THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Bioplastics from Biomass - Acetylation of Xylans with Green Chemistry

AGNES M. STEPAN

Biopolymer Technology
Department of Chemical and Biological Engineering
CHALMERS UNIVERSITY OF TECHNOLOGY
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AGNES M. STEPAN

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Biopolymer Technology
Department of Chemical and Biological Engineering
Chalmers University of Technology
SE-412 96 Gothenburg
Sweden
Telephone + 46 (0) 31-772 10 00

Cover: Picture of a piece of spruce wood and cereal grain as a renewable resource. Candy wrapped in xylan acetate and a thermal processed container of acetylated xylan filled with water.

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ABSTRACT

There is social, environmental and increasing economic pressure on the industrial sector to substitute non-renewable resources with renewable ones as the increasing World population is exponentially depleting the fossil fuel supplies of the Earth. Each year about 260 million tons of plastics are produced from crude oil and most of it ends up as waste. Producing biodegradable plastics from renewable resources could be a contribution to a sustainable development. Hemicelluloses are the second most abundant biopolymers on Earth, with about 60 billion tons biosynthesized each year by plants. Xylans are the largest group of hemicelluloses and were designed by nature to primarily function as a matrix in plants. So far, they are unutilized valuable biopolymers with broad potential applications. Many xylans are acetylated in nature, and the degree and pattern of acetylation influences the material properties of the plant cell wall. Controlled chemical esterification is a powerful tool for tailoring the structure and material properties of hemicelluloses as a renewable raw material for bio-based plastic production.

First, chemical acetylation was carried out on corncob arabinoxylan (CCAX), and the films were then compared with the unmodified pure CCAX films. There was clear improvement of the thermal and water resistant properties of the xylan films after acetylation. In following work, the structure-property analysis showed a positive effect of arabinose side chains on the elongation at break, the thermal stability and glass transition temperature of the acetylated xylan.

To make hemicellulose acetylation more sustainable, green chemistry was applied. The first approach included the utilization of ionic liquids (IL) as reaction media. Rye AX and spruce AGX were both fully acetylated while maintaining a high degree of polymerization (DP) in a very fast reaction. Another green chemistry alternative is to use enzymes. In this thesis the surfaces of rye AX films were acylated with acetate and stearate using lipases and cutinases in a water-free environment. The advancing contact angle of the surfaces was increased, the stearated surface being most hydrophobic.

Finally, AcAX was combined with spruce nanofibrillated cellulose (NFC) to form films. The thermal properties, stiffness and stress at break of the composite were superior to the neat AcAX films. The uniqueness of this composite is the water resistance and high elongation at break, even at a 10 % NFC content.

This work is a contribution to the designing of novel xylan based materials and their feasible and environmentally friendly production. Acetylated arabinoxylans have a potential to replace many of the oil based plastics and become a future bioplastics.

Keywords: *xylan, arabinoxylan, arabinoglucuronoxylan, acetylation, acylation, ionic liquid, enzymatic surface modification, composite, water resistance, glass transition temperature*

LIST OF PUBLICATIONS

This thesis is primarily based on the work published or submitted to journals in the following papers, referred to by their Roman numerals in the text. The papers are appended at the end of the thesis.

Paper I. **Corncob arabinoxylan for new materials**
Itziar Egüés, Agnes M. Stepan, Arantxa Eceiza, Guillermo Toriz, Paul Gatenholm, Jalel Labidi
Submitted to Carbohydrate Polymers

Paper II. **Arabinose Content of Arabinoxylans Contributes to Flexibility of Acetylated Arabinoxylan Films**
Agnes M. Stepan, Anders Höije, Henk A. Schols, Pieter de Waard, Paul Gatenholm
Journal of Applied Polymer Science (2012), 125, (3), 2348–2355

Paper III. **Fast and highly efficient acetylation of xylans in ionic liquid systems**
Agnes M. Stepan, Alistair W.T. King, Tia Kakko, Guillermo Toriz, Ilkka Kilpeläinen, Paul Gatenholm
Cellulose (2013), DOI: 10.1007/s10570-013-0028-y

Paper IV. **Lipases efficiently stearate and cutinases acetylate the surface of arabinoxylan films**
Agnes M. Stepan, George E. Anasontzis, Teresa Matama, Artur Cavaco-Paulo, Lisbeth Olsson, Paul Gatenholm
Journal of Biotechnology 167 (2013) 16– 23

Paper V. **Nanofibrillated cellulose reinforced acetylated arabinoxylan films**
Agnes M. Stepan, Mohd Farhan Ansari, Lars Berglund, Paul Gatenholm
Submitted to Composite Science and Technology

CONTRIBUTION REPORT

Paper I. Co-author. Took active part in planning the experiments, participated in the experimental work of the evaluation of film property measurements.

Paper II. Main author. Took active part in planning the experiments and performed most of the experimental work.

Paper III. Main author. Took active part in planning the experiments and performed most of the experimental work.

Paper IV. Main author. Took active part in planning the experiments and performed the experimental work.

Paper V. Main author. Took active part in planning the experiments and performed all of the experimental work. Some measurements were made together with the co-authors.

RELATED PUBLICATIONS NOT INCLUDED IN THE THESIS

Paper VI. **Flexible oxygen barrier films from spruce xylan**
Alfredo Escalante, Ana Goncalves, Aase Bodin, Agnes Stepan, Corine Sandstrom, Guillermo Toriz, Paul Gatenholm
Carbohydrate Polymers (2012) 87, 2381-2387

Paper VII. **Spruce glucomannan: preparation, structural characteristics and basic film forming ability**
Yujia Zhang, Jiebing Li, Mikael E. Lindström, Agnes Stepan and Paul Gatenholm
Nordic Pulp and Paper Research Journal (2013) 28 (3), xxx-xxx
DOI: 10.3183/NPPRJ-2013-28-03-p323-330

Paper VIII. **Assembly of debranched xylan from solution and on nanocellulosic surfaces**
Toon J. Bosmans, Agnes M. Stepan, Guillermo Toriz, Scott Renneckar, Erdem Karabulut, Lars Wågberg, and Paul Gatenholm
Submitted to Biomacromolecules

ABBREVIATIONS

Ac	Acetylated
[amim]	1-allyl-3-methylimidazolium
AX	Arabinoxylan
AGX	Arabinoglucuronoxylan
B	Bleached
CCAX	Corncob arabinoxylan
DB	debranched
[DBNH]	1,5-diazabicyclo[4.3.0]non-5-enium
DMA	Dynamic mechanical analysis
[emim]	1-ethyl-3-methylimidazolium
ESCA/XPS	Electron spectroscopy for chemical analysis/X-ray photoelectron spectroscopy
HSQC	Heteronuclear single quantum coherence spectroscopy
IL	Ionic liquid
Mw	Weight average molecular weight
NFC	Nanofibrillated cellulose
NMR	Nuclear magnetic resonance
OP	Oxygen permeability
RH	Relative humidity
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
TGA	Thermogravimetric analysis
TOF-SIMS	Time of flight secondary ion mass spectrometry
PLA	Poly(lactic acid)
VAc	Vinyl acetate
VSt	Vinyl stearate
W	Washed

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Chapter 1

Introduction

This thesis is a contribution to the development of new biomass derived materials that can be used as binders, coatings and packaging materials targeting the replacement of some fossil fuel based plastics. For example, polyethylene or polypropylene might be replaced in certain applications. The replacement of starch based plastics, poly(lactic acid) or cellulose acetates is also desirable, since the raw material of some of these bio-based plastics have food-ethical issues or could be utilized in other forms with higher value, all of them being expensive.

This research was carried out in the framework of the Wallenberg Wood Science Center (WWSC), which is a joint research center between the Royal Institute of Technology, Chalmers University of Technology, Stockholm University, Innventia, Swerea and others. The WWSC is a long term research program targeting the development of novel value added materials from trees. The major materials produced today from trees are pulp and paper based materials. Since the pulp and paper market is stagnant or even declining world-wide in recent years due to the competition of digital information, it is crucial to find alternative uses for forest products. This has even more emphasis in countries such as Sweden, where the forest industry plays a significant role in the economy.

Today, bio-based plastic are more expensive than the fossil fuel based plastics, but, as non-renewable reserves of oil are rapidly becoming depleted, more and more emphasis is placed on their commercialization. Bioplastics are also considered carbon neutral because they do not cause a net increase in atmospheric carbon dioxide at the end of life decomposition. The biodegradability of plastics is also crucial to decrease the rate of waste accumulation in the world.

Plant polysaccharides are a group of biopolymers that make up most of plant biomass, thus offering a significant amount of renewable resources. The most relevant polysaccharides are cellulose, hemicelluloses, starch and pectins. Even though hemicelluloses are the second most abundant polysaccharides on Earth after cellulose, they are a highly underutilized and inexpensive source of raw material/biomass and only recently are receiving more research attention.[1]

Xylans are the largest group of hemicelluloses on Earth. They constitute a high percent of annual plants, such as crops and grasses. They are also about 30% of hardwoods and 10% of softwoods, giving an average of about 20% of all wood on the Earth, as the two types of trees have a similar abundance. Many of the xylans are in agricultural side products and burnt or used as animal feedstock. Unfortunately, a significant amount of xylans is burnt as a low energy fuel as a side product of forestry and paper industry, after the wood has been processed. Large volumes of xylan in diverse industrial side streams are left unutilized. Xylans, among other hemicelluloses, have a great potential to replace some plastic products, especially in the market of coatings, binders and packaging. Not only are the xylan films excellent oxygen barriers, but they can also be made thermoplastic by full acetylation. The thermoplasticity and higher thermostability gained from full acetylation of xylans facilitates a range of thermo-processing technologies to be applied for product processing.

The lack of economically feasible large scale extraction methods of hemicellulose has made xylans, as a raw material, an extremely expensive resource. This has probably contributed to the lower commercial interest in xylan derivatization as compared to cellulose and starch derivatization. On the other hand, a financially viable xylan extraction is running on a pilot scale, in a company that is first in the world to aim at commercializing a packaging material based on xylan. Now that these abundant biopolymers are possibly becoming available in larger quantities and reliable quality, their future applications and derivatives should gain increasing attention and relevance. The industrial scale availability and high purity are critical factors in motivating further research on xylans. However, established basic knowledge of the material properties of xylan derivatives is also crucial for motivating the development of industrial scale extraction of xylans. *As with the “chicken and the egg”, the one is necessary for the initiation of the other.*

The conversion of xylans into value added materials could also contribute to initiate the transformation of industrial establishments of forestry and agriculture into biorefineries.

Acetylation of polysaccharides has been performed previously. However, it was only cellulose acetates that received sufficient attention to make it to the market in real life applications. This might be due to the fact that many other polysaccharide esters do not exhibit material properties like those of the cellulose acetates. Cellulose and its derivatives are broadly utilized in very diverse industrial branches. Cellulose acetate is the major ester derivative, and has applications in textiles and fibers, coatings, cigarette filters, plastics, frames for eyeglasses, optical films, laminates and separation membranes [2, 3] depending on the degree of substitution (DS) of acetyl groups. Hemicelluloses have a more complex structure and composition as compared to cellulose. Nevertheless, based on the literature on polysaccharide esters, it is expected that hemicellulose acetates will obtain thermoplastic properties and be more flexible with increased water resistance. These properties could make them suitable for film, binder and laminate applications. Some hemicellulose esters have been prepared in the past[4-8] but, in contrast to cellulose acetates, they never made it to the market.

It is not only environmentally friendly materials that are necessary for a sustainable development, but also to have environmentally benign processes in industry. A philosophy of chemical research, where the processes aim at the reduction or recyclability of the chemicals involved, is called “green chemistry”. The use of ionic liquids and enzymes is most commonly considered a green chemistry approach towards processes.

Aim of this work

The aim of this work was to study the structure property relationships and material properties of acetylated xylans, which are summarized in the first two papers of this thesis.

