pressure range of 0-136 bars and temperature range of 0-305 °C (**II - IV**). The potential was recorded with a MeterLab® PHM220 lab pH meter (Radiometer analytical, Lyon, France) high-input impedance voltmeter and logged to a computer. A Dulcometer® D1Cb mV controller (ProMinent, Heidenberg, Germany) was connected to the pH meter (**III, IV**). The controller regulated a HPLC pump pumping 0.5 M NaOH into the reactor. With this setup it was possible to determine a voltage set point (in mV) for any extraction that the controller with the HPLC pump and the alkali would maintain. A protective net was used inside the reactor (Figure 3.1) to shield the electrodes against the swirling wood particles (**II, III**). Later, the wood was inserted into a cylindrical net cage attached to the stirring rod (Figure 3.2) to avoid both damaging the electrodes and as well as not to contaminate the pH electrode with small wood particles (**IV**). No external pressure was applied during the extractions so the pressure was the water vapor pressure at the different extraction temperatures; about 8 bar at 170 °C and 10 bar at 180 °C.

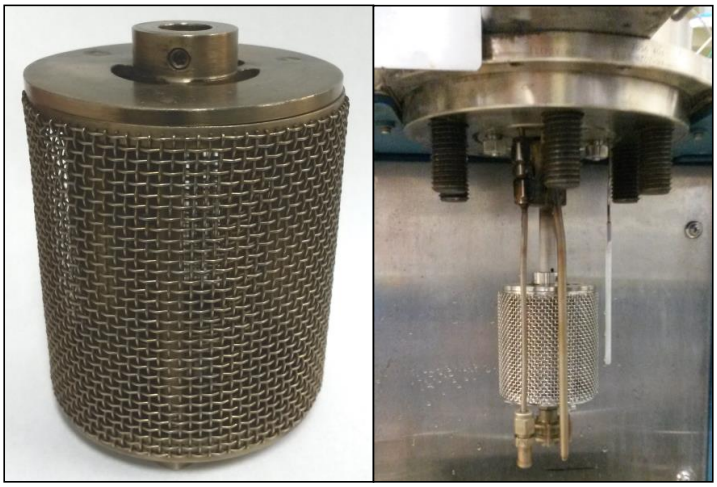

Figure 3.2 Net cage used in pH controlled extractions. Close up on the net cage (left) and the net cage mounted on the stirring rod (right).

The bark samples were extracted with an accelerated solvent extractor (ASE - 300) apparatus (Dionex, CA) (Figure 3.3).

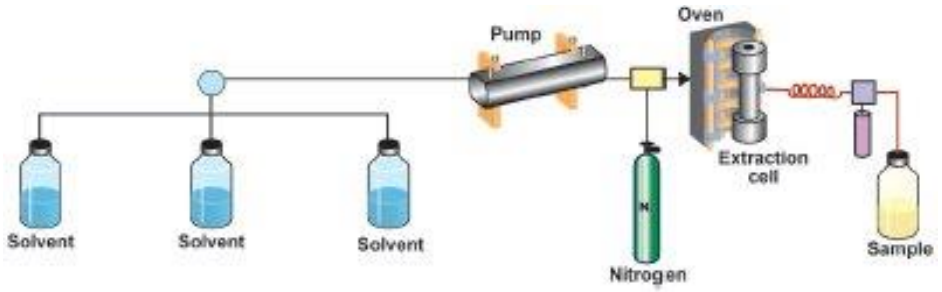

Figure 3.3 ASE set-up.

Extraction with ASE enabled automated sequential extractions with different solvents (polar and non-polar). This made the extraction very reliable and the repeatability was very good. The pressure during an ASE extraction was pre-set to ~103 bar (1500 psi).

3.1.3 Chemicals

Potassium hydrogen phthalate, $C_8H_5KO_4$ (Merck KGaA, Damstadt, Germany), potassium dihydrogen phosphate, Na_2HPO_4 (VWR, Leuven, Belgium), and sodium monohydrogen phosphate, H_2KPO_4 (J.T.Baker, Deventer, The Netherlands) were used for the calibration solutions. All salts for the calibration solution preparation as well as the NaOH pellets (J.T.Baker, Deventer, The Netherlands) used for the alkali solution were of analysis grade.

3.2 Methods

3.2.1 Extraction

Wood

For the particle size study (**I**), the 500 mL reactor with pre-heating was used. Approximately 9 g of air dried wood along with 300 mL distilled water was used for these experiments resulting in a liquid-to-wood ratio (L/W) of 33 mL/g. The whole extraction setup was purged with argon gas before heating. With pre-heating 200 mL of the water, the heating time was about 15-20 minutes and when the temperature reached the desired extraction temperature

(170 °C), an initial sample was taken. With the initial sample the extraction experiment was assumed to start although some wood components had probably been dissolved during the heating phase as well. No external pressure was applied leaving the extraction pressure at about 8 bars. Wood particle sizes of 1.25-2 mm (**II**), 2-4 mm (**III**), and 2-4 and chips (**IV**) were used in the pH measuring and control studies. The wood amounts used were approximately 21.5 g (L/W: 33 mL/g) (**II**), 20 g (L/W: 35 mL/g) (**III**), 17.5 g (L/W: 40 mL/g) (**III**), and 10 g (L/W: 70-75 mL/g) (**IV**), respectively. Since no pre-heating was used in these studies, the heating time to the desired extraction temperatures was slightly over 40 minutes. The potential controller was switched on as soon as the temperature reached 100 °C and the potential readings from the pH electrodes stabilized and dropped below the desired mV set point (**III, IV**). When the samples had cooled down to room temperature, pH was measured with a conventional glass electrode pH meter.

Bark

An ASE apparatus (ASE-300, Dionex, CA) was used for the bark study (**V**). This instrument enabled successive extraction with different solvents in a convenient way. Two different extraction schemes were used (Figure 3.4). The first extraction scheme (A) was used to determine the chemical composition of the barks, while the second extraction scheme (B) was used to compare the extraction of spruce bark components with hot-water at different high temperatures.

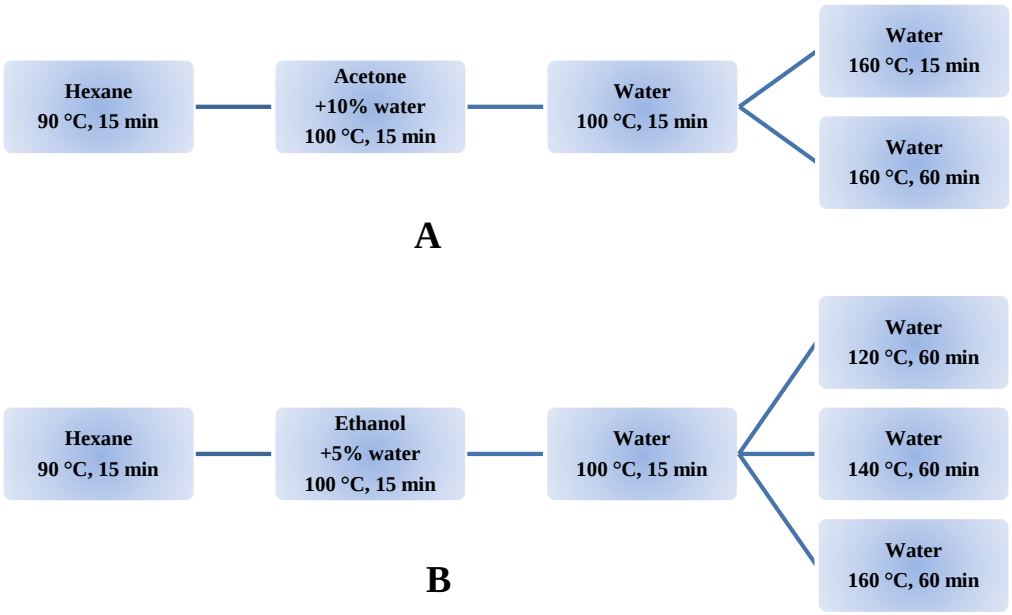

Figure 3.4 Sequential bark extraction schemes.

Extraction scheme A. The inner and outer bark powders were first extracted with hexane at 90 °C, followed by acetone-water (9:1, v/v) at 100°C, both for 15 min (3 × 5 minutes static cycles), to obtain the lipophilic and hydrophilic extractives, respectively. Thereafter, the barks were extracted with water at 100°C for 15 min (3 × 5 minutes static cycles), and after that at 160°C for both 15 min (3 × 5 minutes static cycles) and 60 min (3 × 20 minutes static cycles), in parallel.

Extraction scheme B. After extraction with hexane at 90 °C, extraction was continued ethanol-water (95:5, v/v) at 100 °C for 15 min (3 × 5 minutes static cycles) for both inner and outer bark. After this, the barks were extracted with water at 100 °C for 15 min (3 × 5 minutes static cycles), and finally with water at 120 °C, 140°C and 160 °C for 60 min (3 × 20 minutes static cycles), in parallel.

ASE was used for the bark extraction due to the convenient possibility of sequential extraction of the same sample with different solvents, different temperatures, and high

pressure. However, due to the dimensions of the ASE extraction cell, mounting of the in-line high-temperature pH electrodes was not possible.

3.2.2 High-temperature pH calibration

Because the working range of the commercial high-temperature pH electrode used in this work is from 90 °C to 200 °C, it was not possible to calibrate the system with buffers at room temperature, as is normally done with conventional laboratory pH meters. Since the potential response is very temperature dependent, calibration at room temperature would not be valid at higher temperatures anyway.

Nernst equation (eq. 2) was used for calculating pH values from the measured potential. To be able to do so, E^0 and S should be known and to determine E^0 and S for the system one should have solutions with known and stable pH at the used temperatures. Therefore, buffers with different pH were tested at 160 °C, 170 °C, and 180 °C. The different buffer solutions were 0.05 mol/kg potassium hydrogen phthalate with a pH of 4.005 at 25 °C, 0.025 mol/kg disodium hydrogen phosphate + 0.025 mol/kg potassium dihydrogen phosphate with a pH of 6.857 at 25 °C. These buffers gave stable mV readings at the studied temperatures. The pH values for these buffers at the high temperatures were taken from Galster (1991) in accordance to the electrode manufacturer. Galster (1991) reported pH values for the phthalate buffer up to 150 °C so an extrapolation was made with good fit to provide the pH values for 160, 170, and 180 °C. The pH values for the phosphate buffer was reported for 100, 125, 150, 175, 200, 225, and 250 °C, a model was fitted to the plotted curve to obtain the pH values at 160, 170, and 180 °C for the phosphate buffer as well. With these acquired values, it was possible to plot the known pH values against the measured potential of the phthalate and phosphate buffers at different temperatures to acquire the calibration curves.

Inserting the y-axis intercept (E^0) and the slope (S) from the calibration curve with the potential (E) measured during a wood extraction into equation 2 gave the in-line pH value during the extraction.3.2.3 Analytical procedures

TDS and pH (**I – V**)

Exactly two mL of the extract was freeze-dried and weighed to determine the total dissolved solids (TDS) in the extracts (**I, III - V**). The off-line pH was measured with a SCHOTT Instruments handylab pH 12 meter (SI Analytics, Maintz, Germany) directly after the extract had cooled down to room temperature (**I – V**).

Total non-cellulosic carbohydrates (**I, III - V**)

The non-cellulosic carbohydrates, i.e., the hemicelluloses and pectins, were determined by freeze-drying the extracts, subjecting them to acid methanolysis and finally by analysis with GC (Sundberg et al., 1993; Willför et al., 2009). The original wood as well as the extraction residues was analyzed in a similar manner with acid methanolysis and GC. The results were calculated and presented as anhydro-sugar units if not stated otherwise. The non-cellulosic carbohydrates are hereby referred to as simply hemicelluloses.

Monomeric sugars (**I, III - IV**)

Monomeric sugars in the extracts were determined by freeze-drying an aliquot of the extract, silylating the dry sample, and analysis by GC (**I**).

Oligomers (**I**)

One mg of solid sample, calculated from TDS, was taken from the aliquot, freeze-dried, and then re-dissolved in a 1:1 mixture of pyridine and acetic anhydride. The sample was kept in the dark for 5 days and shaken daily. Ethanol was added and then the mixture was evaporated under a stream of nitrogen until dryness. More ethanol was added and the sample was dried again under a stream of nitrogen and subsequently in a vacuum oven to ensure dryness. The dry sample was then dissolved in tetrahydrofuran (THF) to an approximate concentration of 1 mg/mL, filtered with 0.22 µm polytetrafluoroethylene (PTFE) syringe filter, and analyzed for oligomers with gel permeability chromatography high-pressure liquid chromatography (GPC-HPLC). Samples with 1 mg/mL acetylated glucose and cellobiose, respectively, were used for calibration.

Molar mass (**I, III, IV**)

Molar mass, weight-average molar mass (M_w) and number-average molar mass (M_n), and the molecular weightdistribution (MWD) of dissolved hemicelluloses in the extracts were determined by high pressure size exclusion chromatography (HPSEC) in an on-line combination with a Multi-Angel Laser Light Scatter (MALLS) instrument (miniDAWN, Wyatt Technology, Santa Barbara, USA) and a refractive index (RI) detector (Shimadzu Corporation, Japan). Astra software (Wyatt Technology, Santa Barbara, USA) was used to analyze the data.

Cellulose (**I, IV, V**)

The cellulose contents in wood and the different extraction residues were determined by acid hydrolysis and GC. Original wood and extraction residue were weighed in test tubes. 72 % sulfuric acid was added. After two hours, 0.5 mL distilled water was added and after further four hours, another 6 mL was added. The next day the samples were autoclaved at 125 °C for 90 minutes. A few drops of a bromocresol green solution were added as indicator and barium carbonate was used to neutralize the acid in the samples. One mL of 5 mg/mL sorbitol solution was added as an internal standard and the samples were centrifuged to obtain a clear liquid phase to transfer into pear shaped flasks. The samples were dried and silylated as described above and analyzed to determine glucose with GC (Browning, 1967).

Klason lignin (**I, IV, V**)

Klason lignin was determined on the wood and extraction residues by a modified Klason lignin procedure (Schwanninger and Hinterstoisser, 2002). One gram was used in study (**V**) and (**I**) whereas 100 mg was used in (**IV**) with downscaling the acid and water volumes accordingly.

Acetic and formic acid (**I, III – V**)

Free acetic and formic acid in the extracts, released during the wood extractions (**I**,**III**,**IV**), were analyzed by HPLC (Agilent 1260 series, Waldbronn, Germany) with a Synergi Hydro-PR 80A HPLC column (250 mm×4.6 mm, 4 µm, Phenomenex®, CA, USA) RI detector (Agilent, Waldbronn, Germany). 20 mM KH_2PO_4 was used as eluent. The pH of the eluent

was adjusted to about 2.7 with 85 % *ortho*-phosphoric acid and the eluent was filtered through a 0.1 µm filter. 1 mL of sample was mixed with 1 mL of 40 mM KH_2PO_4 to adjust the pH to 2.7-2.9. The mixture was filtered through a 0.22 µm nylon filter and put into an auto-sampler vial (Song et al., 2008).

For analysis of acetic and formic acid in the solid bark samples (**V**) approximately 2 g of inner or outer bark powder was put into large test tubes with 35 mL of distilled water, after which pH was adjusted to 11 with 1 M NaOH and the total volume was adjusted to 40 mL with distilled water. The samples were kept in an oven at 70 °C for two days. During the treatment, pH was measured twice and re-adjusted to 11 with 1 M NaOH. After hydrolysis, the samples were centrifuged; 0.5 mL of liquid phase was taken, and was then freeze-dried and re-dissolved in 4 mL distilled water. 0.5 ml of 0.5 mg/ml iso-butyric acid solution was added as internal standard. The samples were eluted through a column with Dowex 50WX8 50-100 mesh ion-exchange resin (Acros Organics) to remove cations and ensure that the acids were in protonized form. The pH was then raised to 8 with 0.08 M tetrabutylammonium hydroxide (TBAH) to ensure that the acids were in salt form. The water was evaporated in a rotary evaporator and the residues further dried in a vacuum oven for 30 min to remove all water. The residues were then benzylated with 0.4 mL benzyl bromide in 1.5 mL acetone with shaking for 2 h. The liquid phase was transferred to autosampler vials and analyzed by GC (Alén et al., 1985). Four calibration samples were prepared, with 0.1, 0.2, 0.4, and 0.8 mg acetic acid, and 0.5 mg iso-butyric acid as internal standard, and were treated and analyzed by GC in a similar manner as above to determine calibration coefficients.