As the acetylated xylan proved to be a promising material for bio-based coatings, binders and packaging, the research then aimed at developing alternative and more environmentally benign ways of preparing these materials. One paper discusses the acetylation in ionic liquids and another in a solvent free reaction aided by enzymes.

Finally, the aim was to further improvement the material properties of acetylated arabinoxylans and to demonstrate its compatibility with other bio-based materials to broaden the field of possible application.

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Chapter 2

Background

Plant cell walls

The total terrestrial carbon in all types of vegetation is estimated to be 650 billion metric tons on Earth. This biomass has a turnover of typically less than a century.[9]

Plant cell walls are the major source of biomass in plants and thus the most important renewable source of materials, biofuels and chemical feedstock. The chemical composition of plant cell walls can vary between species. Nevertheless, cellulose, hemicellulose and lignin are the most common biopolymers in dry-land plants, and pectins, proteins and waxes are also common plant cell wall components in addition to a significant amount of water.[10-12]

Both trees and annual plants are multi-component biocomposites created by nature and have very attractive properties as construction materials. In the plant cell walls, the cellulose is responsible for the stiffness and the rigidity of the material through reinforcement, while the surrounding hemicellulose and lignin form a compliant matrix around it.[13] These three components will be discussed in more detail below. However, it is important to mention that structural proteins are common in the primary cell walls along with other pectic polysaccharides.[14]

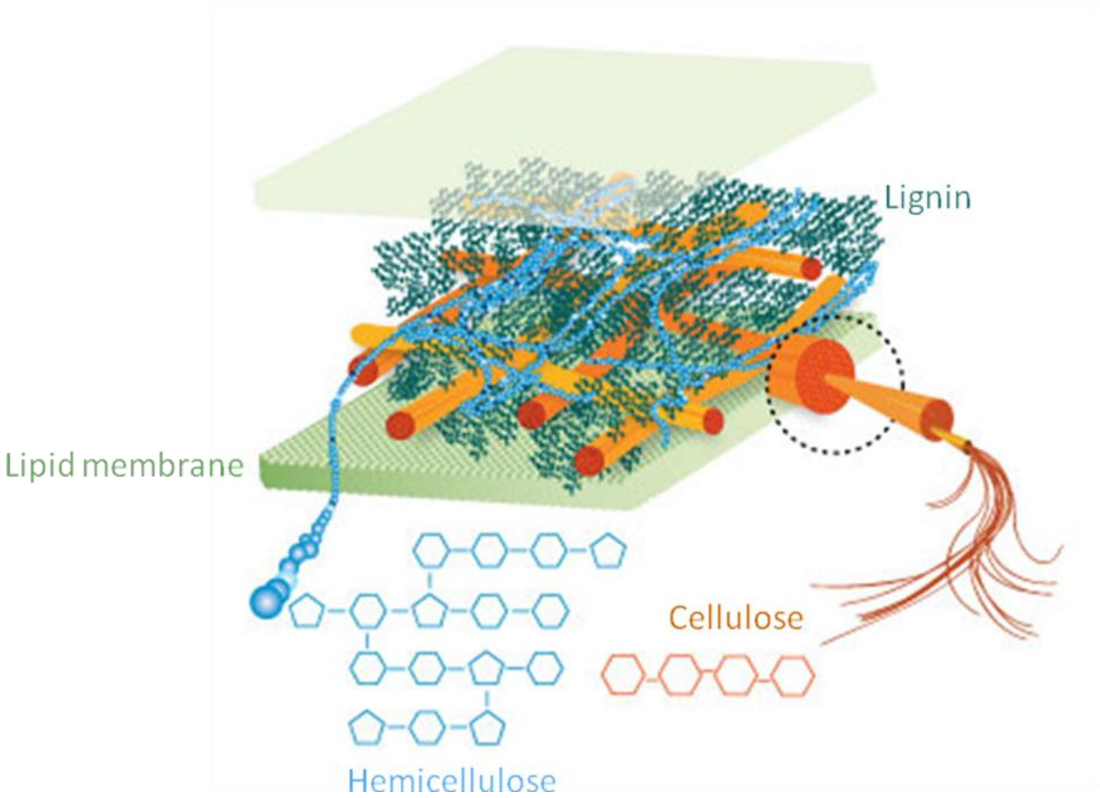

Figure 1 Schematics of a plant cell wall (picture adapted from reference[15])

Cellulose

Cellulose, which is the most abundant biopolymer on Earth, is the major structural component of plant cell walls[16]. Cellulose is a polysaccharide that also has crystalline polymorphs, which contributes to the strength and stiffness of plants. Depending on the species, cellulose composes about 40-45% of the cell walls.[14, 16, 17] Primary and secondary cell walls both contain cellulose, but most of the cellulose is in the secondary cell walls. The cellulose molecule is a linear polysaccharide built up of β-1,4 linked glycopyranosyl units. Since this type of linkage form requires each glycosyl unit to be 180 ° compared to its neighboring residues, the repeating unit is often declared a cellobiose.[12, 14, 18, 19] This zigzag arrangement of sugar units places the macromolecule in the ribbon shaped family.[20] The degree of polymerization (DP) gives information about the number of sugar monomer units in the molecule. The DP of cellulose molecules can range from a few thousand up to 15000 depending on the source in the plant.[21]

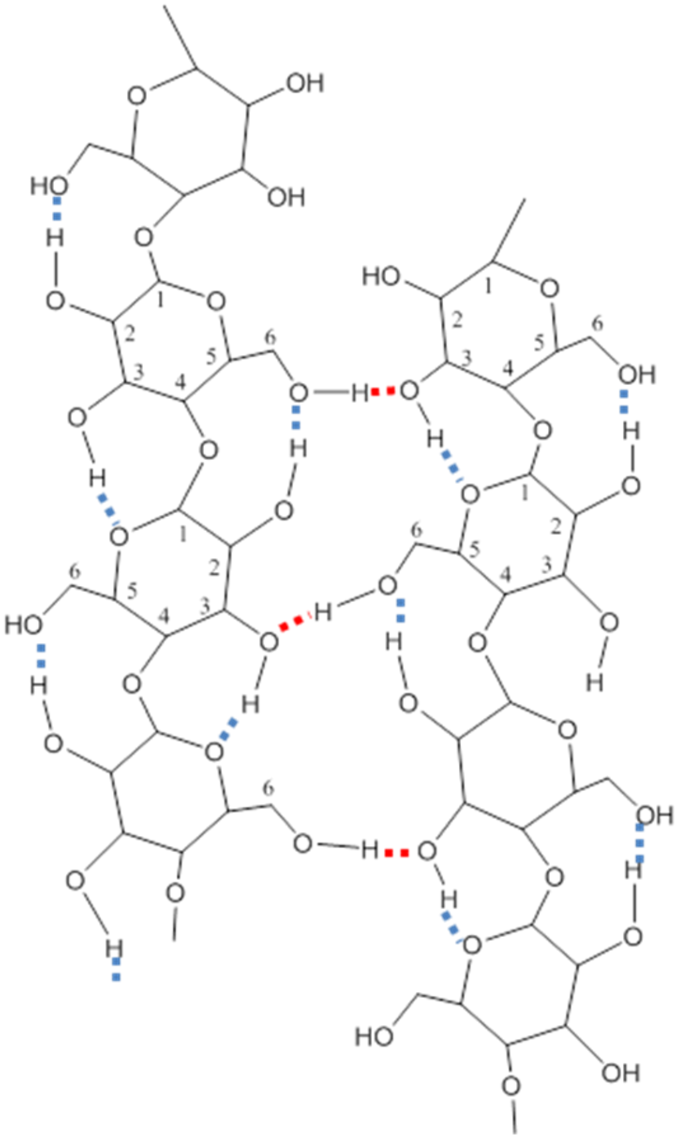

Figure 2 Cellulose I with intra- (blue) and intermolecular (red) hydrogen bonds(adapted from reference[22])

In trees and plants, 36 β-glucan chains form a cellulose microfibril with a diameter of 3-5 nanometers with both crystalline and amorphous regions.[23] These fibrils are held together by strong intra- and intermolecular hydrogen bonds, giving them their significant strength, stiffness and notable solvent resistance compared to other similar polysaccharides.[12, 24, 25]

The literature distinguishes between different crystalline polymorphs of cellulose (cellulose I-IV).[18, 26]

In the plant cell walls, the crystalline regions are often disrupted by amorphous ones, which are proposed to critically influence the physical properties of the microfibrils in the cell walls.[27]

Nanofibrillated cellulose (NFC)

Herrick et al. and Turbak et al. used a high-pressure homogenizer to treat wood fibers in multiple cycles to produce microfibrillated cellulose (MFC) as early as in the 1980s.[28, 29] The materials produced were opened to their substructural microfibrils, which generally consist of elementary fibril bundles on the order of tens of nanometers in thickness but several micrometers in length (aspect ratio >>1). [30] These fibrils constitute a fiber network. In the literature, MFC is often referred to as nanofibrillated cellulose (NFC).[31, 32] The high energy consumption of the NFC preparation has been reduced by combining the high-energy mechanical shearing with chemical and enzymatic pretreatments. These methods also provide more uniform NFC.

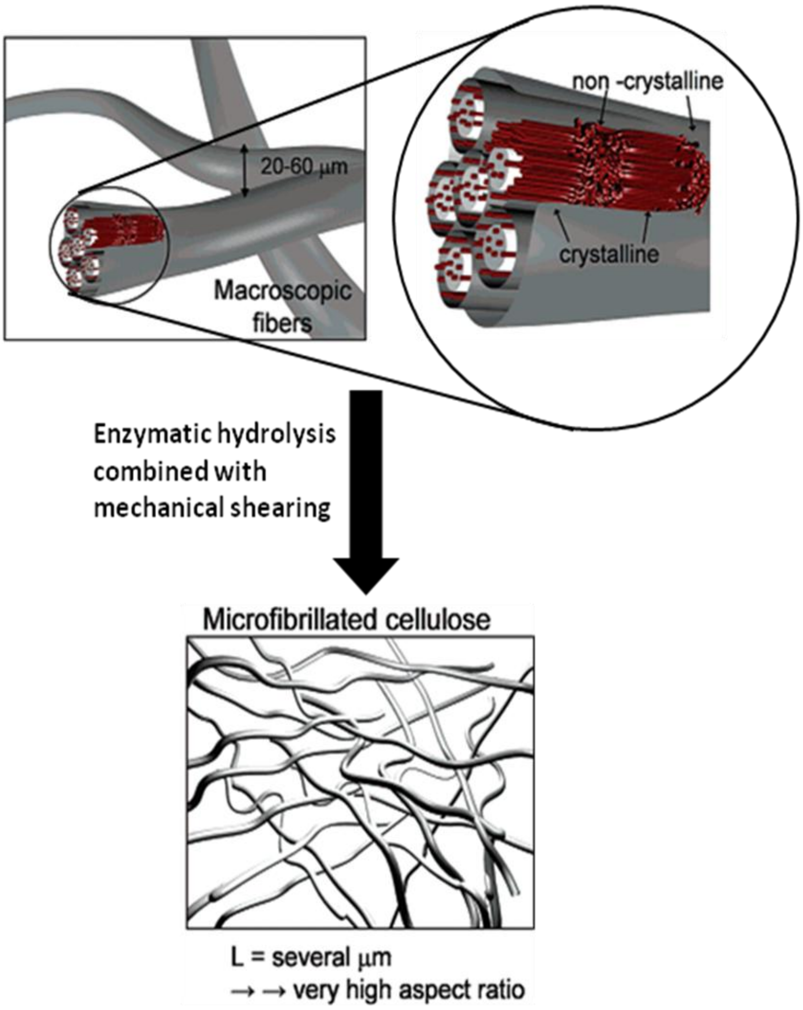

Figure 3 Schematics of preparing NFC, often also termed micro fibrillated cellulose because its length is in the micron range (picture adapted from Paakko, M. et al. *(2007)* [33]*)*

Nanocellulose receives much interest as a reinforcement in composites due to its unique structural and physical aspects such as high strength and stiffness combined with low density, biodegradability and renewability.[34] Furthermore, due to their nanoscale dimensions, they present high light transmittance when incorporated into films.[34] These properties combined with their potential economic advantages can offer to prepare lighter, stronger materials with improved durability.[35]

Hemicelluloses

Hemicelluloses were discovered almost half a century later than cellulose. They were first described as wood gums by Thomsen in 1879. Schulze was the first to name this group of polysaccharides hemicelluloses in 1891.[36, 37] Schulze considered these polysaccharides to be chemically and structurally related to cellulose. However, it was later established that they are a group of heterogeneous polysaccharides that have a complex composition and structure with a biosynthesis independent from that of cellulose.[12, 38] In general, hemicelluloses are polysaccharides that can be extracted with water or alkali from plant tissue.[39, 40] The most common monosaccharide units that build up hemicelluloses are: D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid (and its 4-O-methyl derivative) and D-galacturonic acid.[41-43] Depending on their type and origin, these heteropolymers have an average DP of around 100-2000.[12, 38, 42, 44] Hemicelluloses are often highly branched and have different substitution patterns and frequencies of sugar substituents and/or acetyl or feruloyl groups. This diverse composition and structure prevents hemicelluloses from crystallizing. The amorphous state and lower DP causes hemicelluloses to have less chemical and thermal stability than cellulose.