Extractives (**V**)

Aliquots of the hexane and aqueous-acetone extracts (**V**) were dried, redissolved in pyridine and silylated with *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and Trimethylsilyl chloride (TMCS) at 70 °C for 30 min. The silylated samples were then analyzed by GC with both short and long columns (Willför et al., 2003; Örså and Holmbom, 1994). The short column (6 m) was used for determination of component groups including the high-molar-mass steryl esters and triglycerides, while a normal column (25 m) was used for detailed determination of low-molar-mass components such as fatty and resin acids, diglycerides, lignans, stilbene glucosides etc.

Tannin analysis (**V**)

Aliquots of the 100 °C water extracts and the 160 °C water extracts (**V**) was subected to acid butanol assay according to Gessner and Steiner (2005) to determine the condensed tannins in the extracts. A reference tannin sample was prepared from a hot-water extract of spruce bark. The tannin fraction was isolated by adsorption from an 80 % ethanol solution on Sephadex LH-20 and desorption with 70 % acetone. 200 µL of an acetone solution of the tannin (1.15 mg/mL) was dried and then dissolved in 500 µL water. The reference sample was treated the same way as the extract samples. After cooling, the absorbance at 550 nm was measured with a Perkin-Elmer Lambda 40 ultraviolet-visible (UV-Vis) spectrometer. An additional reference sample was prepared with water, acid n-butanol and iron reagent (2 % ferric ammonium sulfate in 2 N HCl) and treated the same way as the samples to adjust the UV-Vis spectrometer to zero.

Pyrolysis-GC-MS (**V**)

Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) analyses were performed on a filament pulse Pyrola 85 pyrolyzer (Pyrol AB, Lund, Sweden) connected to a HP 5890 GC (Hewlett-Packard Comp., USA) and a HP 5970 quadrupole mass selective detector (EI, 70 eV) using a HP-1 capillary column. For thermally assisted thermolysis and methylation, 10 % aqueous TMAH was added and the filament was heated at 360 °C for 2 s (Pranovich et al., 2005).

Suberin (**V**)

Exactly 1 mg of cholesterol in acetone solution was pipetted into a test tube as internal standard and the acetone was evaporated under a stream of nitrogen. Dried bark (**V**) was weighed into the test tube and 0.5 M KOH in ethanol/H_2O (9:1, v/v) was added for alkaline hydrolysis. The solution was kept at 70 °C for 1.5 h and shaken from time to time. After the hydrolysis the solid phase was left to sediment and the clear liquid phase was transferred into a new test tube. Water was added as well as a bromocresol green solution as indicator. To acidify the solution 0.25 M H_2SO_4 was added until the color changed to yellow (pH below 3.8). Methyl *tert*-butyl ether (MTBE) was added and the test tube was shaken thoroughly. After phase separation, as much of the MTBE phase as possible was transferred into a new test tube and the extraction was repeated three times to ensure that all suberin-derived fatty

acids were transferred into the MTBE phase. The combined MTBE phase was then washed with water. The acid-free MTBE phase was transferred into a new test tube, evaporated under a stream of nitrogen, and re-dissolved in 50 mL pyridine. The sample was silylated with 100 µL BSTFA and 25 µL TMCS and kept in an oven at 70 °C for 45 min. A clear liquid phase was then transferred into autosampler vials and analyzed by GC (Ekman and Reunanen, 1983)

Metal ions (**V**)

Metal ions and inorganic compounds were analyzed at the Finnish Forest Research Institute (Metla) with inductively-coupled plasma – atomic-emission spectrometry (ICP-AES, TJA Corp. Massachusetts) after microwave wet digestion in a nitric acid/hydrogen peroxide solution.

4. Results and discussions

4.1 High-temperature pH measurement

To be able to rely on any measurements and especially pH measurements, the equipment used needs to be calibrated against known and relevant values. Calibrating conventional pH electrodes at room temperature is nowadays a standard procedure and is done regularly. Besides calibration it is also important to validate the measuring equipment in ways of sensitivity, stability, and repeatability.

4.1.1 Calibration

In order to calibrate the high-temperature pH measuring system with a two-point calibration method, it was necessary to obtain pH values for phthalate and phosphate buffers at high temperatures. Values were taken from Galster (1991) and extrapolated with good fit to acquire pH values for the temperatures used. Figure 4.1 shows the curves based on literature data, as well as the extrapolation.

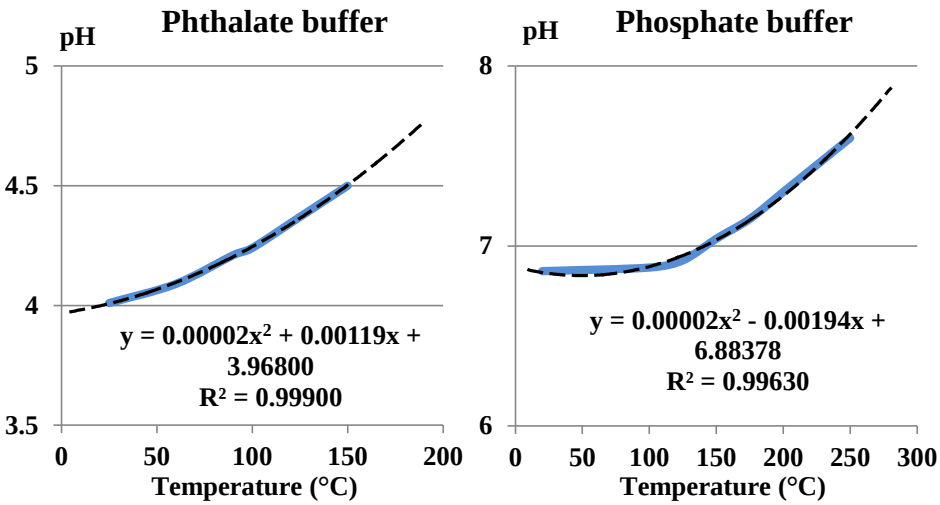

Figure 4.1 pH values at different temperatures for 0.05 mol/kg potassium hydrogen phthalate buffer (left) and 0.025 mol/kg disodium hydrogen phosphate + 0.025 mol/kg potassium dihydrogen phosphate buffer (right). The thick blue lines are values from literature (Galster, 1991) while the dotted line is an extrapolation of the literature values.

The extrapolated models fitted well with the values from literature. The coefficient of determination (R^2) was 0.999 for the phthalate buffer and 0.996 for the phosphate buffer. Using the acquired equations from the extrapolation, an estimation of the pH values for 100 °C, 160 °C, 170 °C, and 180 °C for both buffers were calculated. The results are presented and discussed later in this thesis.

Several experiments were made with the phthalate and phosphate buffers at different temperatures (100 °C, 160 °C, 170 °C, and 180 °C) (Figure 4.2). The main reason was to obtain the potential response at the mentioned temperatures to plot a two-point calibration curve. The system was at the same time also tested for its stability, signal noise, and response to and relaxation time after temperature changes.

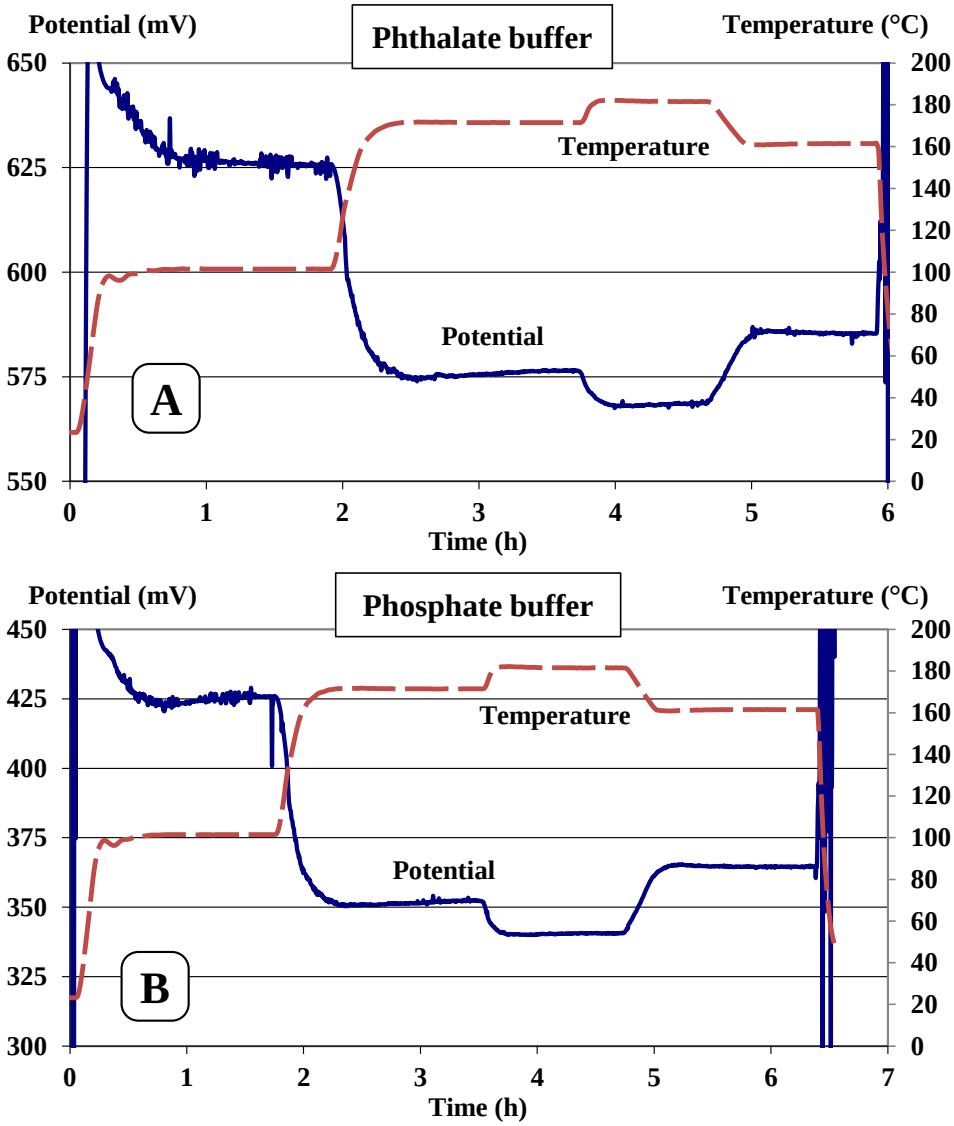

Figure 4.2 The pH electrode potential response (mV) for 0.05 mol/kg potassium hydrogen phthalate (A), 0.025 mol/kg disodium hydrogen phosphate and 0.025 mol/kg potassium dihydrogen phosphate (B) at 100 °C, 170 °C, 180 °C, and 160 °C.

As Figure 4.2 shows, the electrode potential response for the phthalate and phosphate buffers was instant when changing the temperature and the signal was very stable at constant temperature for higher temperatures (160 °C, 170 °C, and 180 °C). The signal noise (SN), the

deviation from the signal average when the temperature is constant, at these temperatures was less than 1 mV showing very good stability and thus, giving very accurate readings. At 100 °C, the potential response was slower and the SN higher, probably because this temperature was close to the electrodes lower working limit (90 °C) and therefore the electrodes were not as sensitive as at the higher temperatures.

Parallel experiments with the buffers were made to test the system repeatability. The relative standard deviation (RSD) of the electrode potential between the experiments at 100 °C was as expected the highest, being 4.5 mV for the phthalate buffer and 3.8 mV for the phosphate buffer (Table 4.1). The SN was on average 1.78 mV for the phthalate buffer and 3.07 for the phosphate buffer, which was much higher than the SN at 160 °C, 170 °C, and 180 °C. The reason for the larger deviation was, as mentioned before, that the temperature was close to the lower working limit of the electrodes.

Table 4.1 pH electrode potential response for phthalate solution (pH 4) and phosphate solution (pH 7) at 100 °C, 160 °C, 170 °C, and 180 °C. The signal noise (SN) is the deviation from the average value for the potential measured from a single experiment when temperature is constant.

pH		**100 °C**		**160 °C**		**170 °C**		**180 °C**	
		mV	**SN**	**mV**	**SN**	**mV**	**SN**	**mV**	**SN**
4	1	627	1.98	585	0.32	576	0.62	569	0.53
	2	620	1.57	585	0.39	575	0.70	567	0.24
	Average	**624**	1.78	**585**	0.35	**575**	0.66	**568**	0.39
	RSD	4.5		0.6		0.8		0.8	
7	1	425	1.93	365	0.34	352	0.67	340	0.21
	2	430	4.20	364	0.23	350	0.74	339	0.28
	Average	**427**	3.07	**364**	0.29	**351**	0.71	**340**	0.25
	RSD	3.8		0.3		0.9		0.8	

Both the RSD and the SN of the electrode potential decreased significantly when the temperature was increased to 160 °C, 170 °C, and 180 °C. The signal deviation between the parallel samples was only 0.9 mV as max (pH 7 at 170 °C) and as good as 0.3 mV at best (at 160 °C) (Table 4.1) showing very good repeatability. The SN was well below one mV for all the high-temperature experiments.

With the estimated pH values from the extrapolation and the measured potential values from the buffer experiments it was possible to plot a two-point calibration curve for the different temperatures (Figure 4.3).

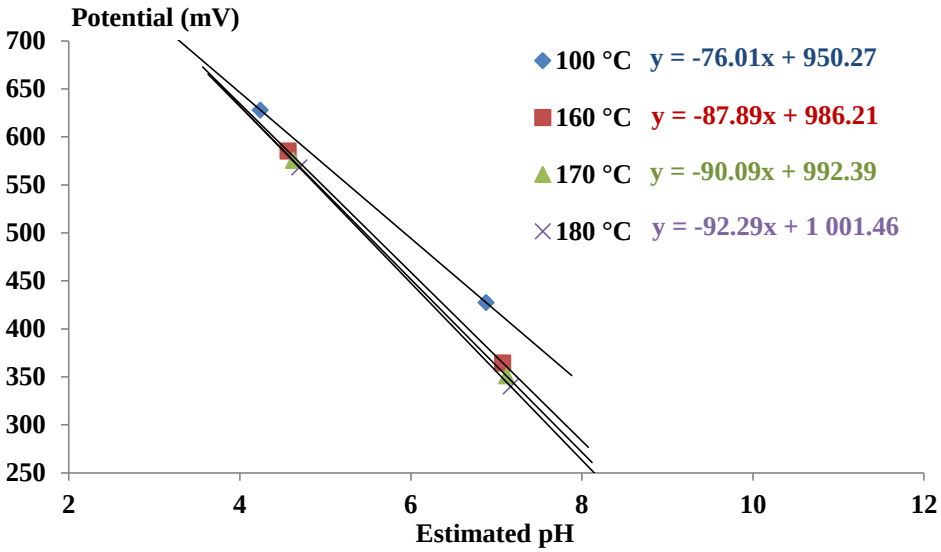

Figure 4.3 Calibration curves for the pH electrodes at 100 °C, 160 °C, 170 °C, and 180 °C with the slope factor and intercept described in the line equations for the different temperatures.