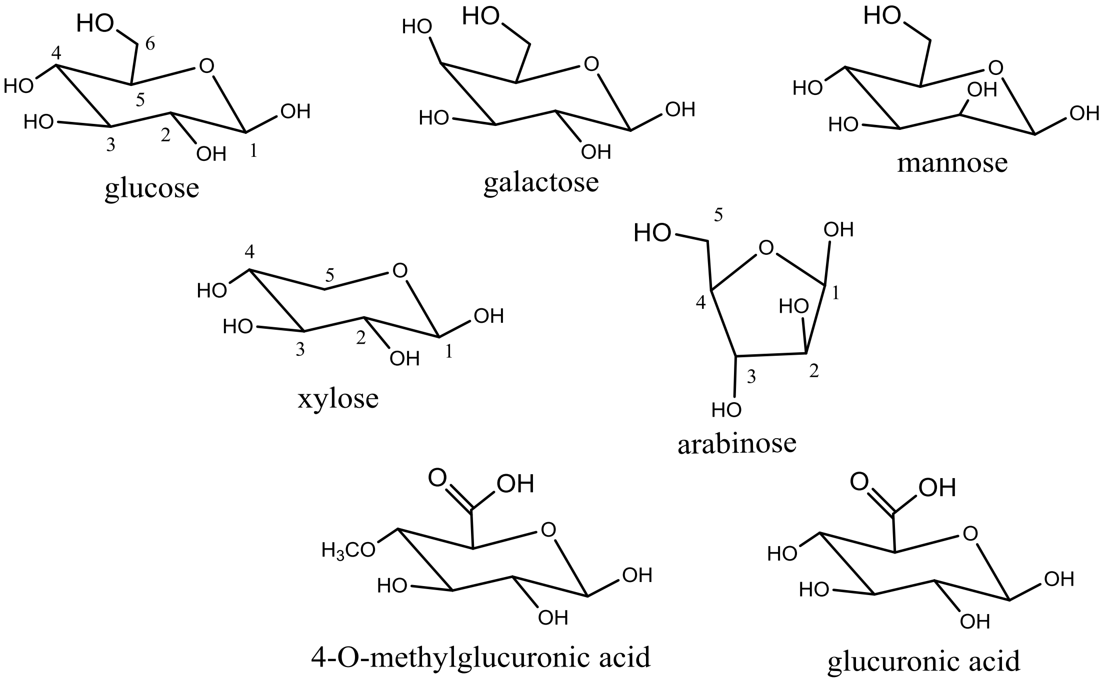

Figure 4 Most common sugar monomer units of hemicelluloses

Biosynthesis of all polysaccharides starts from photosynthesis, which is the ultimate source of glucose (Glc) in a plant cell.[19, 45] The uridine diphosphate glucuronic acid (UDP-GlcA) is the sugar nucleotide serving as a base for all sugar monomer synthesis. NAD$^-$ (nicotinamide adenine dinucleotide) and NADH are coenzymes that are the proton donors and acceptors of the conversions.

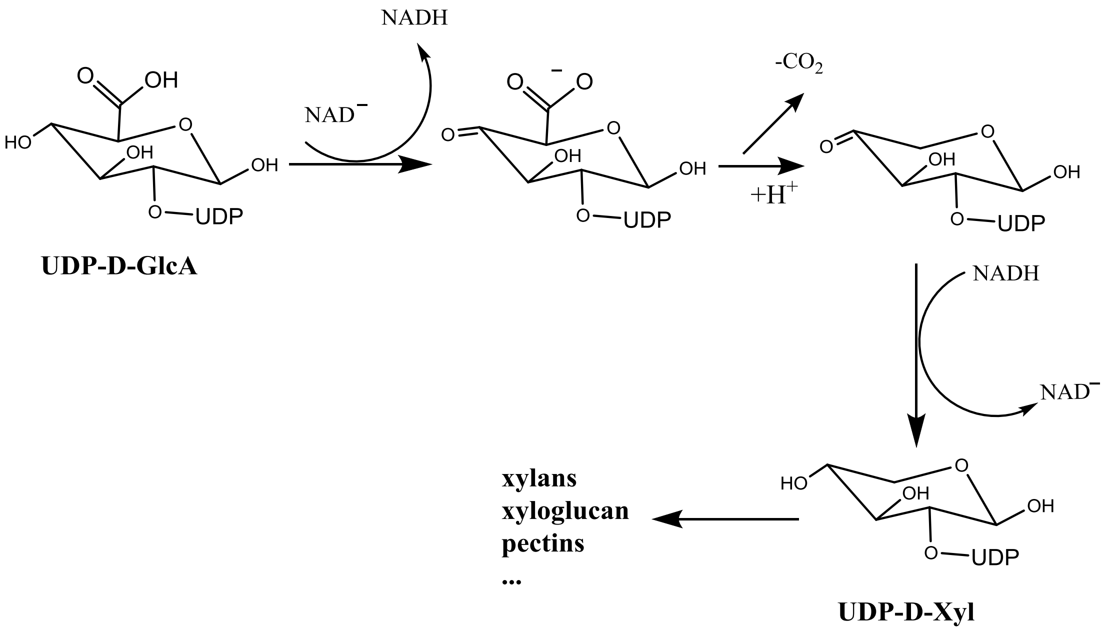

Figure 5 The proposed path for the biosynthesis of the xylopyranose monomer (adapted from Harper and Bar-Peled[46])

After the glucose has been converted to a sugar nucleotide, it can be used directly for cellulose synthesis at the outer cell membrane to construct the cell wall or can be transported to the Golgi apparatus as a precursor for other sugars in different polysaccharides.[45] Hemicelluloses are synthesized in the Golgi apparatus and transported to the plasma membrane in vesicles where they are excreted into the extracellular matrix to assemble together with the newly synthesized cellulose fibrils.[11, 47, 48] There are four major steps of hemicellulose synthesis: backbone initiation, propagation, side chain addition and termination.[49] Several enzymes involved in the biosynthesis of hemicelluloses have been identified, but the detailed mechanisms have not yet been revealed. Some of the hemicelluloses relevant to this thesis are discussed in more detail below.

Glucomannan and Galactoglucomannans

The glucomannans and O-acetyl-galactoglucomannans are the main hemicelluloses of softwoods (about 20%).[12] These hemicelluloses have a backbone composed of alternating β-1,4 linked D-glucopyranosyl and D-mannopyranosyl units. They are partially acetylated on C2 and C3 depending on the tissue source. The glucomannans are a linear polysaccharide while the galactoglucomannans have D-galactopyranosyl substitution on the C6 of the glucose units.

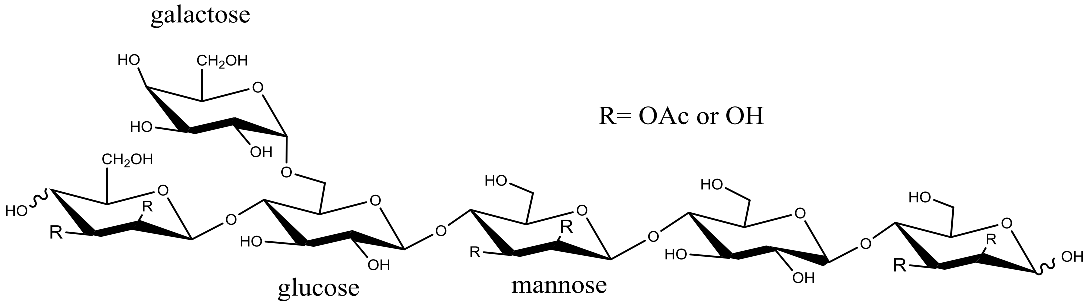

Figure 6 Example of possible galactoglucomannan structure

Xylans

Xylans are the most common type of hemicelluloses found mainly in hardwood and annual plants. They are closely associated with cellulose in the cell walls, and lignin carbohydrate complexes are also assumed to be formed between lignins and xylans.[50] This group of hemicelluloses has a linear backbone of β-1,4 linked D-xylopyranosyl and a variety of side chains based on L-arabinofuranosyl, 4-

O-methyl-D-glucuronopyranosyl, D-glucuronopyranosyl, D-xylopyranosyl, D-galactopyranosyl, acetyl and feruloyl, groups. The structure of xylans varies significantly between different plant species. For example, substitution on both the C2 and C3 positions of a backbone xylopyranosyl and disaccharide side chains is also common in certain cereal xylans.[51-54]

Arabinoxylans

Arabinoxylans are most common in annual plants such as corn, rye, rice, wheat and barley, having predominantly α-L-arabinofuranosyl mono or disubstitution on a linear xylose backbone (Figure 7). However, these are the xylans that, depending on the source, can have a very broad variety of substituents. The DP of these polymers is also source dependent and is in the range of 100-2000.[12, 44] Rye AX has an arabinose to xylose ratio of around 1:2.[51]

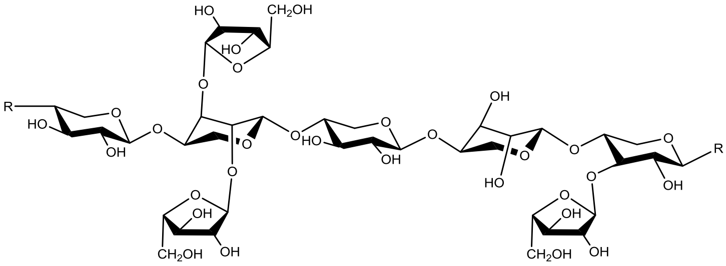

Figure 7 Structure of arabinoxylan from rye

Glucuronoxylan and arabinoglucuronoxylan

Glucuronoxylans are typical for hardwood species such as birch, aspen, beech and eucalyptus, composing about 15-30% of the tree depending on species.[12] The linear xylan backbone has approximately every 10th backbone unit substituted with 4-O-methylglucuronic acids. In addition to the sugar side groups, the xylopyranosyl units also have O-acetyl substitutions on C2 and/or C3. Native aspen xylans have about a 0.45-0.7 degree of substitution (DS) of acetyl groups, but these are removed if the xylan is isolated through alkali extraction.[40, 55] Due to reported possible O-acetyl migrations, the native acetyl substitution pattern of wood xylans is not straightforward.[56]

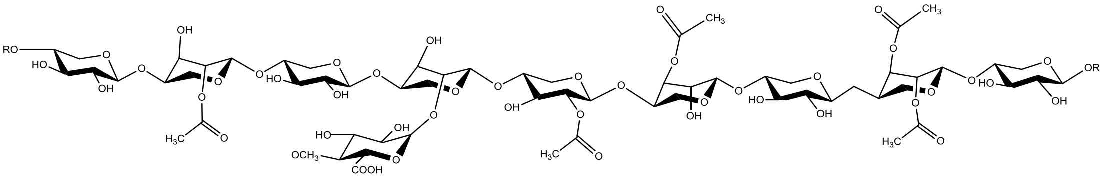

Figure 8 Structure of glucuronoxylan from aspen

Arabinoglucuronoxylans (AGX) are one of the two main hemicelluloses of softwood along with the glucomannans. AGX composes about 8-10% of softwood species. On the xylan backbone, on every 11th backbone unit, there are about two of the 4-O-methylglucuronic acid side groups and one arabinofuranosyl side group.