From the line equations in Figure 4.3, both the slope (S) of the lines and the offset or intercept with the y-axis (E^0) were determined. These values, S and E^0, were then used in Nernst equation when calculating the pH during hot-water extractions. All the values of the estimated pH, the recorded potential, the calculated slope and intercept are presented in Table 4.2.

Table 4.2 Estimated pH of buffers, electrode potential response, slope factor and intercept for the different buffers at 100 °C, 160 °C, 170 °C, and 180 °C, as well as the theoretical slope at the same temperatures.

Temperature, °C	Buffer	pH	mV	Slope (S)		Intercept (E^0)
				Measured	Theoretical*	
100	phthalate	4.24	628	76.0	74.0	950
	phosphate	6.88	427			
160	phthalate	4.56	585	87.9	85.9	986
	phosphate	7.07	364			
170	phthalate	4.63	575	90.1	87.9	992
	phosphate	7.12	351			
180	phthalate	4.70	568	92.3	89.9	1001
	phosphate	7.17	340			

* Calculated from 2.303 × (RT/F).

The measured slope values for the different temperatures were between 2.0 and 3.2 mV/degree units higher than the theoretical slope value. This corresponds to a difference in percentage of between 2.5 % and 3.5 %. This was to be expected and fully acceptable since no pH meters show absolute theoretical values due to factors explained in section 3.2.2.

4.1.2 High-temperature pH measuring

Two parallel preliminary pH measurements with wood were performed to check the stability and repeatability of the electrode potential signal when extracting hemicelluloses at 170 °C with plain water. The system responded well to the extraction and stable signals were recorded throughout the whole extraction time. The potential signal was then recalculated to pH according to the simplified Nernst equation (eq. (2) in section 3.2.2) with S and E^0 from

the buffer experiments (Fig 4.4).

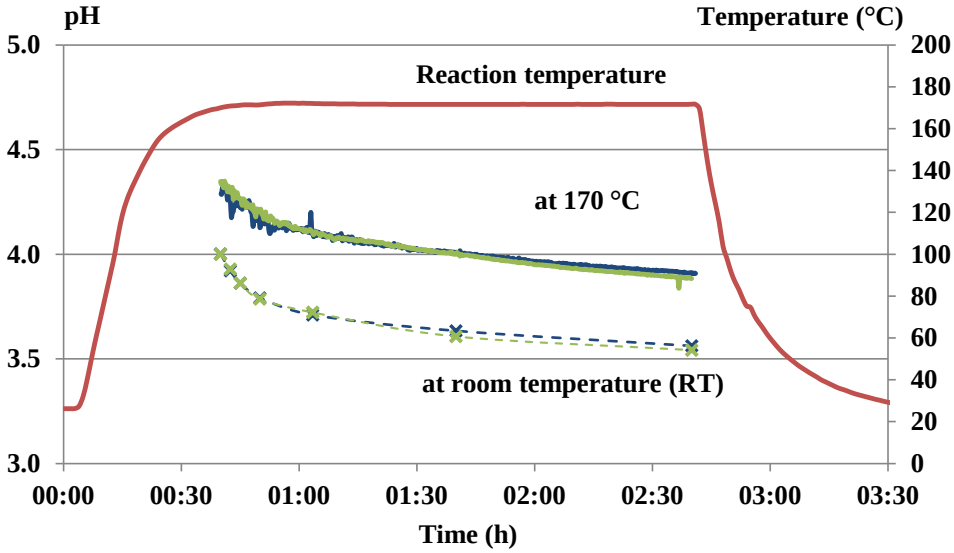

Figure 4.4 In-line pH measurements during two 170 °C hot-water extractions of ground spruce wood. Solid lines in blue and green represent in-line measurements and dotted lines pH measured with a conventional glass electrode at room temperature.

The difference of the electrode potential signals between the parallel experiments were in average only ~0.5 mV, which showed that the repeatability was very good. One important observation from the study was that the measured in-line pH follows in parallel the pH of the samples when measured with a conventional pH meter after the samples had cooled down to room temperature although situated at different levels. This was a strong indication that the calculation was correct and the system was working and could be used to measure pH at high-temperature wood extractions. The results showed that the in-line pH values were about 0.35 pH units higher than the pH values measured at room temperature. One explanation for this could be that the dissociation of acetic acid, one of the main contributors to the low pH in wood extractions like these, is exothermic and pK_a increases at higher temperature (Fisher and Barnes, 1972). The acid dissociation equilibrium was therefore shifted towards the protonated form of the acid at high temperatures, which resulted in less free hydrogen ion in the solution and thus a higher pH.

4.2 High-temperature pH control

In order to be able to not only measure but also to control the pH during hot-water extraction to any desired pH, a potential controller with a pump was added to the system. Connected to the in-line measuring electrodes, the controller compared the measured potential to a determined set point and regulated a pump adding alkali into the reactor to meet the set point. In these experiments, when the pH during wood extraction decreased, the controller was used to maintain a desired pH. Since the electrodes actually measure potential and the potential increases during extraction, the controller was set to start pumping alkali when the potential exceeded a chosen set point.

4.2.1 pH control

Three different set points were tested as well as an extraction without pH control as a reference. The set points were 550 mV, 575 mV, and 600 mV, since the previous pH measurements showed that the potential during the 170 °C extraction was in that range.

Buffer experiments were made before the wood extractions to establish S and E^0 (which, as explained in 3.2.2, changes over time) for accurate pH calculations. S and E^0 were determined to 89.09 mV/degree and 982 mV, respectively. The corresponding pH for theses set points at 170 °C were then calculated to about 4.85, 4.60, and 4.30, respectively.

The results from the pH controlled wood extractions clearly showed that it was possible to control and maintain a desired pH level throughout the extraction (Figure 4.5).

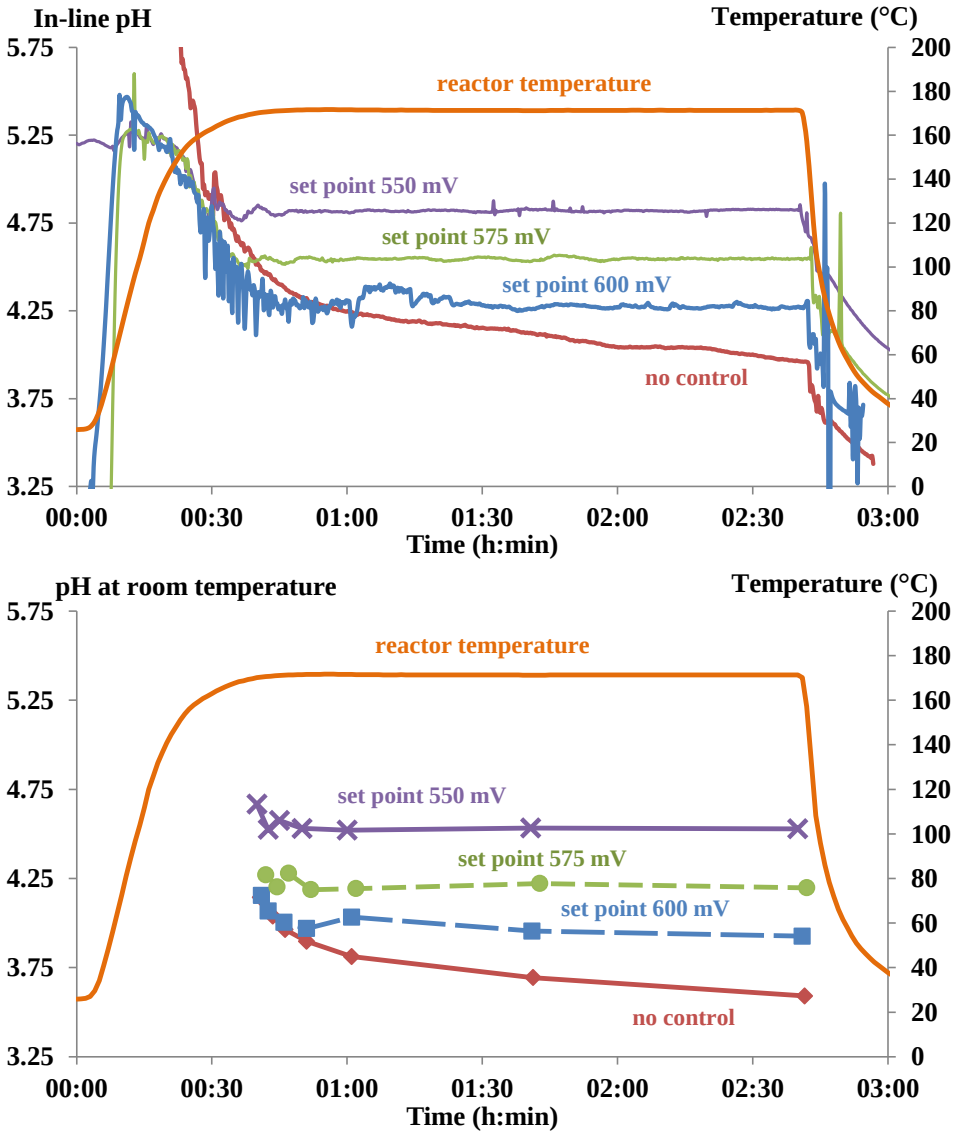

Figure 4.5 pH profiles from hot-water extraction with different set points; 550 mV, 575 mV, 600 mV, as well as a reference extraction with no pH control. Upper graph: actual in-line pH during a 170 °C hot-water extraction of spruce wood. Lower graph: pH of extracts measured with a conventional glass pH electrode at room temperature.

The extraction at pH 4.3 (set point 600 mV) showed some signal disturbance slightly before the controlling started (between 00:30 and 00:45) and a pH overshoot (actually a potential

undershoot). The two other pH controlled extractions were much more stable and the overshoot was much smaller and during shorter times. After these initial problems, the system managed to maintain the desired pH throughout the whole extraction within acceptable variations. Some explanations to this overshoot and relaxation time could be that the system had difficulties to counter the rapid pH drop at the beginning and/or not completely optimized controller parameters. There will probably always be small variations when working with biomass as material and a complete and precise control of the process will be a challenge.

4.2.2 Extracted material

Total dissolved solids (TDS)

The sum of all extracted, or dissolved, material from the extraction, TDS, provided a good overview of the extraction process. Figure 4.6 shows the results from the TDS analyses.

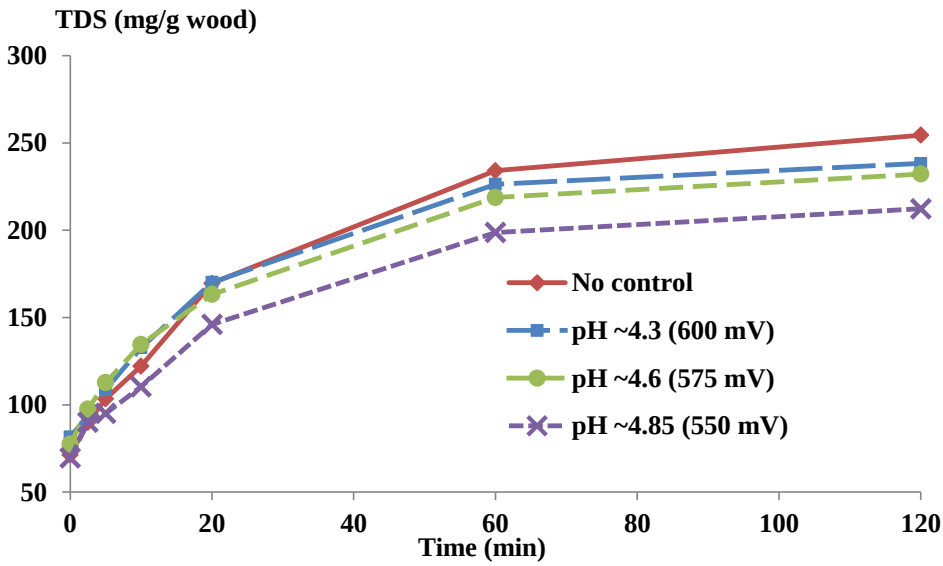

Figure 4.6 Total dissolved solids (TDS) in spruce wood (2-4 mm particles) extracts from extractions with and without in-line pH control at 170 °C.

The TDS values from the extraction with no control were similar to the one in an earlier study (Figure 4.6, **I**). After two hours, approximately 25 % of the wood had been dissolved. Controlling the extraction pH to 4.30 and 4.60 only decreased the extracted material with 10

% and mainly during the last extraction hour. A 10 % lower TDS yield could be observed already at 20 minutes into the extraction when pH was controlled to 4.85 and 20 % at the end compared to the no pH control extraction.

Hemicelluloses

The major part of the dissolved material in the extracts consisted of non-cellulosic carbohydrates, and basically all of these originated from the wood hemicelluloses and a small portion of pectins. Therefore, hereafter the extracted carbohydrates are referred to as hemicelluloses.

The same trend in extraction yield could be seen for the hemicelluloses as for the TDS regarding the different potential set points (Fig. 4.7). Between 60-70 % of the total extracted material was hemicelluloses. The hemicellulose yield from the controlled extraction at pH 4.30 was basically the same as for the extraction with no pH control. The yield at pH 4.30 was slightly, but not significantly, higher at the first 20 minutes. The controlled extraction at pH 4.60 had similar yield at the first 20 minutes as the extraction at pH 4.30, but decreased to 85-90 % of the no pH control extraction yield at the last hour of the extraction. The largest difference in yield was as expected from the controlled extraction at pH 4.85. The yield was lower throughout the whole extraction; at 20 minutes it was 82 % of the no control yield and at 60 minutes and 120 minutes this percentage was 87 % and just over 70 %, respectively.

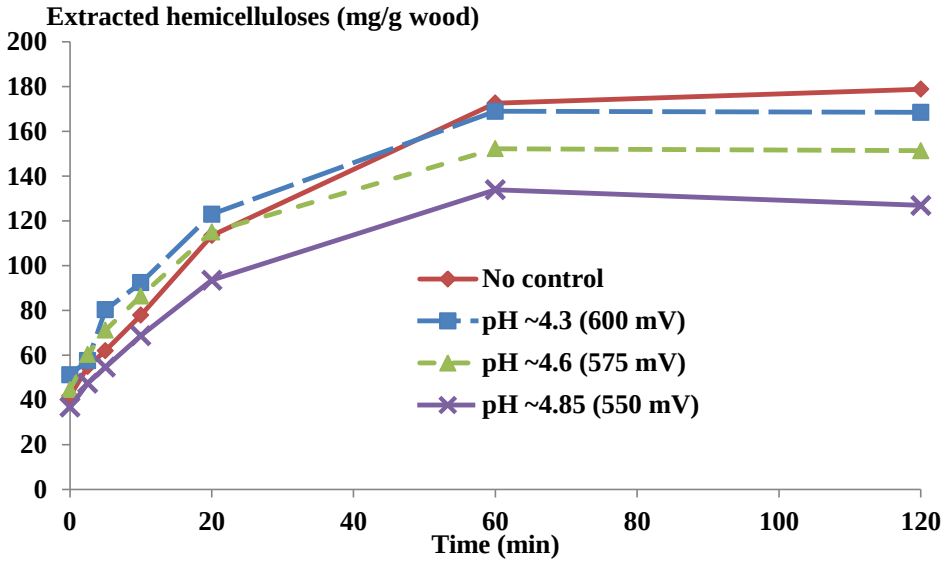

Figure 4.7 Dissolved hemicelluloses in extracts from extractions with and without in-line pH control at 170 °C.

In general, these results showed that less amounts of hemicelluloses were extracted at higher pH and subsequently relatively low pH was required for complete hemicellulose extraction. One explanation to this phenomenon could be that acid hydrolysis cleaved the glycosidic bonds within the hemicellulose chains when the hemicelluloses were still attached to the cellulose-hemicellulose-lignin wood matrix, and not only the bond between the hemicellulose and wood matrix. At higher pH, the acid hydrolysis would naturally be weaker with less chain splitting and less dissolved hemicelluloses. In addition, at higher pH more deacetylation will take place with resorption of the hemicelluloses back onto the fibers as the outcome (Hannuksela et al., 2003), resulting in lower yield of dissolved hemicelluloses.

Monomeric sugars

Sugar monomers were formed during the hot-water extraction and the results from the monomer analysis are described in Figure 4.8.

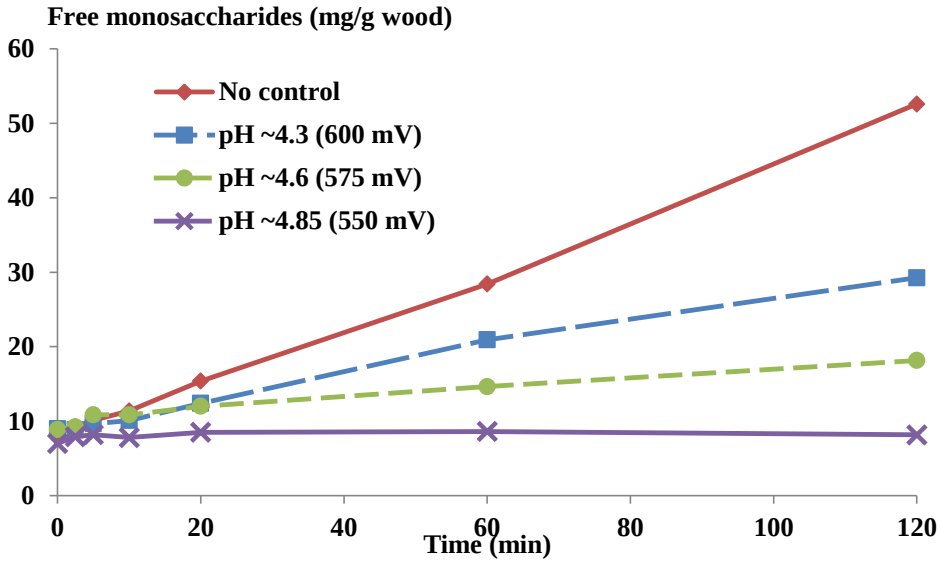

Figure 4.8 Monomeric sugars in extracts from extractions with and without in-line pH control at 170 °C.

A small part of the carbohydrates in the spruce wood were present in the form of natively occurring monomers and dimers, and therefore, the monomeric sugar concentration in Figure 4.8 started at slightly below 10 mg/g wood and not at zero. Some sugar monomers were probably also released during the heating time, contributing to the reference value at zero minutes. In the reference extraction, with no pH control, the sugar monomer concentration increased as shown previously at these conditions (**I**; Song et al., 2008). With increasing extraction pH, the sugar monomer concentration decreased, probably due to milder hydrolytic conditions, which suppressed the degradation of hemicelluloses. The same phenomenon was observed when aqueous phthalate buffers were used for passive pH control (Song et al., 2011). This might indicate that the hydrolysis on the already dissolved hemicelluloses in the liquid phase to monomers was controlled and prevented with sufficient pH control. However, this does not mean that the hydrolysis was prevented; the dissolved hemicellulose chains were still cleaved into shorter oligomers and probably also dimers. This conclusion was further verified by the results from the molar mass analysis (Figure 4.9).

Molar mass

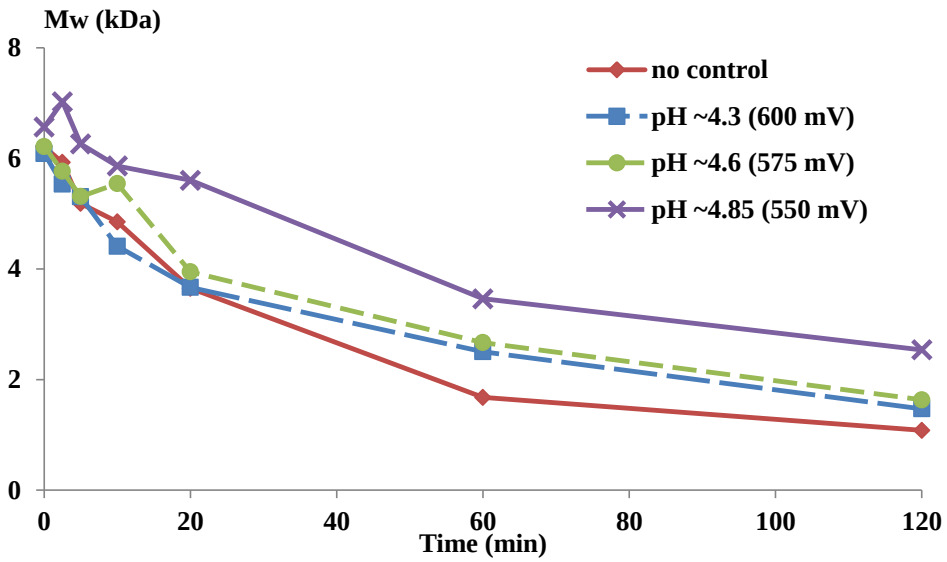

Figure 4.9 The average weight molar mass of the extracted hemicelluloses with and without in-line pH control from the extraction at 170 °C.

The degradation of the hemicellulose chains due to acid hydrolysis are clearly seen in Figure 4.9 with a decreasing molar mass throughout the extraction time. The hemicellulose molar mass in the no pH control extraction followed similar pattern as reported earlier in literature (**I**; Song et al., 2008) and decreased below 2 kDa after 60 minutes of extraction. When controlling the extraction pH to 4.30 and 4.60, a 50 % increase in the extracted hemicellulose molar masses was observed after 60 minutes, and the difference was approximately the same throughout the rest of the extraction time. With an extraction pH of 4.85, the molar mass of the extracted hemicelluloses was significantly higher that of the hemicelluloses in the other extracts, about 50 % higher after 20 minutes and increased to as much as 135 % at 120 minutes. Unfortunately, the hemicellulose yield did decrease with higher pH as seen in Figure 4.7. Nevertheless, these results showed that in-line pH control plays a vital role for the molar masses of the extracted hemicelluloses and it was possible to partly prevent hydrolytic cleavage of the extracted hemicelluloses during the extraction by controlling the pH. Even higher extraction pH might completely prevent degradation of the hemicellulose chains, but

more severe deacetylation at higher pH will lead to a much lower solubility of the hemicelluloses and a large yield loss as result.

Acetic acid

Free acetic acid, originating from acetyl groups in the hemicellulose, was a contributor to the decrease in pH during the hot-water extractions. Figure 4.10 shows the results from an analysis of free acetic acid in the extracts.

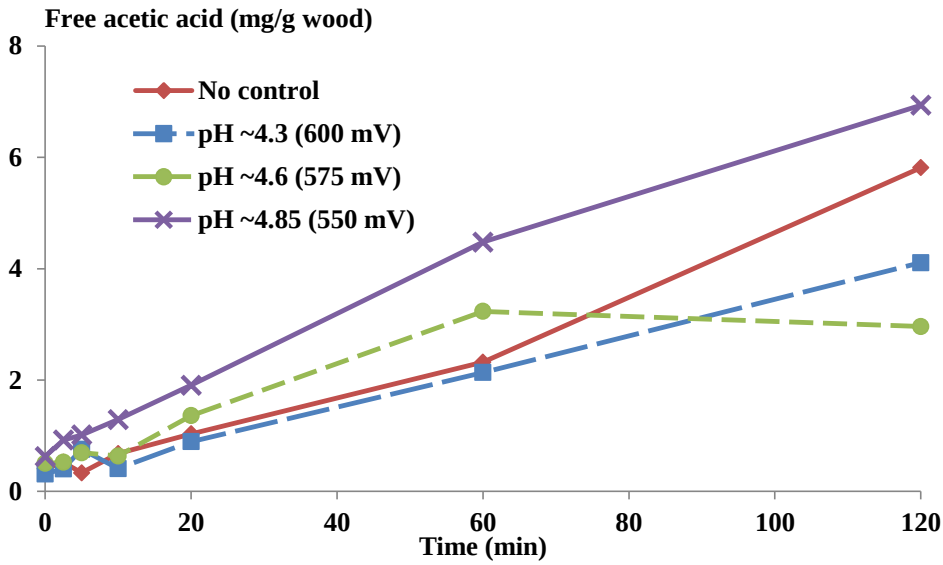

Figure 4.10 Free acetic acid in extract from extractions with and without in-line pH control at 170 °C.

The extraction without pH control, and the pH 4.30 and pH 4.60 controlled extractions all had similar acetic acid profiles, steadily increasing as the extraction proceeded. The acetic acid amount in the pH 4.85 extraction was by far the highest (Figure 4.10). No clear trend between the different pH extractions, except for the one at pH 4.85 which had the highest concentration, regarding released acetic acid could be established as for the TDS and dissolved hemicellulose results.

4.3 Temperature effect during pH control

The temperature is one of the easiest extraction parameters to control and adjust, and at the same time maybe the most important. With increasing extraction temperatures, the yield usually increases. One simple reason for this is, as stated earlier in section 2.3.1, that the energy in the extraction increases, which will break the different chemical bonds between the wood components and thus release/dissolve the components into the water. Another reason to the increased yield might be swelling of cell walls, which would facilitate the diffusion of already dissolved molecules. However, the increase in energy will also activate unwanted reactions, such as hydrolysis of the hemicellulose chains, degradation of the monomers into furfural, hydroxymethylfurfural (HMF) etc., and lignin condensation to name a few. Therefore it is not as straightforward to just increase the temperature in order to improve the extraction of high-molar-mass molecules. With control of other extraction parameters, e.g., pH or particle size, the drawbacks that follow increased temperatures could be avoided or at least minimized.

4.3.1 Extracted material

The following results compare 170 °C extraction with 180 °C extraction, at pH set point 4.85 and without pH control. Other pH set points were also tested (4.30 and 4.60). Since the results at 4.85 showed the largest difference against the extraction with no pH control and the results from pH 4.30 and 4.60 extractions followed a logical pattern between the extraction without pH control and the extraction at pH 4.85, these results are not shown in this summary. For more details, see (**III, IV**)

TDS

With increased temperature, the TDS yield also increased as seen in Figure 4.11. The enhancement was most pronounced at the beginning of the extraction, during the first 20 minutes.

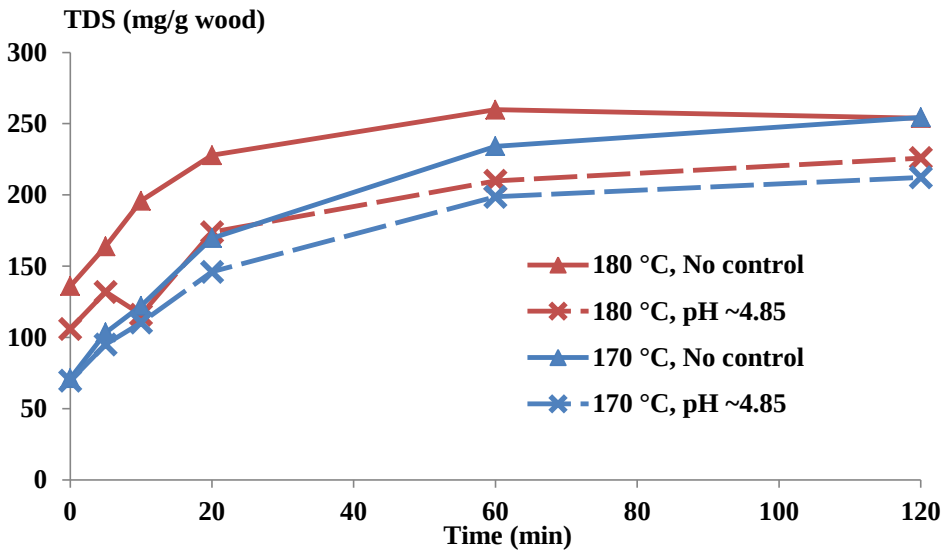

Figure 4.11 Comparison between TDS from a 170 °C extraction and a 180 °C extraction, with and without pH control.

At first, 100 % more was extracted at 180 °C than 170 °C, while the percentage was decreased to 35 % at 20 minutes. After one-hour extraction only a 10 % increase in TDS was noticed and at 120 minutes the yield was the same for both temperatures, approximately 250 mg/g wood. Three different pH set points were tested and the result from the highest set point (in-line pH~4.85) is presented in Figure 4.11. As stated earlier in section 4.2.2, less material was extracted at 170 °C when the pH was controlled to higher than without in-line pH control. The same was true for the extraction at 180 °C and the difference was even more pronounced at this temperature. Comparing the two pH controlled extractions, the difference was not as pronounced as for the extractions without pH control. About 50 % more was extracted at the beginning and 20 % at 20 minutes. Throughout the rest of the extraction the difference was only about 10 %. These results indicates that with an extraction pH of 4.85 about 200 mg/g wood can be extracted after two hours almost regardless of the temperature within the 170-180 °C range.

Hemicelluloses

A comparison between the amount of extracted hemicelluloses from the 170 °C and 180 °C extractions of spruce wood are presented in Figure 4.12.

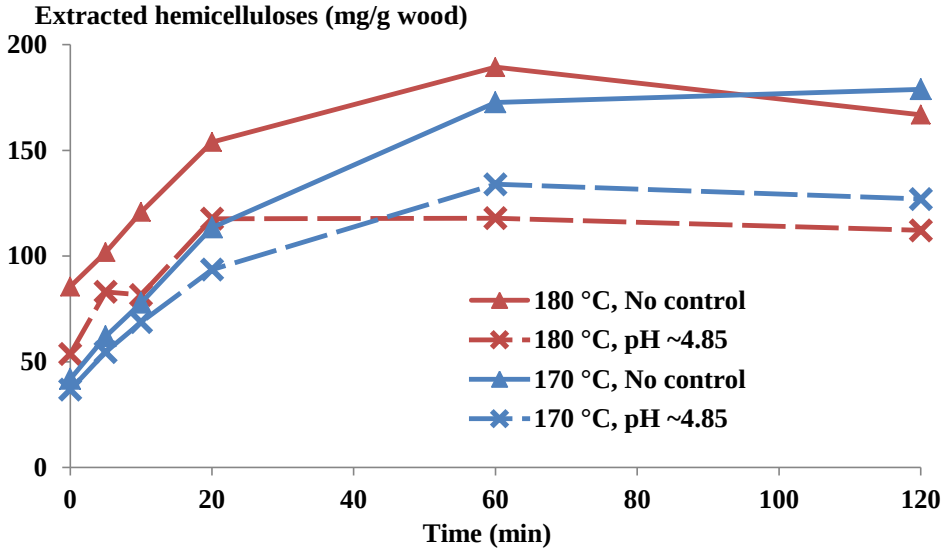

Figure 4.12 Comparison between extracted hemicelluloses from a 170 °C extraction and a 180 °C extraction, with and without pH control.

At the first 20 minutes, the amounts of the extracted hemicelluloses followed the same pattern as the TDS both regarding the different temperatures and the controlled pH set points; more hemicelluloses were extracted at higher temperature. At the end of the extraction, the yield of hemicelluloses from the 180 °C extraction without pH control decreases and was actually lower after two hours than for extracted at 170 °C. This was due to sugar degradation, as explained at the beginning of this chapter. Interestingly, the yield from the 180 °C, pH 4.85 controlled extraction completely levelled out at about 115 mg/g wood. This stagnation was also noticeable for the 170 °C, pH 4.85 controlled extraction but first occurred after one hour extraction and the yield was approximately 130 mg/g wood. The reason for this phenomenon is unknown but it might be related to degradation of hemicelluloses or resorption onto fibers.

Monomers

About 50 % more monosaccharides were formed, evenly along the extraction during the extraction at 180 °C without pH control than at 170 °C (Figure 4.13). When pH was controlled to 4.85, the extraction at 180 °C showed the same behavior regarding sugar monomer formation as the extraction at 170 °C described in section 4.2.2. In both extractions, no additional monosaccharides were formed besides the initial ~10 mg/g wood. A conclusion from this comparison was that pH control was as an effective monomer formation inhibitor at 180 °C as it is at 170 °C.