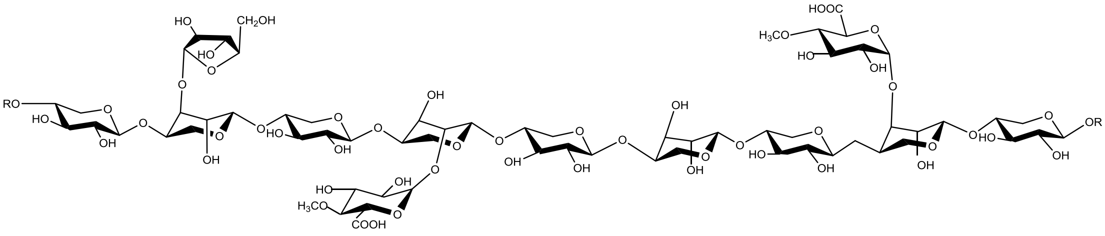

Figure 9 Structure of arabinoglucuronoxylan from spruce

Structure-property relationship in xylans

The unsubstituted β-1,4 linked xylan has been reported to form crystals hydrated by water. Two antiparallel xylan chains with a threefold screw form a unit cell with one water molecule per xylose monomer.[57, 58] However, the diverse substitutions on the xylans prevent the molecules from tight stacking into crystallites, as cellulose does, thus significantly influencing the properties of xylans through the distribution of the substituents. This is what leads to the amorphous structure and water solubility of the xylans. For example, it has been shown that the increase or decrease of arabinose substitution directly influences the solubility of arabinoxylans in water.[59, 60] Side groups determine not only the xylan morphology and solubility in water but also the interactions with other cell wall components.[61] For example, parts of the xylan backbone with low substitution will closely interact with cellulose and are less water soluble.[62, 63] Furthermore, other material properties such as the mechanical performance of film casted from xylans are also influenced by the side groups.[60]

The side groups play an important role in solubilization in water, whether they are a sugar unit or acetyl or ferulic acid group. A certain level of acetylation can increase the solubility of xylans in water by preventing strong interactions, such as hydrogen bonds that lead to tight stacking of the molecules, causing aggregation.[55] However, a chemically induced high degree of substitution with acetyl groups also results in the removal of the hydrophilic substitutions, allowing the polysaccharides to dissolve in more hydrophobic or aprotic solvents, whereas precipitation occurs in water.[64]

Acetylation of polysaccharides

In 1865 Paul Schützenberger was the first to report on the preparation of cellulose acetates by reacting them with acetic anhydride.[65, 66] Cellulose triacetate, soluble in chloroform, did not receive much attention until George Miles discovered in 1904 that partially hydrolyzed cellulose acetate is soluble in other solvents such as acetone. World War I further boosted acetate lacquer production and, in the early twentieth century, cellulose acetate yarns were spun which quickly became an important material in the textile industry. In 1934 Cellulose acetate started to replace cellulose nitrate in film applications as a non-flammable thermoplastic. However, hemicellulose acetates have not been commercially utilized.

Acetylation of oligo- and polysaccharides involves the replacement of available hydrophilic OH groups with more hydrophobic acetyl groups. Today there are numerous publications on various acetylation methods, resulting in various degrees of substitution and/or pattern.[5-7, 64, 67-84] However, the most basic reaction to achieve acetylated hemicelluloses is still based on acetic anhydride as the reagent. Depending on the targeted degree of substitution, different reaction conditions can be used. The basic reaction mechanism in this case starts with a nucleophilic attack of the lone electron pair of the alcoholic sugar OH group on the carbonyl carbon atom of the acetic acid anhydride molecule. Due to the consequent split-off of acetic acid, acetylated carbohydrates are formed. This reaction is often performed with acid or base catalysis.

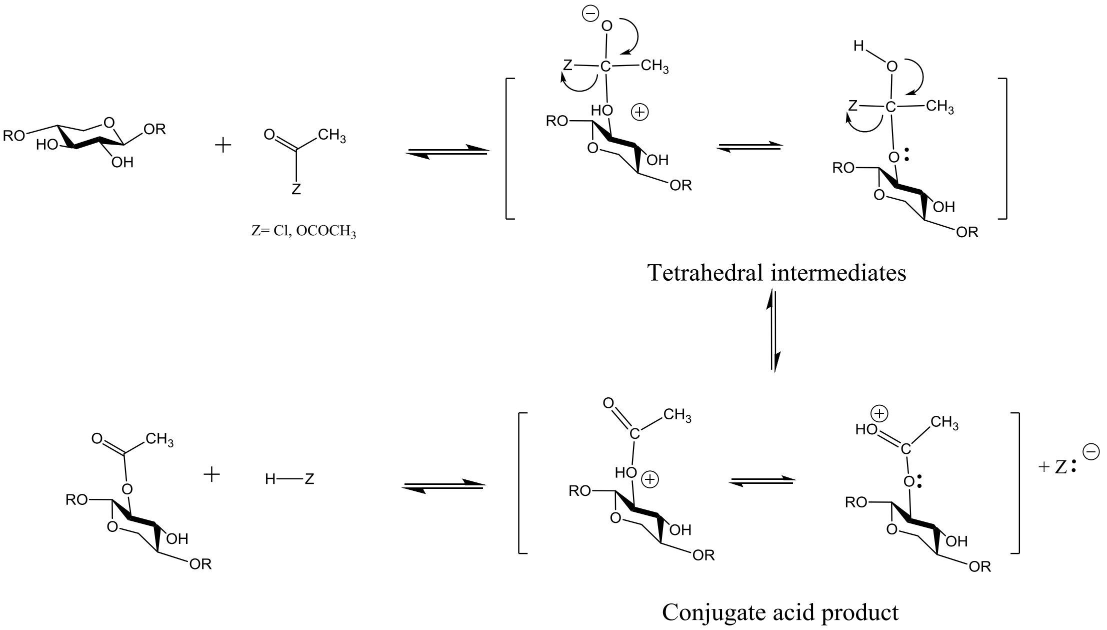

Figure 10 Reaction mechanism of the acetylation of xylan (based on a general scheme for acetylation of wood[85])

Gas phase surface esterification, plasma treatment assisted surface esterification or pretreatment of sample with glacial acetic acid are also common methods to achieve different levels of acetylations.[86] Reaction in DMAc often uses LiCl as the catalyst.[78, 87]

Under the pressure to achieve new products in an environmentally friendly way, several other acetylation methods are being studied. Two major modern approaches will be summarized below:

The use of ionic liquids (IL) as a solvent for polysaccharides is an emerging field that is providing new opportunities for various cellulose and hemicellulose modifications.

Enzymatic esterification of polysaccharides is also gaining significant attention now in the biotechnological revolution.

Ionic liquids

Ionic liquids are a heavily researched group of salts that melt below 100 ºC. They recently became an attractive polysaccharide solvent due to some of their intriguing properties. They have negligible vapor pressure, low flammability, high viscosity along with high thermal and chemical stability, which promote them as environmentally benign compared to several other reaction media. Their physicochemical properties are tunable, they have high loading capacity and, potentially, high recovery rates after use, which makes them desirable in chemical reactions.[76, 88-95] They are assumed to be recyclable; however, depending on the field of application, long-term recyclability of many ILs still remains a challenge due to currently employed purification steps. Most common ILs used for cellulose modifications are alkyl- imidazolium based compounds.[89] There are extensive studies and numerous reports on cellulose, and somewhat fewer on hemicellulose modifications in ionic liquids. However there is an emerging group of ionic liquids that contain a double aromatic ring as the positive ion of the salt.

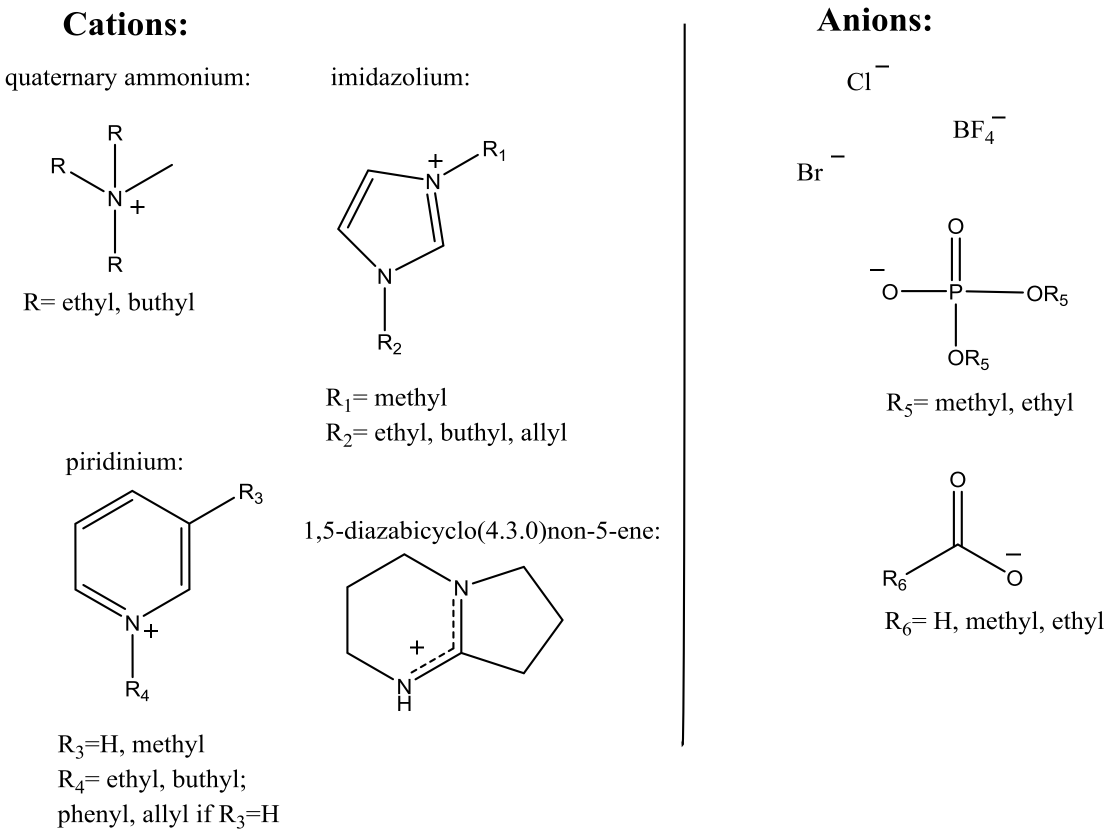

Figure 11 Some common cations and anions of ionic liquids for the dissolution of cellulose

Enzymes

The increasing environmental consciousness and sustainable technologies also promote the modification of carbohydrate and polysaccharide aided by enzymes.

Enzymes are the proteins that are responsible for the catalysis of the thousands of chemical inter-conversions that sustain life. They are highly selective catalysts that greatly accelerate the rate and increase the specificity of metabolic reactions in living organisms.[96] They can be applied broadly once purified and they are a modern industrial tool with several applications. Enzymes are an excellent alternative to classical organic techniques in the selective transformation of complex molecules for several reasons. Their major advantages are the high specificity, decreased side reactions and most of them react at ambient conditions. The recyclability of immobilized enzymes also makes them an attractive tool. In many cases the chemical and energy consumption is lower compared to conventional chemical conversions, since many industrially applied enzymes act under mild conditions and produce less toxic side streams from processes.[97] Moreover, the enzymatic modifications are less invasive than most chemical treatments.

Lipases and cutinases are enzymes that, among other activities, in the presence of water, hydrolyze ester bonds with different alkyl chain length specificities. Enzymes with such activity are often called esterases. Their reaction specificity is based on recognizing the alkyl chain participating in an ester bond. The lipases act on longer alkyl chains as substrate, while the cutinases also react with both longer and shorter alkyl chains. Some of these enzymes were shown to have activity on esterified monosaccharides, oligosaccharides and polysaccharides and derivatives.[82-84, 98]

It is possible to tune the reaction conditions such that enzymes catalyze the reactions in a chosen direction. For example, while the presence of water is essential in the hydrolysis reaction, esterification on various substrates has been demonstrated using esterases in non-aqueous or non-polar media, such as ionic liquids, organic solvents, acetonitrile, etc.[69, 83, 99-101] The enzymatic esterification of polysaccharides and their derivatives has come to the focus of research only in the last decade and until now has been limited to celluloses.[83, 84, 102, 103]

Outline of the Research

Structure-property relationships of acetylated arabinoxylans

- The effect of acetylation on the material properties of arabinoxylan films was investigated and compared to the native arabinoxylan (Paper I)
- Structure-property relationships were elucidated by studying the effect of arabinosyl substitution on the material properties of acetylated xylans (Paper II)

Green chemistry for the acetylation of xylans

- Acetylation in ionic liquids (Paper III)

  With the use of ionic liquids the challenge of the full acetylation of spruce xylans was addressed.
- Enzyme aided acylation in a solvent free reaction (paper IV)

  The enzymatic surface acylation targeted the preservation of the good oxygen barrier properties while hydrophobizing the surface of films.

Acetylated arabinoxylan – nanofibrillated cellulose composite films

- Paper V

  The fifth paper in this thesis targeted the demonstration of these acetylated xylans as a matrix for nanocellulose reinforced composite films. Thermal, mechanical and surrounding relative humidity related properties of these films were evaluated.

Future Work

- The possible continuation of this work is discussed by applying the new knowledge presented in this thesis in combination with other novel related findings in the field.

Chapter 3

Materials and methods

The most relevant starting materials and methods are discussed in this chapter.

Materials

Forestry and agriculture offer two major sources of xylans (and glucomannans) as renewable raw material. Xylans are non-food polysaccharides and thus an attractive and competitive resource for materials avoiding food ethical issues.

The starting material for the experiments using rye (Secale cereal L.) AX was purchased from Megazyme (Ireland) (~95 % purity, LOT 20601a). The arabinose to xylose ratio was 0.52 as determined by ion chromatography. The structure is shown in Figure 7 and is also described in the literature.[104] The molecular weight fluctuates between batches, and the weight average molecular weight was found to be between 150 000 – 232 000 g/mol.

The starting material for experiments using spruce GM and AGX were from the “Wallenberg spruce”, a single log of Norway spruce (Picea abies) dedicated to be a uniform raw material for the 5+5 year Wallenberg Wood Science Center projects.

The AGX was extracted from Norway spruce by alkali extraction based upon the methods published by Wise and Timell.[105-107] The resulting material obtained had a 1:2:11 ratio of A:GlcA:X sugars, respectively, as determined by NMR and supported by ion chromatography. The structure of the spruce xylan was elucidated through NMR, Figure 9 [105]. The weight average molecular weight was measured to be around 12000 ^{VI} and 35 000 g/mol ^{III}.

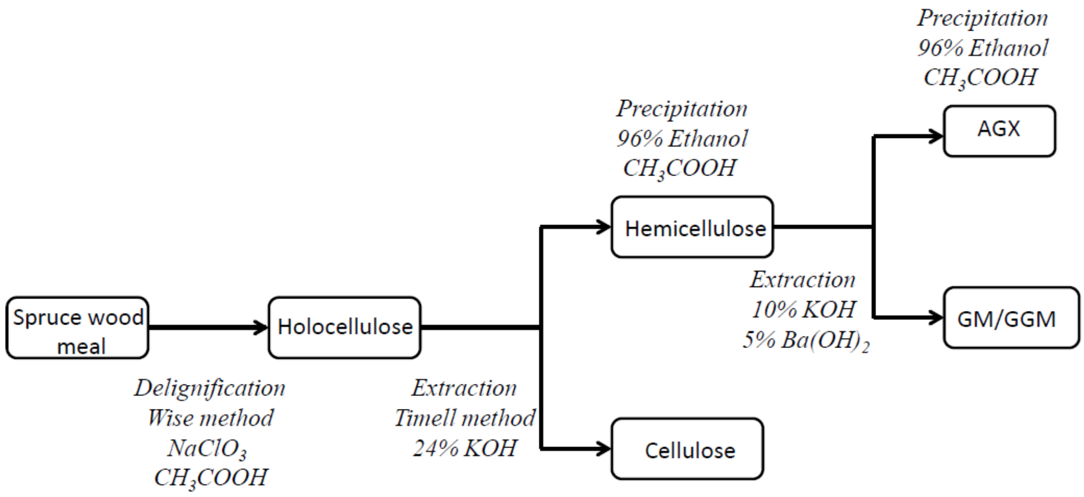

Figure 12 Scheme of AGX extraction

The IL 1-Ethyl-3-methylimidazolium dimethylphosphate ([emim][Me_2PO_4]) and 1,5-diazabicyclo[4.3.0]non-5-enium acetate ([DBNH][OAc]) were synthesized to high purity, as described below. 1-allyl-3-methylimidazolium chloride ([amim]Cl) was prepared according to a previous publication.[108]

The IL [DBNH][OAc] was synthesized from 1 g of DBN and 0.6 g of acetic acid (1:1 molar ratios).The IL ([emim][Me_2PO_4]) was synthesized as described elsewhere.[109]

Lipases were purchased from Sigma-Aldrich: lipase from *Candida rugosa* (L1754), lipase from *Rhizopus oryzae* (86012) and lipase from *Mucor javanicurs* (L8906). Cutinase from *Fusarium solani pisi* was produced as described earlier.[110]

The cellulose used for NFC preparation was from never-dried softwood sulfite pulp (13.8% hemicelluloses and 0.7% lignin, Nordic paper – Sweden).

All further chemicals were purchased from different providers; details can be found in the respective publications.

Experimental methods

This thesis contains five major papers and complimentary data from one further related publication which can be grouped according to their main goal. This is schematically presented in Figure 13.

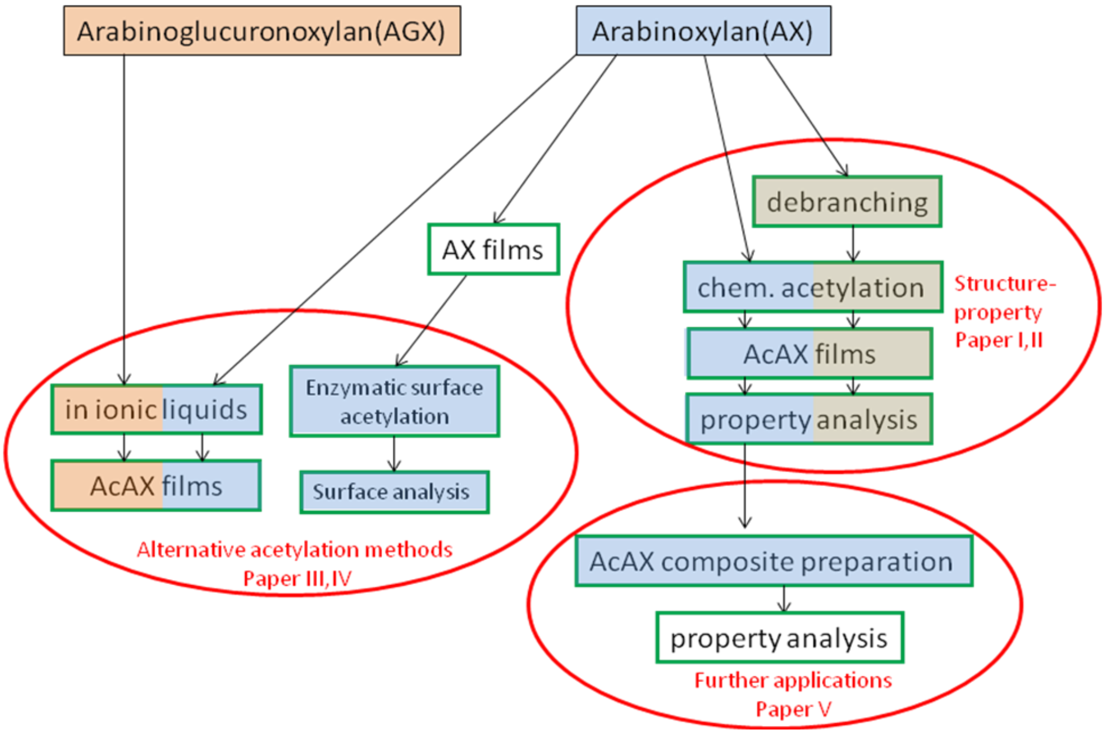

Figure 13 Scheme of materials and methods of the thesis, where color filling of boxes refers to the materials, the green frame refers to methods and the red circles refer to the aim of the work in the respective papers

Debranching AX

Chemical debranching was performed using protocols described by Sternemalm.[60] The different levels of debranching were done in parallel experiments using 1g of rye AX for each batch. Dry powder AX was added to 0.1 M oxalic acid water solution, and the reaction took place at 50 ºC for different periods of time. After precipitation in ethanol and drying, the samples were characterized and further derivatized. More information on this method can be found in paper II.

Acetylation

The first four papers of this thesis focus on the acetylation of xylans. The acetylation was achieved in three different ways. The first two papers use a classic chemical acetylation, which are common in the literature for acetylating polysaccharides.[81] The third paper uses ionic liquids as a potential green solvent as reaction media. The fourth paper focuses on a surface esterification of xylans catalyzed by enzymes.

Chemical acetylation

Esterification of arabinoxylan was carried out using experimental conditions based on the literature.[81] Dry AX samples were dispersed in formamide, pyridine was then added, and this was followed by a slow stepwise addition of acetic anhydride (Ac_2O). After a total of 30 hours, the reaction mixture was precipitated in excess of 2% ice-cold hydrochloric acid. The precipitate was filtered and washed with deionized water, methanol and diethyl-ether. The samples were then dried in vacuum at 40ºC overnight. Further details can be found in papers I and II.

Acetylation in ionic liquids

Dissolution and acetylation of rye and spruce xylans in [emim][Me_2PO_4]

Acetylation of both the rye AX and the spruce AGX was carried out in the same way in [emim][Me_2PO_4]. In these systems, the reaction was done under an Argon blanket. 5% of xylan was first dissolved in the IL, pyridine was then added as an acid scavenger, and then the reagent was added, which was acetyl chloride (AcCl). Chloroform was subsequently added as a cosolvent. After 5 minutes of mild heating (50 ºC), the reaction product was precipitated with water and ethanol. Further details can be found in paper III.

Dissolution and acetylation of rye and spruce xylans in [DBNH][OAc]

Acetylation of both the rye AX and the spruce AGX in [DBNH[OAc] was carried out as follows: 5% xylan was dissolved in the freshly synthesized IL [DBNH][OAc]. Additional DBN was then added and thoroughly mixed before the reagent, Ac_2O, was added. After 5 minutes at 50 ºC, the product was recovered by precipitation in ethanol and washed with water and ethanol. Further details can be found in paper III.