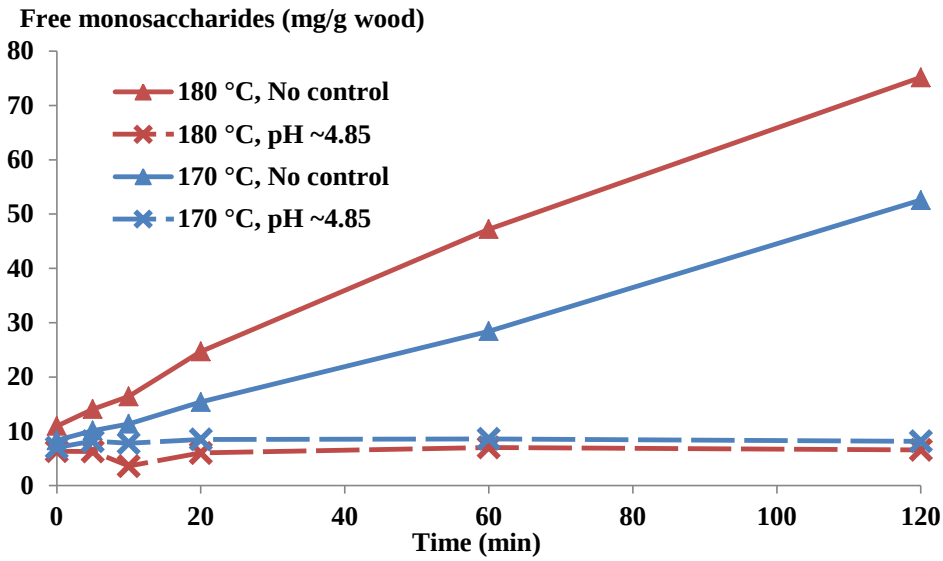

Figure 4.13 Comparison between monomeric sugars released during extraction of spruce wood at 170 °C and 180 °C, with and without pH control.

Molar mass

The molar mass of the extracted hemicelluloses decreased faster and were overall lower, 30-40 %, for the 180 °C extraction without pH control than for the corresponding extraction at 170 °C (Figure 4.14). This was because of the more severe conditions at 180 °C leading to lower pH and therefore stronger hydrolysis and consequently also shorter hemicellulose chains.

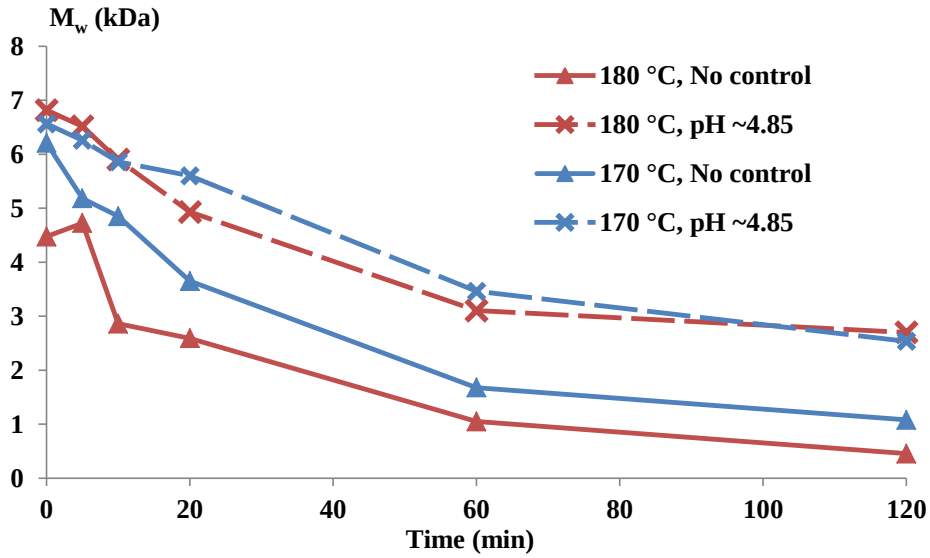

Figure 4.14 Comparison between the average molar masses of the extracted hemicelluloses sugars released during extraction of spruce wood at 170 °C and 180 °C, with and without pH control.

When the extraction pH was controlled to 4.85 for both 170 and 180 °C, a higher molar mass was achieved with no significant difference between the two temperatures. Considering that more hemicelluloses could be extracted faster at 180 °C than at 170 °C at the beginning of the extraction and the fact that the molar mass could be preserved by controlling the pH to 4.85, this method opens up for optimization regarding not only high hemicellulose yield, but also of high-molar-mass hemicelluloses. Since the molar masses were more or less the same at both temperatures, it seemed that the active in-line pH control could minimize the hydrolysis to the same level as 170 °C at 180 °C although the autohydrolysis rate was more pronounced at the higher temperature.

Acetic acid

Due to hydrolysis of the ester bonds between the acetyl groups and sugar units in the hemicellulose chain, acetic acid was released into the water during hot-water extraction. Figure 4.15 shows the amount of free acetic acid in the extracts during 170 °C and 180 °C hot water extractions, with and without in-line pH control.

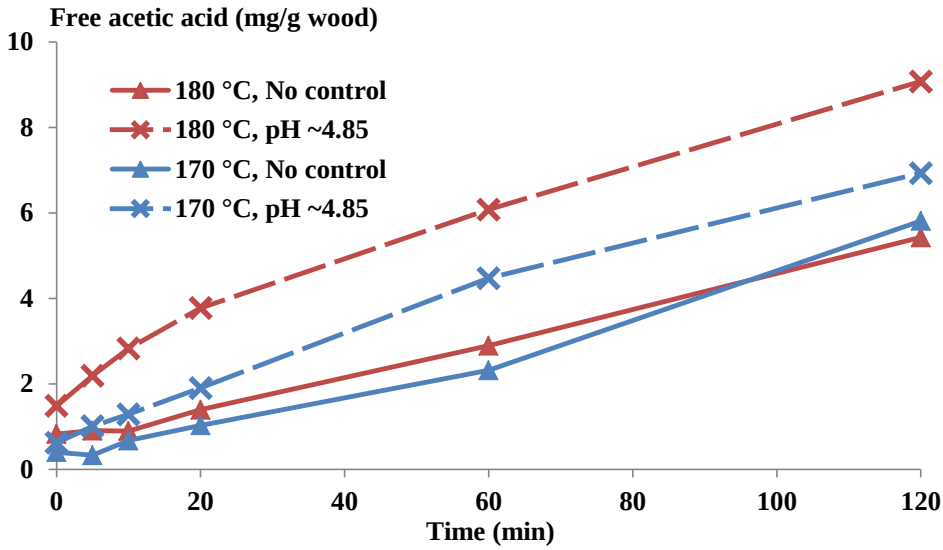

Figure 4.15 Released acetic acid during extraction of spruce wood at 170 °C and 180 °C, with and without pH control.

Without active in-line pH control, the acetic acid concentrations in the extracts obtained at 170 °C and 180 °C were quite similar. Both extractions gave a quite linear increase in acetic acid that reaches about 5.5 mg of acetic acid per g wood after a two-hour extraction. For the pH controlled extractions there was a significant difference between the two temperatures, more than 100 % acetic acid was released at 20 minutes during the 180 °C extraction compared to the 170 °C extraction. After the two-hour extractions, the acetic acid concentration in the 180 °C extract was 9.1 mg/g wood and the acetic acid concentration in the 170 °C extract was 6.9 mg/g wood.

4.3.2 Residue

In a biorefinery concept it is extremely important to also consider other components than hemicelluloses in the wood. The core idea with a biorefinery, as described in more detail in section 2.1, is that all, or at least as much as possible, of the material is used as efficiently as possible and eventual waste streams are kept to a minimum. Therefore, it is important to consider the cellulose and lignin remaining in the wood when designing the optimal hemicellulose hot-water extraction. Figure 4.16 shows the total amount of both cellulose and

lignin in the wood and how much is removed during hot-water extraction at 170 °C and 180 °C without any pH control.

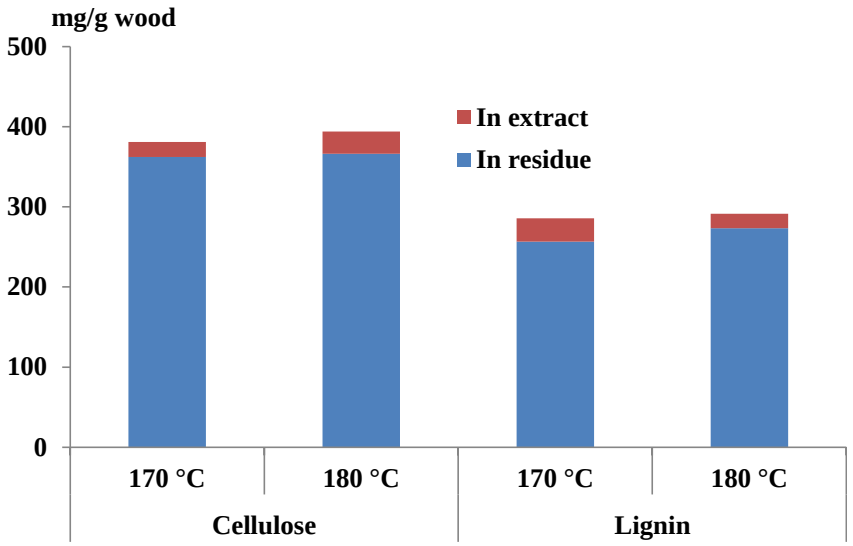

Figure 4.16 Extracted and residual cellulose and lignin after two hour extractions at 170 °C and 180 °C of 2-4 mm wood particles.

The results showed that the cellulose was not much affected by the hot-water extraction at these temperatures (Figure 4.16). Only about 5 % of the total cellulose was removed after a two hour 170 °C water extraction. At 180 °C, about 7 % was removed; still a rather small amount of the total cellulose. The reason for the higher removal at 180 °C was probably that the more severe conditions at the higher temperature affected the amorphous regions in the cellulose to a larger extent. The conditions at 180 °C might even damage parts of the crystalline structure in the cellulose and by that creating more easily hydrolyzed amorphous regions. For the lignin, the results showed an opposite trend. About 10 % of the total lignin was extracted at 170 °C, but only 6 % at 180 °C. An explanation could be that the released lignin at the higher temperature was condensed and became insoluble.

4.3.3 Alkali consumption

The consumption of alkali necessary to control the extraction to a desired pH is important, both regarding the plant infrastructure and process economy, especially when considering

scaling up to industrial processes. Therefore, the alkali consumption was recorded gravimetrically. The recording was first made manually (170 °C extraction) and later on logged automatically to a computer (180 °C extraction). The results are presented in Table 4.3.

Table 4.3 Alkali consumption during hot-water extractions of wood particle size of 2-4 mm at 170 °C and 180 °C water extractions with pH set points ~4.6 and ~4.5, respectively.

		170°C	**180 °C**
pH set point		~4.6	~4.5
Pumping time (min)		195	130
Total (mL)		11.2	6.87
	(mg)	224	137
Average flow rate	(mL/min)	0.057	0.053
	(mg/min)	1.15	1.06
Wood used* (g)		20.7	17.5
Alkali/wood ratio	(mL/g)	0.54	0.39
	(mg/g)	10.8	7.8

* Calculated as dry wood.

For actively controlling and maintaining the pH to ~4.6 at 170 °C for about 195 minutes, 11.2 mL 0.5 M NaOH was used. A total of 6.87 mL was consumed at 180 °C and a pH set point of ~4.5 for 130 minutes. These numbers might be somewhat misguiding when comparing the different extraction temperatures and times, so a more comparable number was calculated by dividing the total alkali amount used with the amount wood used in the extractions. This ratio was 0.54 for the 170 °C extraction and 0.39 for the 180 °C extraction indicating that less alkali was needed in the higher temperature extractions. Important to consider is, of course, that the pH set point for the 170 °C extraction was 0.1 pH units higher and the pumping time longer, which naturally increases the alkali consumption.

The water amount used in the extraction was 700 mL, so the added alkali was only 1.0 and 1.6 % of the total liquid in the extraction. The low percentages are good when scaling up to

larger, industrial volumes. Unfortunately, the liquid to wood ratio (L/W) in these experiments were 35-40, which are too high for industrial applications. More alkali is probably needed to maintain a chosen pH set point if the L/W ratio is lowered to a more realistic value.

4.4 Particle size effect

While the temperature and pH influences the detachment, dissolution, and degradation of the hemicelluloses during a hot-water extraction it was vital to diffuse the dissolved hemicelluloses out from the wood and into the liquid phase. The size of the wood particles used in the extraction determined how far the dissolved hemicellulose molecule needed to diffuse out to reach the liquid phase. When still inside the wood, the dissolved hemicelluloses could be resorbed back onto fiber surfaces if they were deacetylated. Furthermore, the probability for the dissolved hemicelluloses to interact with other wood components that would slow down the diffusion increases with increasing diffusion path.

4.4.1 Extraction of different wood particle sizes 0.5 mm – 12.5 mm at 170 °C

A detailed study on different size of wood particles was conducted to systematically optimize the wood particle size in respect of intensification of the hemicellulose extraction. The particle size fractions used were 0.5-0.7 mm, 1.25-2 mm, 2-4 mm, 4-8 mm, and 8-12.5 mm. Commercial wood chips are still larger than this, with dimensions normally of 45 × 30 × 5 mm. Figure 4.17 shows the different wood particle sizes used in this study after milling and sieving.

Figure 4.17 Different wood particle size fractions used in the study after milling and sieving.

TDS

All the different particle sizes were extracted at 170 °C without using pH control and samples were taken regularly throughout the extraction time. In general, the results from a TDS study

of the different extractions showed an increasing yield with decreasing wood particle size (Figure 4.18).

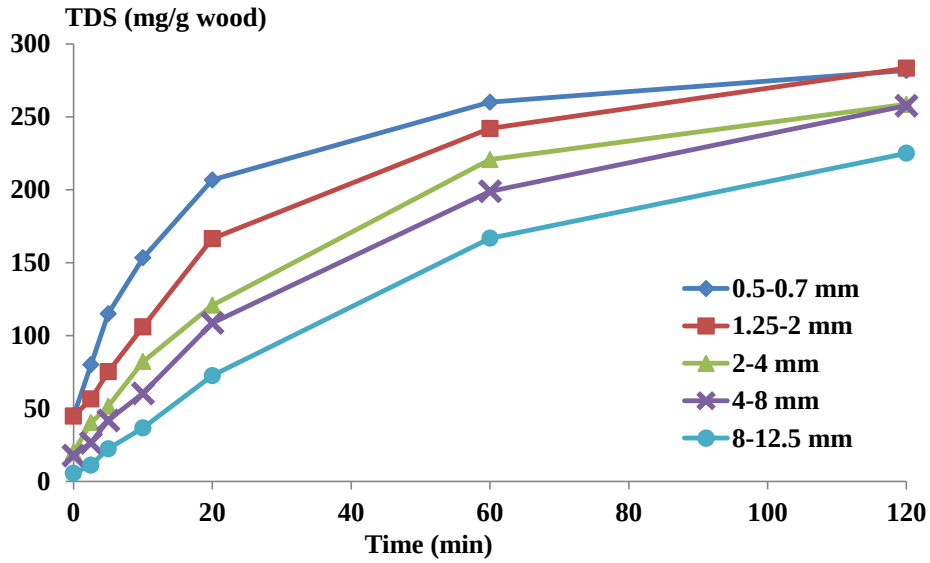

Figure 4.18 Total dissolved solids (TDS) obtained at 170 °C for five different wood particle size fractions, ranging from 0.5 mm to 12.5 mm.

With the smallest particle size, 0.5-0.7 mm, 280 mg/g wood was extracted after two hours extraction. This seems to be close to the maximum of extractable material at these applied conditions since the yield start to level out from one to two hours. The TDS after a two-hour extraction from the 1.2- 2 mm fraction also reached 280 mg/g wood, but the extraction kinetic was somewhat slower. The other particle size extractions had lower end yields, 260 mg/g wood for the 2-4 mm and 4-8 mm and only 225 mg/g wood for the largest particle size, 8-12.5 mm. The speed of the extraction, or the extraction rate, was also different for the different particle size fractions, especially in the first 20 minutes. As much as 207 mg/g wood was extracted from the 0.5-0.7 mm particles after 20 minutes, while the same yield for the 8-12.5 mm particles were only 35 % of that, 73 mg/g wood. The other particle size fraction yields were found between these limits. The extraction pH, measured only at room temperature after extraction, showed a familiar pH profile starting at about 4.9 with a rapid drop in the beginning of the extraction and leveling out at around pH 3.3-3.5 in the end.

Total hemicelluloses

Since most of the extracted material consists of hemicelluloses, the yield profiles of the extracted hemicelluloses (Figure 4.19) were very similar to TDS yield profiles (Figure 4.18).

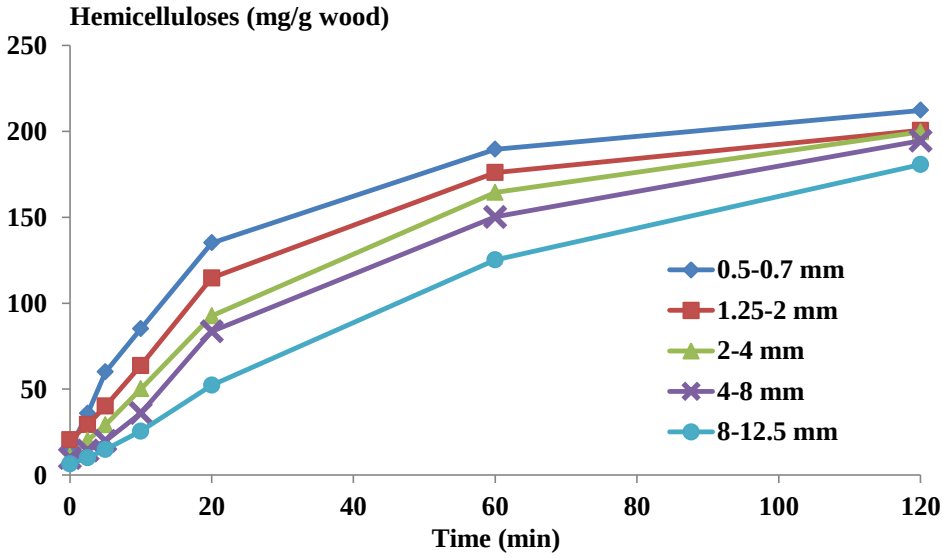

Figure 4.19 Dissolved hemicelluloses in the extracts obtained from extracting different wood particle size fractions at 170 °C (presented as sum of anhydro sugars).

About 212 mg hemicelluloses per g wood were extracted from the smallest particle size fraction after a two-hour extraction at 170 °C (Figure 4.19). This corresponded to about 75 % of the TDS (Figure 4.18). The total hemicellulose content in the spruce wood was determined to be ~250 mg/g, which made the hemicellulose yield from the smallest particle size fraction 85 % of the total hemicelluloses in the wood. When the particle size was increased, both the yield and the speed of the extraction decreased (Figure 4.19). After two-hour extraction of the largest particle size (8 – 12.5 mm), the yield was only 180 mg/g wood, i.e., 72 % of total hemicellulose content. Only 52 mg/g wood had been extracted from the 8-12.5 mm particles after 20 minutes, while the yield from the 0.5-0.7 particles at that time was 135 mg /g wood. The sugar monomer formation was also shown by the increase in monomeric sugars in the extracts from the different particle size fractions (**I**). The increase was more or less linear and increased from below 5 mg/g wood in the beginning of the extraction to about 50 mg/g after

two hours. No significant difference regarding composition of monosaccharides obtained from the different particle size fractions could be observed.

Polymeric hemicelluloses

To roughly estimate the oligomeric and polymeric part of the extracted hemicelluloses, the monosaccharides content was subtracted from the total hemicellulose results. These values showed how much of the carbohydrates were oligosaccharides or polysaccharides. Dividing the result from the subtraction with the monosaccharide result gives a polymeric material/monosaccharides ratio that describes at what time during the extraction most of the extracted sugars were in polymeric form (Figure 4.20).

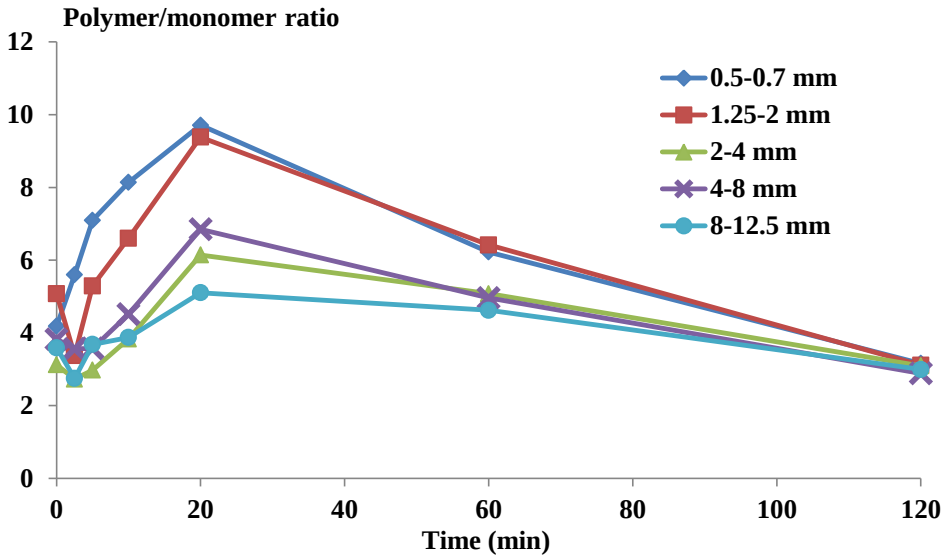

Figure 4.20 Polymeric to monomeric ratio of the extracted carbohydrates from different particle size fractions at 170 °C.

The graph shows a maximum of the polymeric to monomeric ration of the extracted hemicelluloses at 20 minutes for all the different particle size fractions (Figure 4.20). This implied that at 20 minutes of extraction was the best time for extracting the largest portion of polymeric material without intensive formation of monosaccharides, regardless of particle size. It was, however, a large difference on the different particle size ratio value at 20 minutes.

The ratio for the smallest particle size was almost twice as large as the ratio for the largest, further indicating that high-molar-mass hemicelluloses were more easily extracted from smaller particles. After a two-hour extraction the ratio was about 3 for all the different particle sizes, meaning that ⅓ of the extracted sugars were monomeric.

Molar mass

Due to the acid-catalyzed hydrolytic cleavage caused by the auto-ionization and released acetic acid, the hemicellulose molar masses decreased rapidly. The results from the molar mass determination are shown in Figure 4.21.

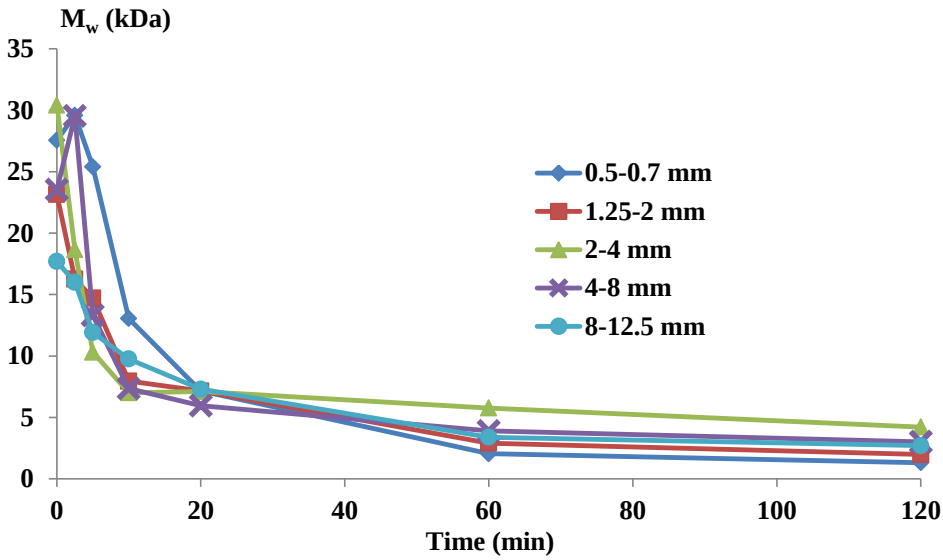

Figure 4.21 Average molar mass of the extracted hemicelluloses at 170 °C from the different wood particle sizes.

It seemed that the decrease in the molar masses of the extracted hemicelluloses during the extraction was not severely affected by the size of the extracted wood particles. Figure 4.21 clearly shows the rapid decrease in molar mass already in the beginning of the extraction. In the extract from the fraction 0.5- 0.7 mm the molar mass of the dissolved hemicelluloses was slightly higher during the first 20 minutes.

Carbohydrate oligomer formation

Since the acid catalyzed degradation of the hemicellulose chains induced during extraction was random and formed besides monomers also oligomers with different chain lengths, the extracts were analyzed with HP-SEC to investigate the oligomer distribution throughout the extraction time. The method was best suited for oligomers with a degree of polymerization (DP) of 7 and less.

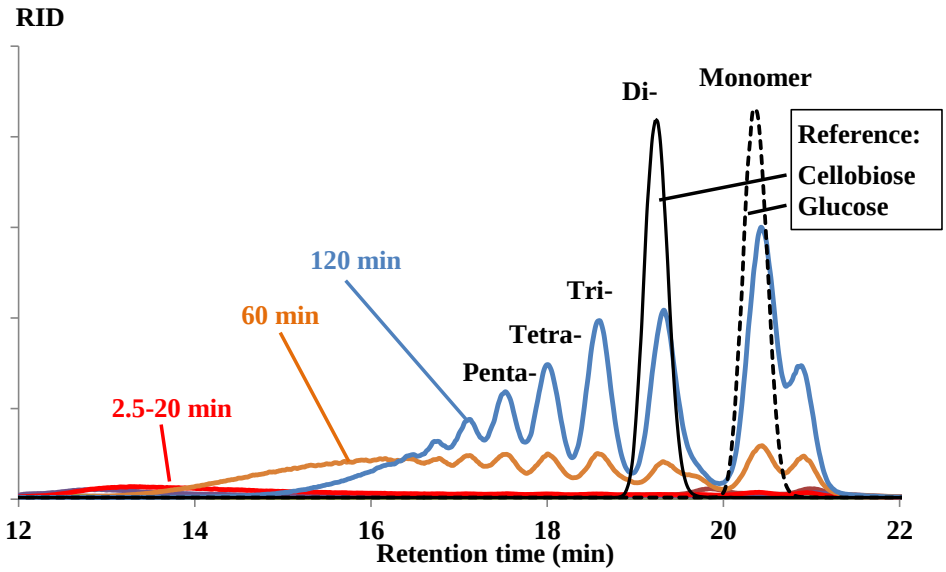

Figure 4.22 GPC-HPLC chromatograms on oligomers formed during a two-hour extraction of 1.25 – 2 mm wood particles at 170 °C. Monomer (glucose) and dimer (cellobiose) were used as reference. All samples were acetylated prior to analysis.

The GPC-HPLC result confirmed the hemicellulose chain degradation into different size oligomers, as well as monomers (Figure 4.22). From the chromatogram it was visible that during the first 20 minutes no smaller oligomers are yet formed (Figure 4.22, red line). After 60 minutes (Figure 4.22, orange line) of extraction, the chromatogram shows also small penta-, tetra-, tri- , di-, and monomers peaks indicating that the degradation of the hemicellulose chains increased. At 120 minutes (Figure 4.22, blue line) most of the

hemicelluloses were in hexa-, penta-, tetra-, tri- , di-, and monomer form with a majority of mono- and dimers.

Acetic acid

The free acetic acid released during the extractions increased quite linearly throughout the extraction time. For the larger wood particle sizes (2-12.5 mm), the total acid concentration after two hours was 4 mg acid per g wood and no significant difference in released acid could be seen for the different larger wood particle sizes. The similarity might indicate that acetic acid release was not wood particle size related and therefore neither internal mass transfer related. However, this requires more studies before any certain conclusions should be made.

4.4.2 Extraction of 2-4 mm wood particles and chips at 180 °C

A more industrially oriented study dedicated on how particle size and temperature effect the extraction was made to investigate the possibilities of upscaling of the process. Extractions were performed with handmade chips (25×20×5 mm, 45° cutting angle), as well as 2- 4 mm particles (Figure 4.23) at 180 °C to evaluate the possibility to use already commercially available wood particle size fractions.

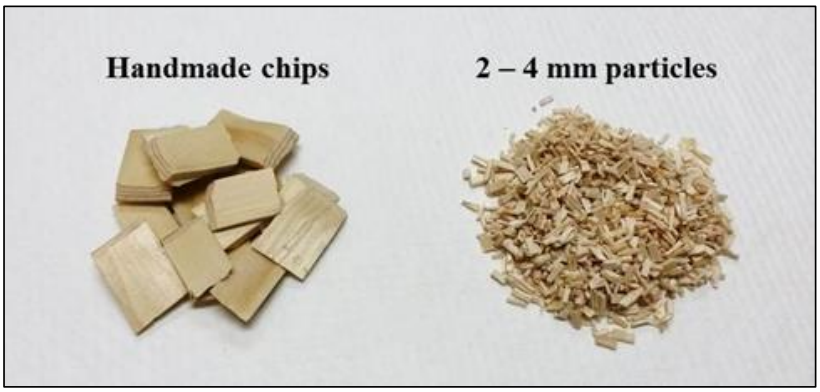

Figure 4.23 Handmade chips modelling industrial size (25×20×5 mm, 45° cutting angle) and 2- 4 mm milled and sieved wood particles.

Extraction of chips has several advantages, besides an already well-established chip preparation process with the entire infrastructure involved at many of the would-be

biorefineries. Producing larger particles require less energy than smaller ones, so larger particles are more economical, and these can be further also used in pulping. Figure 4.24 shows a comparison of the results from several analyses (TDS, hemicelluloses, monosaccharides, molar mass, acetic acid, and pH) between 2-4 mm wood particles and handmade industrial chips at 180 °C.