Enzymatic surface acylation

Rye AX films were cast from water as described above. Films were suspended in the treatment solutions; thus both sides of the film strips were treated. The treatment solutions were the enzyme (cutinase or lipase) suspensions in the reagent (vinyl acetate or vinyl stearate) kept on 40°C. Evaporation of the reagents was prevented by keeping the reaction systems closed. Separate batches were run for different times. The films were then washed with methanol and hexane. Blank samples were treated in pure vinyl acetate (VAc blank) or vinyl stearate (VSt blank) without enzymes. Untreated AX film (AX blank) served as double blank for both treated blank studies. Further details can be found in paper IV.

Preparation of films

Water and chloroform solutions were used to cast films from AX or AcAX, respectively. AX films were cast from 2% water solutions into plastic petri dishes and left to dry at 50% relative humidity (RH). AcAX films were cast from 2% chloroform solutions into glass petri dishes and left to dry at ambient conditions. The films were covered with a perforated aluminum foil to avoid dust accumulation on the films while drying.

Preparation of the NFC

NFC was prepared from never-dried softwood sulfite pulp according to a previously reported method.[111] The process included enzymatic pretreatment and mechanical beating of pulp followed by eight passes through a Microfluidizer (Microfluidics, USA), resulting in a gel like suspension with ca. 2 wt% NFC in water.

Composite preparation

Solvent exchanging the water suspended NFC to DMF

The NFC-water gel was diluted ten times with DMF and the suspension thoroughly homogenized in an Ultraturrax (model D125 Basic. IKA, Germany). The suspension was then centrifuged. This process was repeated four times to ensure complete exchange from water to DMF. Further details can be found in paper V.

Mixing components

Films were prepared with different NFC contents. AcAX was weighed dry, and the NFC was weighed in a suspension in DMF. The samples were mechanically stirred and Ultraturraxed until homogeneity. The samples were then cast in glass petri dishes and degassed, followed by drying steps. These films were stored in a conditioning room with 50 % relative humidity (RH) and 23 ºC for two days. These composite films were then the subject of further analysis. Further details can be found in paper V.

Analytical methods

Carbohydrate analysis

The sugar composition of polysaccharides can be determined by ion exchange chromatography once the polymer is broken down into its monomers. Sulphuric acid hydrolysis was adequate to break the linkages between arabinose and xylose without any further degradation or side reactions. The insoluble fraction remaining after the hydrolysis is Klason lignin, which can be determined gravimetrically. This method is suitable for determining neutral sugars but not acidic sugars, such as 4-O-MeGlcA. Samples containing such monomers are more efficiently analyzed with other methods (papers I and II).

Structure analysis

Nuclear magnetic resonance (NMR)

One of the most efficient ways to study polymer structure today is by NMR. The method consists of aligning the magnetic nuclear spins (polarizing) in a static magnetic field exposing the nuclei to a second oscillating magnetic field. The nuclei absorb and re-emit electromagnetic radiation that is characteristic of each nuclei and its surrounding. ^{1}H-NMR is the most commonly used technique, often combined with COSY, TOCSY and HMQC to obtain more detailed information on structure and linkages (paper II). The degree of acetylation can be determined based on the total integral of sugar peaks and the acetyl peak (papers II and III).

Furier transform Infrared (FTIR)

When a sample is irradiated by infrared radiation, some of the radiation will be absorbed by the material and some will be transmitted. The energy of the absorbed light is equivalent to certain rotational and vibrational energies of chemical groups in the sample. The resulting transmission spectrum is obtained as a function of the wave number, which is like a fingerprint for every compound (paper IV).

Attenuated total reflectance (ATR) IR is a type of IR used for surface analysis. It is based on the total reflection of the electromagnetic wave on the samples surface, and a so called evanescent beam is absorbed in the material (papers II and III).

Molecular weight determination (SEC)

In size exclusion chromatography, the molecules are separated on the basis of their hydrodynamic volume in a certain eluent by filtration through a gel, which has pores with a specific size distribution. The most common eluent for native polysaccharides is water based and, depending on the type of polysaccharide derivative, DMSO or DMA based ones can also be used. In paper III, the pullulan was the calibrating standard for both water and DMSO systems, which is the most commonly used standard for estimating the molecular weight of xylans.

Surface characterization

Electron spectroscopy for chemical analysis (ESCA)

ESCA is often also referred to as X-ray photoelectron spectroscopy (XPS), which is a quantitative surface analytical spectroscopic technique that measures the elemental composition of a substance surface. The method uses X-ray irradiation on the sample, which results in emission of electrons from an inner electron shell of a surface atom. Based on the difference in the energy of the irradiating photon and emitted electron, the binding energies can be calculated. The emitted electron’s kinetic energy is characteristic for the atom and its surrounding. The photo-emitted electrons originate from the top few nanometers of the sample surface.

In the case of xylans, the most relevant atoms are oxygen and carbon. The carbon peak can be divided into four sub-peaks, which correspond to carbons at different oxidation levels.[112] In xylans, these peaks refer to certain bonds and groups formed with oxygen: C1 is for C-C bond; C2 is for C-O- ; C3 is either for C=O or O-C-O; C4 is for COO-. (paper IV).

Thermal and mechanical properties

Tensile tests

The mechanical properties of the films produced were measured with a tensile test machine. Samples were cut into strips, and the thickness was measured on several places and averaged for each specimen (details are given in papers I, II and V). The cross section area and elongation of the films were calculated by software based on the measured dimensions. The straining method was either stretching at a constant rate or at a constant stretch rate. Papers I and V deal with materials also containing components in the samples that are sensitive to humidity.

Dynamic mechanical analysis (DMA)

Dynamic mechanical analysis is most commonly used to measure the viscoelastic properties of polymers. This measurement was used to determine the glass transition temperatures (Tg) of the films by studying the changes in modulus as a function of temperature. The sample is subjected to a sinusoidal strain, with a preset amplitude. The storage modulus, E’, and loss modulus, E’’, can be calculated from the stress-strain relations. When E’ and E’’ are plotted versus the temperature, the storage modulus decreases and the loss tangent shows a peak at the glass transition. Papers I, II and V contain further details on the experimental set-ups.

Interaction with water

Dynamic mechanical testing in changing relative humidity

Papers I and V have moisture sensitive samples and components. Absorbed water can soften the materials, thus affecting the modulus of a material. It is important to study these materials by DMA by varying the relative humidity (RH) and keeping the temperature, frequency and amplitude of the induced strain constant. Similar to the DMA, the E’ and E’’ can be plotted, in this case, against the surrounding relative humidity and a softening can be observed for water sensitive samples. Paper V discusses this method in more detail.

Water vapour sorption

Moisture sorption isotherms were measured gravimetrically and expressed as the percent of water of the total weight of the sample. Different humidity conditions were created in airtight vessels, where the samples were stored until they reached equilibrium. The relative humidities in the vessels were set by using saturated salt solutions, which have specific water vapor pressure above the solution according to ASTM E104-85. The pre-dried samples were first conditioned at the lowest RH and then moved successively to the highest RH vessel, conditioning the samples in each vessel until equilibrium.

Water permeability

Water permeability is a measure of the passage of water through a material. Permeability measurements were performed in diffusion cells using ^{3}[H]-labelled water as a diffusing tracer. Composite films were placed between the donor and acceptor chamber, where the donor chamber contained the labeled water. The acceptor chamber was sampled at certain times. Scintillation liquid was added to the samples, and the radioactivity of the samples was analyzed with a scintillation analyzer (paper V).

Contact angle

The static contact angle of a material measured after a specific time of contact can provide information about the water resistance of the material. This was used in papers I and II to describe interactions of the native and acetylated xylan towards water. Paper IV measured the advancing contact angle of the surface acylated materials, which means that the contact angle was measured immediately after the contact of the sample and water.

Oxygen permeability (OP)

OP, or often termed the oxygen transmission rate (OTR), expresses the ability of the films to let oxygen pass through by diffusion. A Systech 8001 permeation analyzer from Systech instruments was used. The films were mounted in a diffusion cell, where the oxygen was removed by nitrogen from the inner side of the cell, and oxygen was supplied to the outer. Oxygen permeates the test material and is picked up by nitrogen gas flowing through the inner half. The amount of oxygen carried by the carrier gas is measured using an oxygen sensor to determine the oxygen transmission rate. The temperature and relative humidity of the test conditions were 25 ºC and 50% RH. The final transmission rate was recorded when a stable duplicate samples were measured. The area of the samples was 5 cm^2.

X-ray diffraction

The crystallinity of the xylan films was determined using a Siemens D5000 goniometric diffractometer (Munich, Germany). The crystallinity or amorphousness of a material can provide an explanation for several phenomena related to the materials, such as thermal transitions, solubilities and mechanical properties. The film was placed in goniometer using a sample holder (with a 3-cm diameter). Diffractograms were recorded in the reflection geometry using Cu Kα radiation with a wavelength of 1.54Å. The 2θ was varied between 10º and 40º at a rate of 0.5º (2θ) per minute, with a step size of 0.1 (2θ) and the divergence split fixed to 0.5 mm.

Chapter 4

Results and Discussion

This chapter summarizes the most important findings of the research, including the results of papers I to V, some unpublished data and some data from other published work not included but related to the thesis (papers VI-VII).

Structure property relationships of acetylated AX

Effect of acetylation on material properties of AX

The characterization of the material properties of xylan acetates is of critical importance because xylans from different plants have different structures and properties. Two different annual plant (rye and corn cob) xylans were fully acetylated. The thermal, mechanical and humidity dependent film properties were evaluated. Paper I reports that acetylation improved the thermal, mechanical properties along with the water resistance of the films. Paper II supports the findings of paper I by presenting similar results based on a different raw material (rye AX) and elaborates further on the structure property relationships of acetylated AX.

Acetylation of polysaccharides increases the mobility of the chains with respect to each other, since the acetyl groups prevent tight packing of the polymer chains and the formation of strong hydrogen bonds. This theory is also supported by

X-ray diffractograms previously reported in the literature that show the acetylated xylans to be amorphous.[55] However, it is important to note that several hemicelluloses are already amorphous in their native state due to the number and distribution of side chains.

In paper I, CCAX was extracted and purified by two major purification methods: washing (W) and bleaching (B). Both washed and bleached samples were acetylated, but only the bleached sample could be fully acetylated, as shown in the FT-IR spectra in Figure 14 by the total disappearance of the hydroxyl peak around 3100-3700 cm^{-1}. The incomplete acetylation of the washed sample might be due to a higher residual lignin content that promotes formation of supramolecular structures that hinder the acetylation reaction. The poor solubility of the WA in chloroform, and thus the poor film quality in addition to its yellowish appearance, made it unsuitable for further film characterization. Therefore, only the bleached acetylated sample (BA) was subject to further material property analysis. The full acetylation of BA was confirmed by H^1-NMR by comparing the integrals of the anomeric protons of the xylan and the protons of the methyl group in the acetyl substitution.