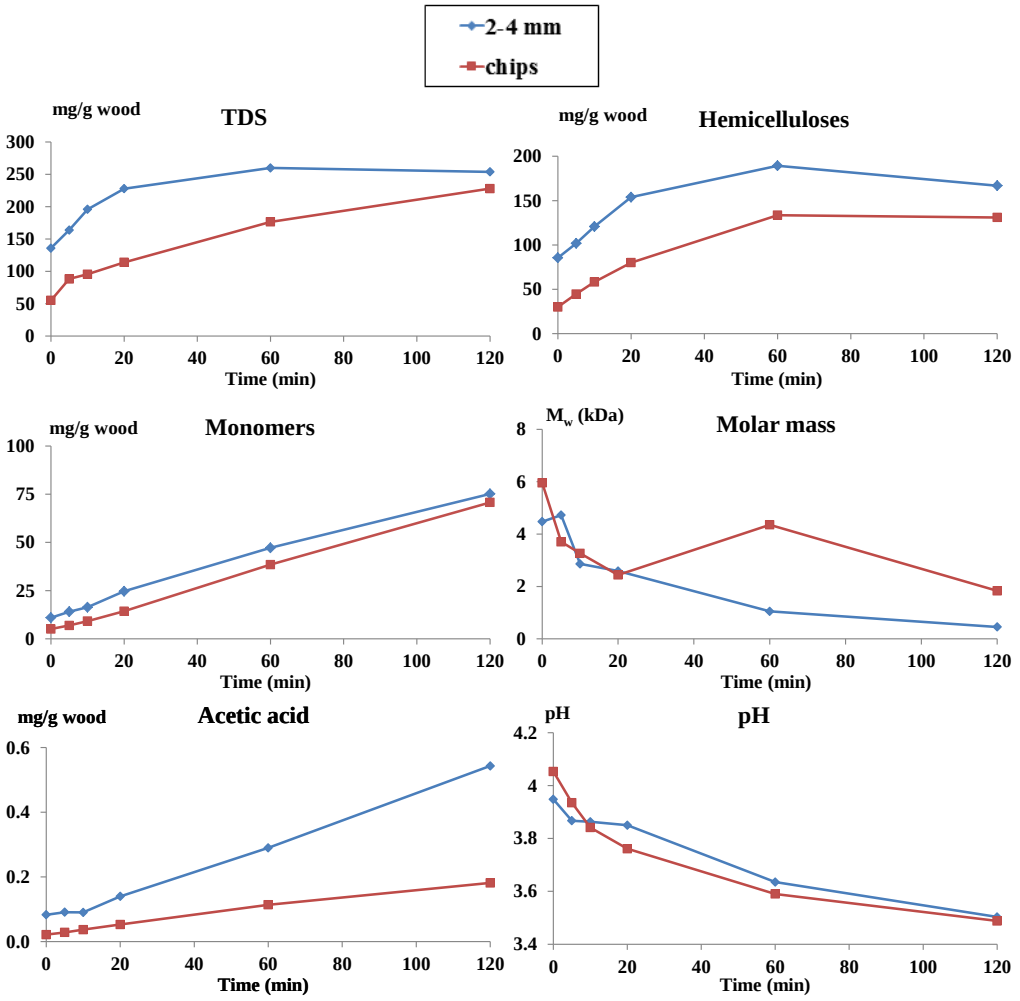

Figure 4.24 Comparison of TDS, total hemicelluloses, monosaccharides, molar mass, acetic acid, and pH (measured at room temperature) obtained from hot-water extractions of 2 – 4 mm wood particles and handmade industrial chips at 180 °C.

Both the TDS and hemicellulose results showed higher yields and faster extractions for the 2-4 mm wood particle than from the larger wood chips (Figure 4.24). The TDS from the 2-4 mm particles leveled out at around 250 mg/g wood, which corresponded to earlier findings (Figure 4.18). The wood chips extraction was much slower and had not yet reached 250 mg/g

wood after two hours. A similar trend was observed for the hemicellulose yield. A maximum yield of hemicelluloses was reached at one hour at 189 mg/g wood for the extraction of 2-4 mm fraction and decreased after that to 167 mg/g wood after two hours. This decrease was, probably due to reprecipitation of deacetylated GGM on the fibers and acid-catalyzed degradation of some monosaccharides due to the higher extraction temperature and lower pH. The hemicellulose yield maximum for the wood chips (134 mg/g wood) was also reached after one hour. No decrease in hemicellulose yield after one hour was observed in the chips extraction. The monosaccharide content increased linearly for both wood particle sizes and in parallel with each other with 5-10 mg/g wood more extracted from the 2-4 mm fraction. The monosaccharide yield after a two-hour extraction was 75 mg/g wood from the 2-4 mm particles and 71 mg/g wood from the chips. The molar mass of the extracted hemicelluloses from the different wood particle sizes followed each other rather accurately and showed the typical decrease at the beginning of the process, as was seen in previous experiments as well. The molar masses of the extracted hemicelluloses were quite low due to the high extraction temperature. The deviation of the molar mass from the chips at one hour from the trend was probably due to an analytical error because the method used for molar mass determination has limitations for molar masses below two kDa. Acetic acid released during the extractions increased for both the 2-4 mm particles and for the chips. For the wood chips, this increase was very small and the amount of the free acetic acid in the extract after two hours was only 1.8 mg/g wood (started at 0.2 mg/g wood). The 2-4 mm particles released almost three times more acetic acid after two hours extraction, with an end concentration of 5.4 mg/g wood. The extraction pH, here measured at room temperature, did not differ much between the two different particle sizes (0.1 in pH or less) throughout the extraction, which was to be expected since the temperature was the same for the two extractions.

4.5 Kinetics

A study was made on the extraction kinetics of the hemicelluloses from different wood particle sizes. Wood particle fractions between 0.5 and 12.5 mm were used with an extraction temperature of 170 °C. The reaction rate of an *n* order chemical reaction in batch is described with eq. (3)

$$-\frac{d[C]}{dt} = k'[C]^n \quad (3)$$

where *C* is the concentration of the reactant, *t* time, *k'* the rate constant and *n* the reaction order. Integrating and rearranging eq. (3) when $n = 1$ and $n \neq 1$gives eq. 4 and 5:

$$-\ln(\frac{C}{C_0}) = k't \quad (4)$$

$$C^{1-n} - C_0^{1-n} = (n-1)k't \quad (5)$$

It has been suggested that the extraction of hemicelluloses from wood can be regarded as a pseudo-first order reaction and thus following eq. (3) (Grénman et al., 2011). Normally, C_0 and *C* represent the concentration of the reactant at the beginning of the reaction and the reactant concentration at a given time (or the end of the reaction), respectively. For hemicellulose extraction, C_0 would mean the original hemicellulose concentration in the wood and *C* the hemicellulose concentration in the wood during extraction. In this study, C_0 represents the hemicelluloses in the untreated wood while *C* was obtained by subtracting the extracted hemicelluloses from the hemicelluloses in the untreated wood.

Figure 4.25 plots $-\ln(C/C_0)$ versus *t* for the different wood particle sizes. The coefficient of determination (R^2) was close to 1 for the three largest particle sizes, indicating that they followed pseudo-first order kinetics.

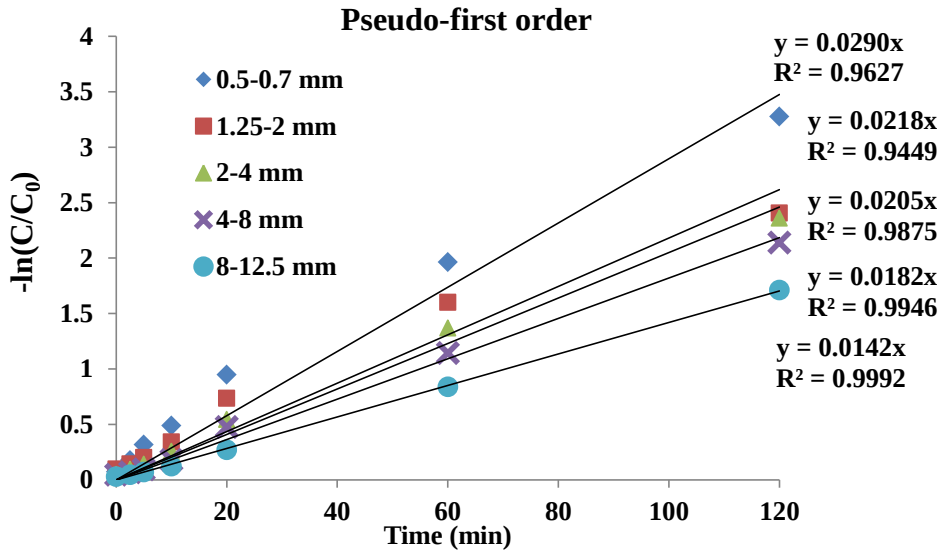

Figure 4.25 Pseudo-first order plot of hemicellulose extraction/dissolution from five different wood particle sizes during hot-water extraction at 170 °C.

Since the plot for the two smallest wood particle sizes did not follow straight lines, eq. (5) was used to test higher reaction orders. It was found that a reaction order of 1.5 gave straight lines with satisfactory R^2 also for the two smaller particle sizes. Eq. (6) describes the reaction order 1.5 and Figure 4.26 the corresponding plot.

$$\frac{\left(\frac{1}{\sqrt{C}} - \frac{1}{\sqrt{C_0}}\right)}{0.5} = k't \qquad (6)$$

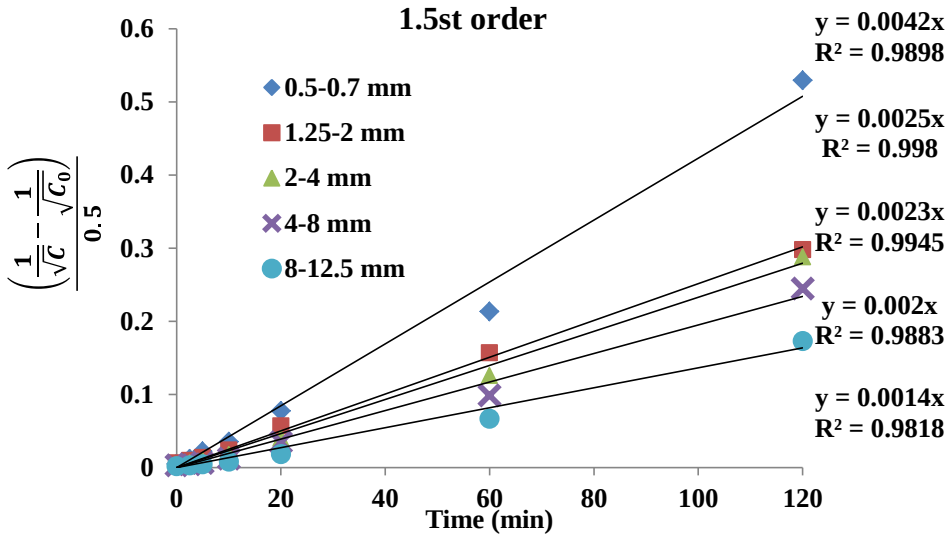

Figure 4.26 1.5st order plot of hemicellulose extraction/dissolution from five different wood particle sizes during hot-water extraction at 170 °C.

The rate constants along with the R^2 for pseudo-first order and1.5st order kinetics are presented in Table 4.4 along with the rates at different times during the extraction.

Table 4.4 Rate constants and extraction rates at different times of hemicellulose extraction from different spruce wood particle size fractions at 170 °C. The unit for pseudo-first order rate constants is min^{-1} and for the 1.5st order is 1/(min * √(mg/g)).

	0.5-0.7 mm		1.25-2 mm		2-4 mm		4-8 mm		8-12.5 mm	
Rate constants	***k'***	**R^2**	***k'***	**R^2**	***k'***	**R^2**	***k'***	**R^2**	***k'***	**R^2**
pseudo- first order	0.0290	0.9627	0.0218	0.9449	0.0205	0.9875	0.0182	0.9946	0.0142	0.9992
1.5st order	0.0042	0.9898	0.0025	0.998	0.0023	0.9945	0.0020	0.9883	0.0014	0.9818
Extraction rates										
Initial	8.09		3.52		3.77		1.74		1.46	
2.5-5 min	9.64		4.33		3.61		2.23		1.88	
5-10 min	5.03		4.71		4.19		3.24		2.14	
10-20 min	5.00		5.10		4.25		4.75		2.67	
20-60 min	1.36		1.53		1.80		1.66		1.82	
60-120 min	0.38		0.41		0.59		0.74		0.92	

Comparing R^2 for the different particle sizes from the pseudo-first order plot and the 1.5st order plot in Table 4.4 shows that the three smaller particle sizes (0.5-4 mm) were best described with 1.5st order kinetics while the two largest (4-12.5 mm) on the other hand followed pseudo-first order. It is although mentionable that the R^2 for the three largest particle sizes does not differ much between pseudo first order kinetics and 1.5st order kinetics. This indicates that the reaction order differs depending on the particle size and therefore, a comparison between the acquired rate constants altogether is not completely accurate. Nevertheless, it was possible to distinguish a trend of larger rate constants for smaller particle sizes, strengthening the idea that particle size had an effect on the general extraction speed. This is also graphically visible in the first 20 minutes in Figures 4.19 and 4.24 for hemicelluloses. Table 4.4 also presents the extraction rates during the 170 °C hot-water extraction. The increasing extraction rate with decreasing particle size further proved that the hemicellulose extraction was faster from smaller wood particles. The smallest wood particle size showed the highest extraction rate at the beginning of the extraction and it was also the highest of all the extraction rates. The other fractions showed a rate maximum between 10 and 20 min. This indicated that some treatment or heating time of the wood in water might be necessary for the larger wood particle sizes to increase the extraction rate due to the longer path the carbohydrates had to diffuse out into the bulk water phase after dissolution from the wood matrix. Another explanation could be that two different dissolution mechanisms of the hemicelluloses take place during a hot-water extraction of the wood. Borrega et al. (2011) suggested that there are two different types of xylan fractions in birch wood, one that was easily soluble and one that was more difficult to dissolve, which could be a possible explanation also in our case. In general, it could be concluded that the extraction kinetics was strongly influenced by internal mass transfer resistance, i.e., the diffusion of the molecules out from the wood structure.

4.6 Bark extraction

For a complete biorefinery concept, other parts of the tree, such as the bark, needles/leaves etc., besides the wood should be utilized as well. Typically 10-15% of the total weight of trees constitutes of bark, depending on species (Fengel and Wegener, 1984d; Sjöström, 1993). The main functions of the bark are (1) to transport water and nutrients and (2) protect the tree from mechanical and microbiological damage, fire and pest insects. Therefore, the bark differs

from the wood from a chemical point of view and consists of, besides cellulose, hemicelluloses and lignin, many different chemically active compounds. These compounds can be of interest due to their diversity and biochemical activity in a biorefinery. A great challenge lies in the isolation of the different compounds due to the heterogeneity of the bark.

Bark contains much more extractives than the wood. The extractives found in wood consists of 2-3 % of the total wood (Fengel and Wegener, 1984f), but in bark the amount can be as much as 20-40 % of the bark, depending on species (Sjöström, 1993). Table 4.5 presents the extractive content from both inner and outer bark extracted sequentially with hexane and acetone/water with an ASE apparatus.

Table 4.5. Lipophilic and hydrophilic extractives in spruce inner and outer bark as analyzed by GC (mg/g dry bark)

	Inner bark		Outer bark	
	Hexane	Acetone	Hexane	Acetone
Fatty acids[L]	0.3	0.7	1.5	0.27
Resin acids[L]	2.8	0.2	10	0.12
Sterols[L]	0.5	0.3	0.4	0.15
Steryl esters[S]	3.0		2.1	
Triglycerides[S]	4.1		3.8	
Aliphatic alcohols[L]	+		0.7	
Diglycerides[S]	0.6		1.9	
Diterpenoids[L]	0.7	+	3.2	+
Flavonoids[L]				2.8
Stilbenes[L]		2.7		0.7
Stilbene glucosides[L]		106		4.8
Mono- and disaccharides[M]		35.5		29.8
Group 1		5.1*		0.5*
Group 2		15*		6.7*
Total	12.0	166	23.5	45.7

+: <0.1 mg/g, L: Analyzed on long GC column, S: Analyzed on short GC column, M: Analysis by GC after direct silylation, *: Compounds with similar elution times as steryl esters and triglycerides, but most probably being other compounds, not yet identified

The amount of lipophilic extractives was two times higher in the outer bark compared to the inner bark, and consisting mostly of fatty acids, steryl esters, and triglycerides. Contrary to the lipophilics, most of the hydrophilic extractives were found in the inner bark, even 3 times more. The largest group of hydrophilic extractives in the inner bark was stilbene glucosides, consisting of 64 % of the total hydrophilic extractives in the inner bark. In the outer bark that percentage was only 10 %. Mono- and disaccharides accounted for a fairly large part of the inner and outer bark, 21 % and 65 %, respectively. Considering that the total bark consists of two-thirds inner bark and one third outer bark (calculated on dry bark basis), the amount of

stilbene glucoside in the whole bark was 86.3 mg/g bark or 8.3 %. The second largest group, mono- and disaccharides, constituted for 33.6 mg/g bark or 3.4 % of the total bark.

To investigate the solubility of the bark hemicelluloses in water the extractive-free bark was further extracted with hot-water with ASE, first at 100 °C and then in parallel at three different temperatures; 120 °C, 140 °C, and 160 °C. Acid methanolysis analysis on the original inner and outer bark showed that inner bark contained 33 % carbohydrates and outer bark contained 25 % carbohydrates (**V**). Some of these carbohydrates are in free mono- and dimer form while others are glucose from the stilbene glucosides. Removing these from the total, the remaining amounts should be in polymeric form and are hereby called hemicelluloses. The hemicellulose content in the inner and outer bark was hence 26.6 % and 22.8 %, respectively. The extractions at different temperatures showed that the inner bark hemicelluloses were best extracted at 140 °C (53 % of total) and the outer bark hemicelluloses at 160 °C (44 % of the total). The dominating sugar units in the inner bark were glucose and arabinose and in the outer bark xylose, glucose, and arabinose. These results suggesed that the hemicelluloses in spruce bark were of different character than the galactoglucomannans in the spruce wood. Considering the composition of the bark hemicelluloses, it indicates that part of them could be pectins (Le Normand et al., 2014).

The cellulose content in spruce bark was determined by acid hydrolysis and glucose determination of the original bark, followed by subtraction of the glucose content obtained by acid methanolysis and GC of the same samples. The glucose in stilbene glucosides, mono- and disaccharides, and starch is included in the methanolysis results because acid methanolysis cleaves all these components. In this way it was found that inner bark contained 225 mg cellulose/g of bark, and outer bark 107 mg/g. Previous studies have also reported smaller amounts of cellulose in bark than in wood (Timell, 1961). As reported by von Dietrich et al. (1978), Norway spruce whole bark contain 19.1% of α-cellulose.

The lignin in the extracted residues, as well as in the original bark was determined with the Klason method. The Klason lignin method has been developed for wood and since the bark differs from wood both in morphology and composition, caution should be taken when the results are reviewed. In the original bark samples, 32 % of the inner bark and 45 % of the outer bark was identified as Klason lignin (Figure 4.27).

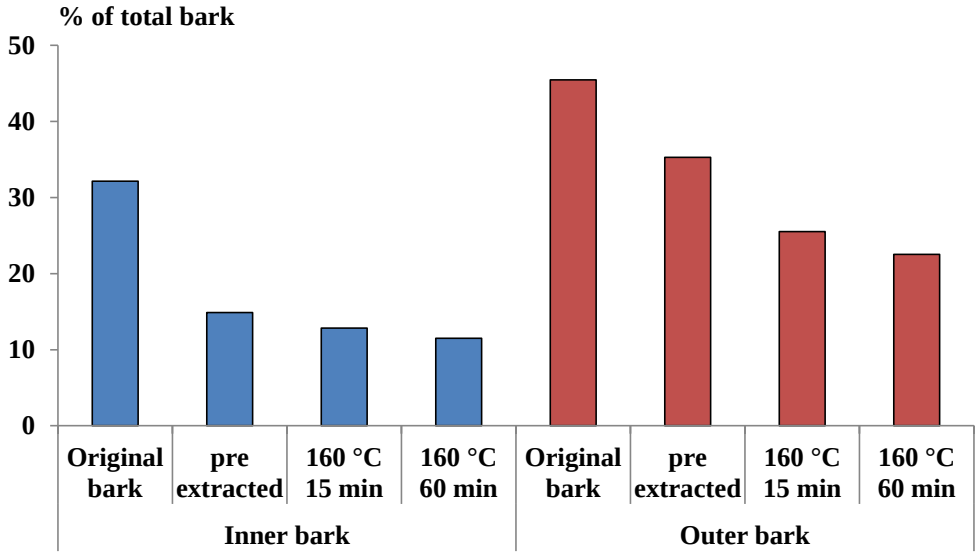

Figure 4.27 Klason residue ("lignin") determined on original barks, pre-extracted barks, and bark residue after 160 °C hot-water extraction (15 and 60 minutes)

After hexane and acetone/water extractions, the percentage Klason lignin decreased to 15 % and 35 %, respectively. This does not necessarily mean that the lignin had been extracted. Since the Klason methods involve strong acid treatment it is more likely that some of the extractives in the original bark, probably the stilbene glucosides and/or tannins, were polymerized and therefore not soluble and were detected as Klason "lignin". Only a small portion of the lignin-like substances was further released when the pre-extracted inner bark was extracted with hot-water at 160 °C, and no significant difference could be noticed between 15 minutes water extraction and 60 minutes water extraction. The "lignin" content in the outer bark decreased from 35 % to 26 % with a 15 minutes 160 °C hot-water extraction. Increasing the extraction time to 60 minutes for the outer bark decreased the "lignin" to 23 %. These results show that there is less lignin, or lignin-like substances, in the inner bark than in the outer bark in general and that the Klason lignin method might not be the best for spruce bark samples. TMAH-Py-GC-MS results on the residues show that besides lignin related compounds, the Klason lignin residue also contains many long-chain fatty acids, probably originated from suberin (**V**). These compounds were mostly found in the outer bark residues. This suggests that suberin is partly condensed and acts as lignin in the Klason lignin analysis.

Metal ion analysis with ICP-AES showed that calcium and potassium were the most abundant metal ions in both the inner bark and outer bark. Other metals with small amounts were, e.g., magnesium, manganese, and zinc. The amount of calcium in the inner bark and outer bark was 8.1 g/kg bark and 11.9 g/kg bark, respectively. The amounts of potassium were 2.46 g/kg bark and 1.56 g/kg bark, respectively. The extraction with organic solvent and water at 100 °C had little effect in the removal of metals from the barks. However, hot-water extraction at 160 °C removed some of the calcium from the inner bark (20 %) and basically all of the potassium from both the inner bark (97 %) and the outer bark (95 %). The potassium removal by the hot-water extraction is beneficial with respect to bark incineration because potassium is known to cause corrosion problems in the burner (Salmenoja and Mäkelä, 1999).

An overall composition of spruce inner and outer bark is presented in Figure 4.28.

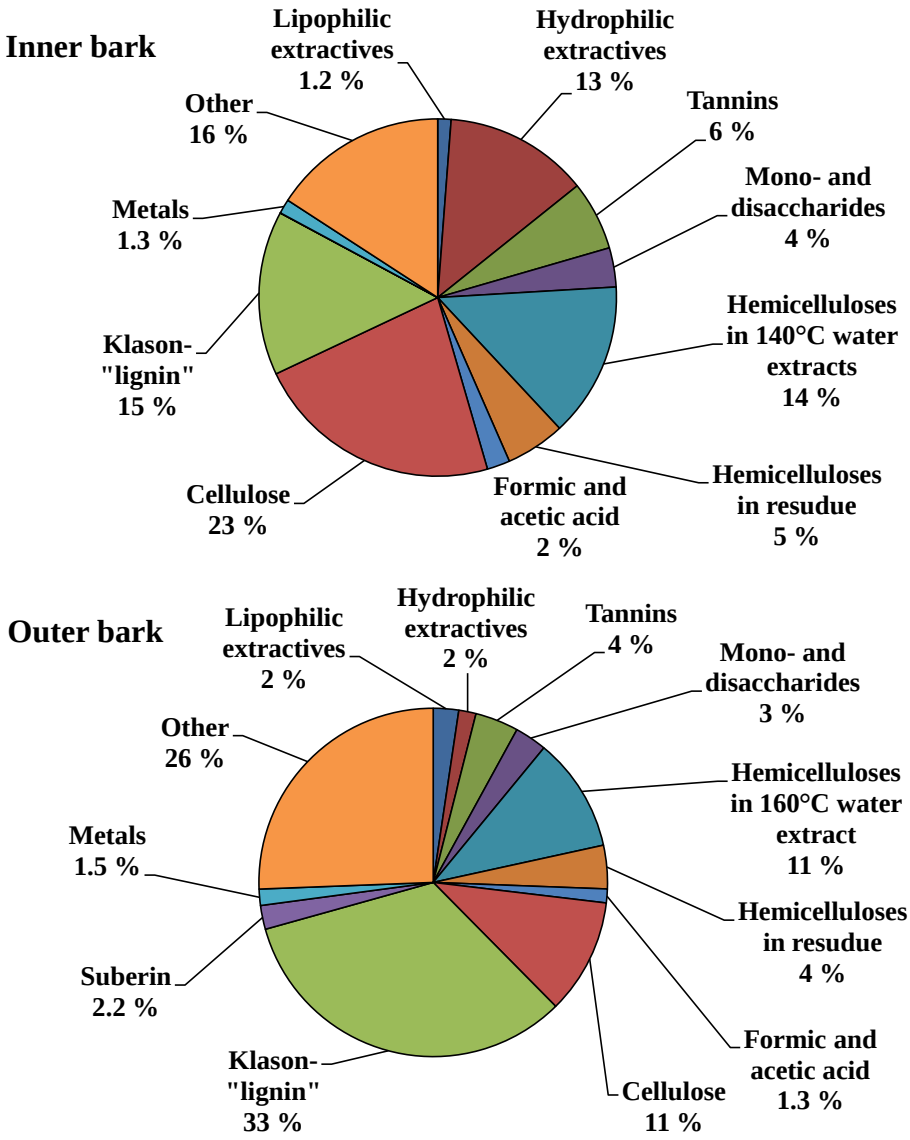

Figure 4.28 Overall composition of spruce inner and outer bark (%). The composition was calculated stepwise as follows: Lipophilic extractives by GC of hexane extract; Hydrophilic extractives in acetone-water (9:1 v/v) extract by GC of other components than mono- and disaccharides; Mono- and disaccharides by GC of acetone-water extract; Tannins by the acid-butanol assay of water extracts and UV-Vis spectrometry; Non-cellulosic polysaccharides extracted with hot water at 140-160^{o}C, by acid methanolysis and GC of sugar units; Non-cellulosic polysaccharide in residues after hot-water extraction by acid methanolysis and GC;

formic and acetic acid by alkaline hydrolysis and GC; Cellulose by acid hydrolysis and GC of glucose, with subtraction of non-cellulosic glucose determined by acid methanolysis and GC; Klason lignin gravimetrically determined on extractive-free residues after acid hydrolysis; Suberin by alkaline hydrolysis of original bark and GC of suberin-derived fatty acids; Metal ions by ICP-AES; Other: part not covered by the previous analyses.

The inner and outer bark differed considerably in composition. Although basically all the different components were found in both the inner and the outer bark, the quantity was different between them. Much more hydrophilic extractives were found in the inner bark than in the outer bark, 13 % versus 2 % respectively. Slightly more tannins and mono- and disaccharides were extracted and identified in the inner bark compared to the outer bark. Hemicelluloses were more easily extracted from the inner bark than from the outer bark, 14 % from the inner versus 11 % from the outer. Interestingly, from the inner bark most hemicelluloses were extracted at 140 °C, whereas from the outer bark the optimal temperature was 160 °C. This indicates that there are different hemicelluloses in the inner and outer bark. This hypothesis is emphasized by the fact that the hemicelluloses extracted have different sugar composition (results not shown here). The inner bark hemicelluloses contained mostly arabinose, glucose and galacturonic acids, indicating pectic material (Le Normand et al., 2014), where the outer bark hemicelluloses consists of arabinose, xylose, mannose, and glucose. Slightly more than two times the amount of cellulose was found in the inner bark compared to the outer bark, 23 % and to 11 %, respectively. The largest part of the outer bark was “Klason-lignin” whereas in inner bark it constituted only 15 %. A small amount of suberin (2.2 %) was identified in the outer bark but basically not at all in the inner bark. The metal content was about the same for both inner and outer bark. Slightly less than 16 % of the inner bark was not covered by these analyses and remains unknown. The unknown part for the outer bark is a bit larger, 26 % of the total outer bark.

5. Conclusions

The main objective of this work was to intensify the hot-water extraction of high-molar-mass hemicelluloses from spruce wood by parameter optimization for better utilization of the woody biomass. The focus was set to pH control during the extraction and particle size optimization. A sub-study was conducted on the spruce bark to characterize the chemical composition and validate if the bark hemicelluloses could be extracted in similar manner as the wood hemicelluloses.

An extensive study of different particle sizes, ranging from 0.5 mm up to industrial size chips (25×20×5 mm), showed that smaller particle sizes increases both the total yield of extracted hemicelluloses as well as the extraction kinetics. Nevertheless, smaller particle sizes are more energy demanding to produce, which will increase the overall cost.

The main challenge when trying to extract high-molar-mass hemicelluloses at temperatures reaching 170-180 °C is that the auto-ionization of the water molecules lowers pH, which in turn will generate acetic acid hydrolyzed from the hemicelluloses. This will drastically lower pH already in the beginning of the extraction with acid catalyzed hydrolysis of the hemicelluloses and lower molar mass as result. Lower temperatures mean less chain-splitting but also lower yield. Controlling the pH during extraction would give the advantage of higher pH without lowering the temperature and thereby maintaining short extraction times.

Normal, conventional glass pH electrodes cannot withstand temperatures over 80-100 °C, therefore a high-temperature pH measuring system was developed for measuring the extraction pH in-line during the wood extractions. The high-temperature pH was measured with a solid, yttria-stabilized Zr/ZrO_2 pH electrode and an Ag/AgCl reference electrode. Phthalate and phosphate buffers were used for a two point calibration of the system. In-line measurements during initial wood extractions revealed that the actual extraction pH was ~0.35 pH units higher than before believed.

In order to control the pH during the extraction, a controller connected with a pump was installed into the system. The controller enabled accurate and precise control to any desired extraction pH. Several different pH set points were tested at 170 °C. Analysis of the extracted

hemicelluloses showed that less hemicelluloses were extracted at higher pH. However, the chain degradation could to some extent be prevented as higher extraction pH yielded higher molar-mass of the extracted hemicelluloses. Furthermore, monomer formation could be avoided at an extraction pH of 4.85 and higher. Increasing the temperature to 180 °C with a pH set point of 4.85, intensified extraction speed without losing hemicellulose molar-mass could be obtained.

For a wood biorefinery further processing of the other components in the wood, i.e., the cellulose and the lignin are of great interest. Therefore, it is important to not only consider the hemicellulose extraction but also how it affects the cellulose and the lignin. The hot-water extractions at 170 °C and 180 °C did not affect the other components in the wood considerably. 5-7 % of the cellulose was removed during the hot-water extractions and slightly more lignin, 6-10 % removed.

A short kinetic study revealed that hemicellulose extraction at 170 °C of larger particle sizes (4-12.5 mm) followed pseudo-first order kinetics while hemicellulose extraction of smaller particle sizes (0.5-2 mm) followed a reaction order of 1.5.

Spruce bark hemicelluloses differs from spruce wood hemicelluloses. Judging from their sugar composition, the main part is comprised of pectic material. Besides hemicelluloses, the bark contained much more extractives than the wood. The most abundant extractive in the bark is stilbene glucosides, and thus might be of interest in a biorefinery perspective. But the total different composition of the bark compared to the wood complicates the bark separation process considerably more than the wood process.

This work shows the importance of pH during hot-water extraction of wood in order to obtain a high yield of high-molar-mass hemicelluloses. A huge advantage lies in actively measuring and controlling the pH in-line during a hot-water extraction which has not been possible before this study. A better understanding and better control of the hot water extraction process that this work brings will undoubtedly be of help in the forest biorefinery future.

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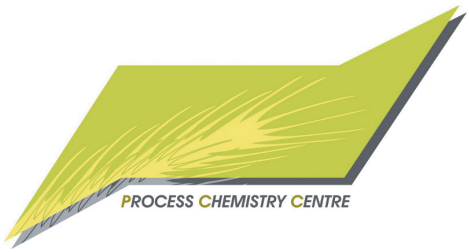

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