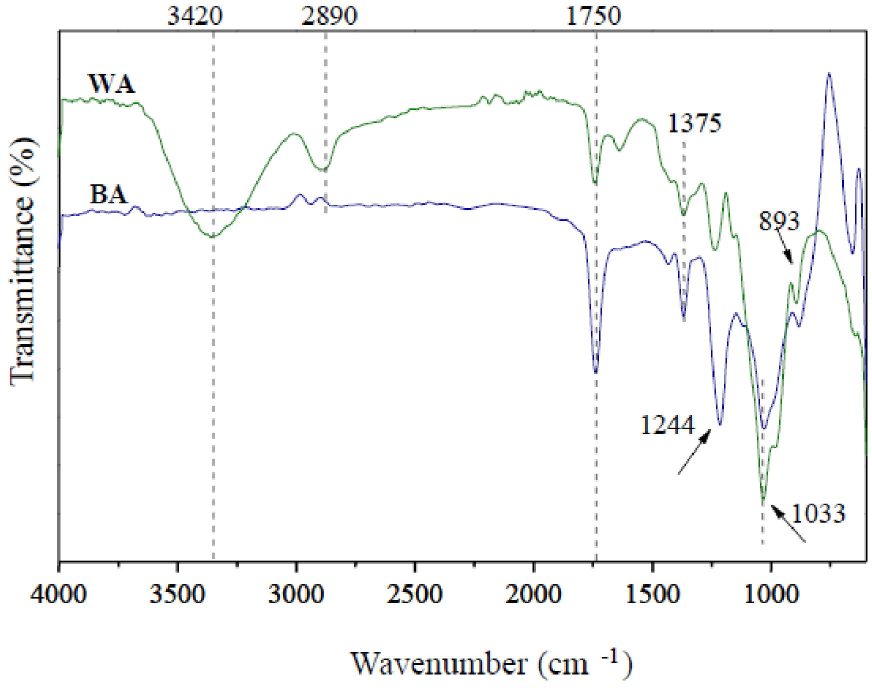

Figure 14 FT-IR of acetylated CCAX after washing (WA) and after bleaching (BA) showing full acetylation for BA and incomplete acetylation for WA

It has been shown before for cellulose and glucuronoxylan that acetylation improves the thermal stability of the polysaccharide, and a more distinct glass transition can be detected for the material with an increasing degree of acetylation.[64] The increase in thermal stability is shown in Figure 15, where we can see the thermal degradation temperature increase from 210 ºC to 325ºC. This is more than a hundred degrees increase in the thermal stability of the bleached sample after acetylation.

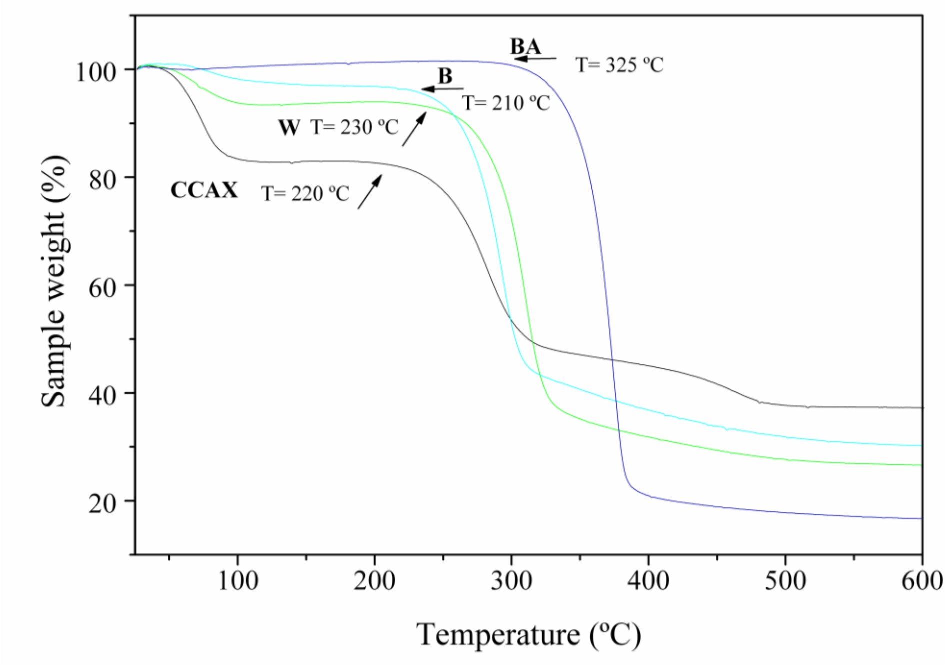

Figure 15 TGA of CCAX, washed (W), bleached (B), and bleached acetylated (BA)

Structural changes in polymers inevitably also cause changes in mechanical properties. Films were cast from water in the case of the native xylans and from chloroform in the case of the acetylated xylan to evaluate further material properties. For films cast from BA, the tensile strength and the Young´s Modulus improved dramatically compared to the purified non-derivatized films, while the tensile strain at break also increased up to 13 %. The tensile strength and Young´s Modulus increased about seven fold in a comparison of the crude CCAX to the BA, reaching 67 MPa and 2240 MPa, respectively (Table 1).

Table 1 Mechanical properties of films

Sample	Stress (MPa)	Strain (%)	Young's modulus (MPa)
CCAX	9.02 ± 0.74	8.10 ± 2.5	293 ± 90
W	53.54 ± 12.8	7.08 ± 1.31	1662 ± 268
B	53.56 ± 4.66	8.17 ± 0.93	1432 ± 437
BA	67.30 ± 12.18	13.4 ± 1.55	2241 ± 353

Water interaction of acetylated xylans

The improved mechanical properties undoubtedly broaden potential applications. However, many biobased plastics are applied in the food or related industries, where moisture resistance is crucial. It is therefore of key importance to recognize the importance of the increased contact angle of the acetylated xylan films towards water. The extracted CCAX had an advancing contact angle of 59º and was eventually water soluble. The purified CCAX already had an increased contact angle towards water at around 78º, but still dissolved with time. The acetylated film had a contact angle above 80º and was water resistant, meaning that it did not dissolve with time (Table 2).

Table 2 Contact angles of films against water

Sample	**Contact angle (º)**	
	t= 0 sec.	**t= 45 sec.**
CCAX	59.0 ± 5.29	21.3 ± 1.68
W	78.8 ± 3.0	67.9 ± 5.0
B	77.6 ± 4.9	74.3 ± 3.9
BA	82.1 ± 5.96	80.5 ± 5.80

This water resistance leads to an important measurement, where the DMA scans show the dependence of the modulus on the surrounding relative humidity. Figure 16 represents the relative storage modulus as the function of RH for native bleached xylan and the bleached-acetylated xylan. It is clear that the native, unmodified, bleached sample still had a modulus that was highly dependent on the surrounding RH, whereas the storage modulus became practically independent after acetylation.

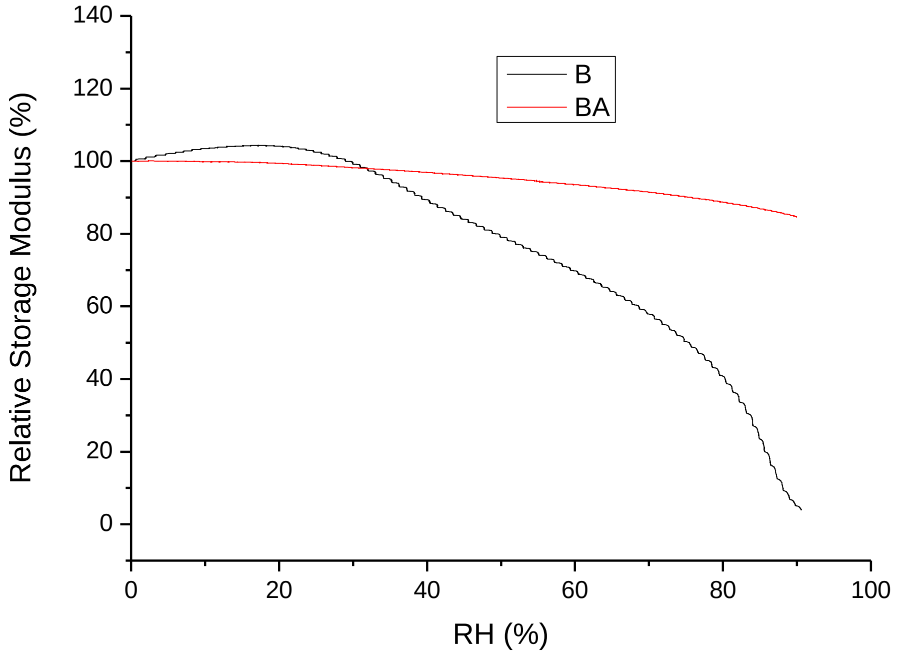

Figure 16 Relative storage modulus of bleached (B) and bleached-acetylated film (BA) against humidity

The second paper is a detailed study on the effect of arabinosyl substitution on the properties of acetylated arabinoxylan (AcAX).

Figure 17 shows an AcAX film from rye, cast from a chloroform solution, which was investigated for morphology, thermal and mechanical properties, along with oxygen and water permeability, which are elaborated in papers II and V.

Figure 17 Rye AcAX film cast from chloroform

Morphology of AcAX films

The lack of crystallinity of the rye AcAX samples was confirmed by XRD, as shown in Figure 18, where no peaks of crystalline regions are visible.

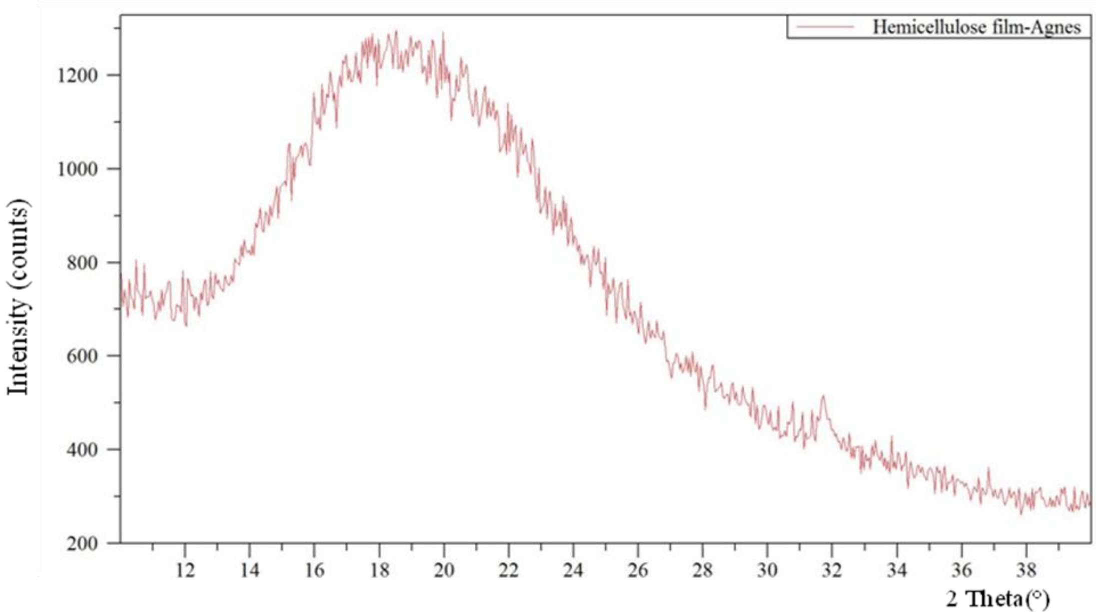

Figure 18 XRD of AcAX from rye

Effect of side groups on material properties of acetylated rye AX

Paper II presents the effect of the amount of arabinose side groups on the properties of AcAX from rye. Rye AX was debranched gradually with mild acid hydrolysis using dilute oxalic acid and different treatment times. The sugar compositions of the native and the debranched samples are shown in Table 3.

Table 3 Sugar composition of native and debranched AX based on HPAEC results

Sample Name	Treatment time	A/X
NDB	0 min	0,52
DB1	7 h 20 min	0,41
DB2	12 h 40min	0,33
DB3	17 h 59 min	0,27

These samples were subsequently acetylated with the same method as reported in paper I. The full acetylation of the native and debranched samples was confirmed by HSQC, 2D-NMR. Films were cast from chloroform solutions and tested for thermal and mechanical properties. The most relevant findings of these measurements reflected the arabinose side groups as acting as an internal plasticizer (Figure 19 and Figure 20).

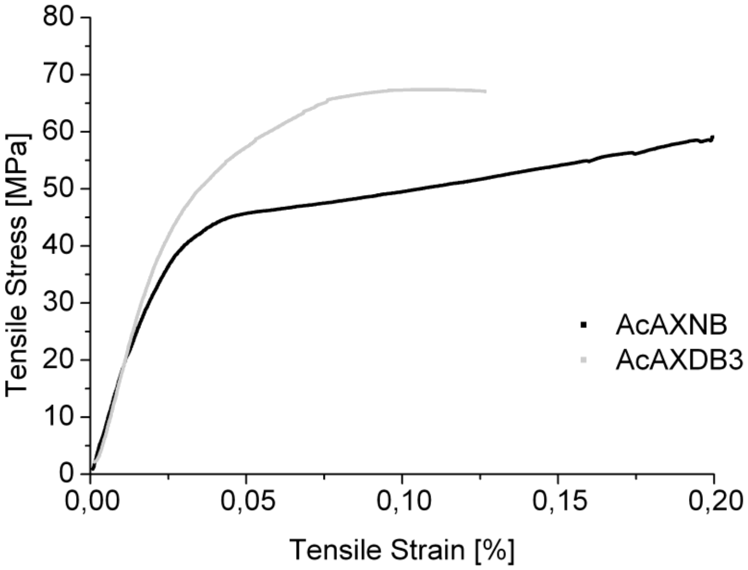

Figure 19 Effect of arabinosyl substitution on the tensile properties of AcAX films

As the arabinose content of the sample decreased, so did the strain at break, as seen in Figure 20. The stiffness of the samples showed a slight trend, as the Young´s Modulus was lower for higher arabinose substitution levels, but the differences were not significant due to the overlapping standard deviations of the measurements. Neither was the strength of the samples significantly affected by the debranching of the arabinose side groups.

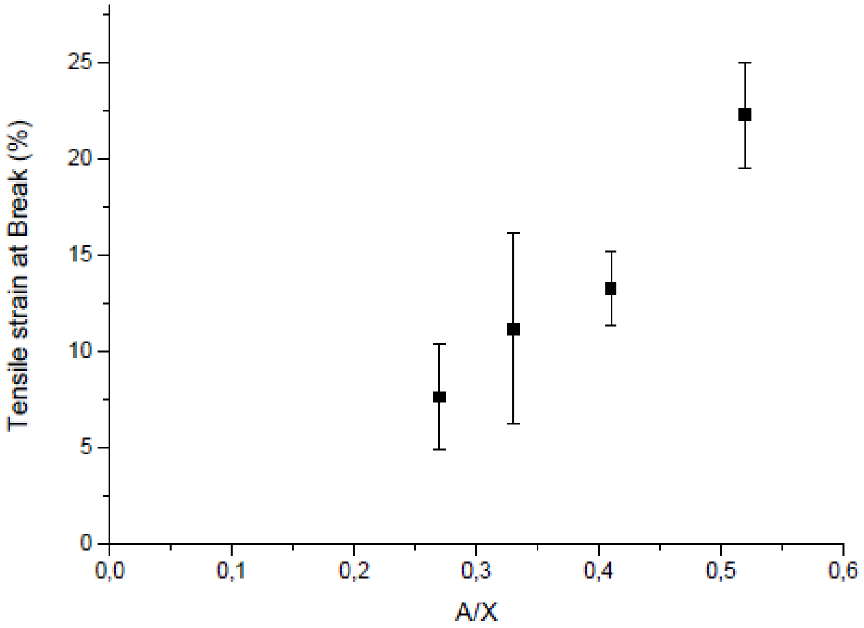

Figure 20 Strain at break for AcAX with different amounts of arabinose side groups

Running a temperature scan, the DMA was used to determine the glass transition temperatures (Tg) of the samples. The results showed a clear correlation between the amount of side groups and Tg. Figure 21 shows the lower Tg of the samples with a higher arabinose substitution.

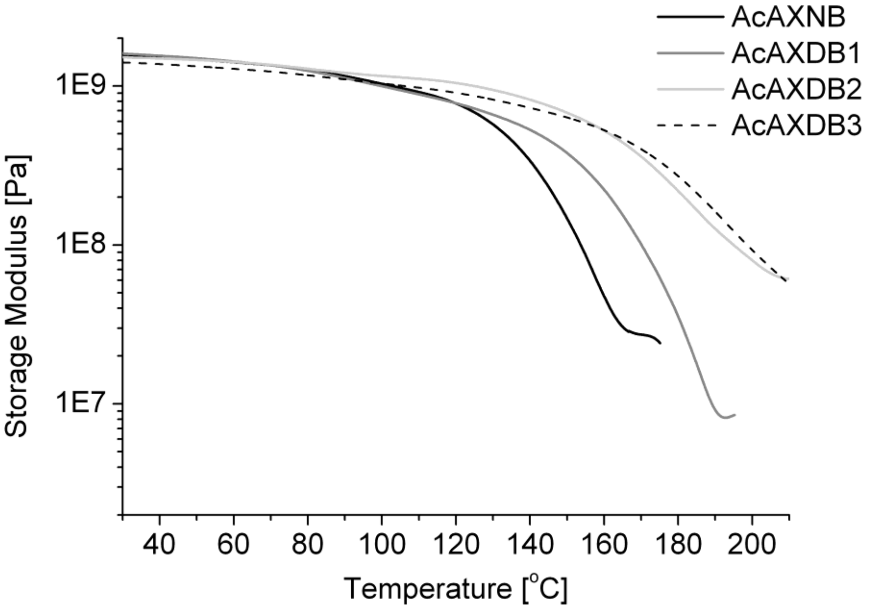

Figure 21 Effect of arabinosyl substitution on the storage modulus of acetylated AX films

The addition of plasticizers has a reported effect of lowering transition temperatures in polysaccharides.[113-115] The measured properties of AcAX with different amounts of arabinose substitution thus correlate well with the assumption of acting as internal plasticizers.

After having discussed the material properties of the acetylated rye AX and acetylated CCAX separately, it is also important to discuss the structural differences between the CCAX and the rye AX. These can lead to some differences in the material properties of the fully acetylated derivatives of the samples, but obvious correlations can be seen between measured properties: for example, it can be mentioned here that, in paper II, the AcAX from rye had a contact angle increase from the native to acetylated AX only from 54º to 66º, but still showed permanent water resistance by staying intact and unchanged when submerged in water just as the BA in paper I. The BA and AcAX also had similar tensile stress at break and Young´s Modulus, but the elongation to break was notably higher for AcAX. This is in good agreement with the model of debranched acetylated AX presenting a lower strain at break for a xylan with fewer substitutions. The most important structural differences are in the side chain substitutions of the native xylans. The bleached corn cob has substitutions of mixed arabinose and a small amount of glucose with a substituent to xylose ratio (A+Glc)/X of about 0.16, while the rye AX has an A/X ratio of about 0.5. It is important to mention that rye AX also has about a three to four times higher molecular weight than the extracted CCAX. This can possibly influence molecular entanglements as well.

When we compare the most debranched acetylated xylan (AcAXDB3), with an A/X ratio of 0.27, to the BA from corn cob, we find the strain at break for these two samples to be 7.5% and 13%, respectively. Although the AcAX has more substituents, these numbers are in a comparable range, and it is important to remember that different types of side groups and their mixture can also have a significant effect on material properties [VI], which are less known. Nevertheless, both cases represent a remarkable strain at break for an acetylated polysaccharide without additional plasticizers.

Oxygen permeability of AcAX films

Xylan films are known for their excellent oxygen barrier properties.[105, 113] This exceptional and attractive property is most likely due to morphology, hydrogen bonds that result in tight packing of the molecules where even the passage of O_2 molecules is extremely hindered.[113] When arabinoxylans are fully acetylated, the distance between the AX chains increases and no hydrogen bond between hydroxyl groups can be formed. This loosening of structure is one of the reasons for the increased flexibility and the lowered Tg and can result in a decrease of oxygen barrier properties.

This has been supported by measurements of the oxygen permeability of rye AX and AcAX films. The permeability increased about three orders of magnitude (from 4.91 to 1039 (cm^3 μm)/(m^2*day*kPa)) after acetylation, thus losing the advantageous oxygen barrier property in favor of mechanical and water resistant properties.

Table 4 Oxygen permeability of native and acetylated AX compared to other xylans and other common plastics in the field

Material	**OP** [(cm^3 μm)/(m^2*day*kPa)]	**condition**
AX (barley husk)	0.16	50% RH[105]
AX (rye)	4.91	50% RH
GX	0.21	35% sorbitol, 50% RH[113]
AGX	0.12	50% RH[105]
AcAX	1039	50% RH
PVA	0.21	50% RH[113]
EVOH	0.1-12	70% VOH, 0-95% RH[113]
LDPE	1870	50% RH[113]

LDPE is a typical non-barrier for oxygen and, unfortunately, the AcAX has similar scale values (Table 4). It is interesting to mention that native xylans from different sources also have different OP values. Most of them are under 1, but rye AX is 4.9 [(cm^3 μm)/(m^2*day*kPa)]. An industry rule-of-thumb is that a film is considered a "high oxygen barrier" if its OTR is less than 15.5 [cm^3 /(m^2*day)]. Native rye AX can still perform as a high oxygen barrier film if the film has a minimum thickness of 32 µm. However, xylans from barley husk, aspen and spruce can be applied in thinner coatings to qualify for this criterion.

As a summary, Table 5 compares the properties of native AX from annual plants and after acetylation. It is clear that, for bio-based film applications, several material properties have been improved, especially thermal and mechanical properties. However, it is important to be aware of poorer oxygen barrier properties if the aim is to produce food packaging.

Table 5 Comparison of properties of AX versus AcAX

Material property	**Arabinoxylan (AX)**	**Acetylated arabinoxylan (AcAX)**
Thermal stability	Low	Improved
Thermoplasticity	None	Heat processable
Solubility	Water	Apolar or aprotic solvents
Film forming ability	Excellent	Excellent
Strength	Good	Good
Stiffness	Good	Improved
Flexibility	Good	Excellent
Contact angle to water	Low; eventual dissolution	Higher
Moisture sorption	High	Low
Water permeability	n.a.	in range of other bioplastics
Oxygen barrier properties	Good/Excellent	Low

When considering the fields of application of AcAX among other bioplastics, the most obvious and logical is to use cellulose acetates as a guide. Table 6 reveals that most of the material properties of AcAX are comparable or superior to cellulose acetates with DS between 2.5 and 3. The properties of poly(lactic acid) (PLA) are also listed in Table 6. PLA it is another major bio-based plastic which has a well established market. The CA and PLA together can provide guidance towards potential fields of applications.

Table 6 Properties of cellulose acetates compared to xylan acetate

Material property	PLA	CA (2,5 ≤ DS ≤ 3)	Xylan acetate
Strength	~48MPa	~48 MPa	~61 MPa
Young´s Modulus	2.7GPa	1.9 GPa	2.1 GPa
Elongation to break	5-10%	2.6%	22.3%
Contact angle (water)	75º	50-55 º	~66º
Moisture uptake in water or at 97% RH	≤1%(moisture)	~5% (water)	~6% (moisture)
Transparency	high	High	High
Crystallinity	37%	25-30%	None
Tg	60-65 ºC	120 ºC	~120-130 ºC
Tm	173-178 ºC	Not apparent enough	Not apparent enough

Different acetylation methods

The WWSC targeted wood as a raw material. Until wood xylans in the project were available, annual plant arabinoxylans were used as a model compound in papers I and II. Once the spruce AGX was isolated and characterized, the same acetylation methods were applied to it as to the annual plant arabinoxylans. The products were colored and not soluble in chloroform, however, most possibly due to the significant amount of glucuronic acid side groups. After using different approaches to acetylate spruce AGX, we turned to the use of ionic liquids, which are a novel green solvent for polysaccharide modification.

Acetylation in ionic liquids

Ionic liquids have been shown to be efficient and a greener approach to modifying polysaccharides. Ionic liquids are claimed to be more environmentally friendly than several other solvents used today because of their low vapor pressure, non-flammable properties and a potential for high recyclability rates. However, up to today, in reality, the reuse of the most commonly used ILs is very limited owing to high energy demands and high costs of recycling. Paper III studied two novel ILs, ([emim][Me_2PO_4] and [DBN][OAc]), from which the DBN based IL has been distilled and recovered in good quality by Parvianen et al.[116]

The literature on cellulose modifications in ILs is vast, which is a significant source for designing new methods for hemicellulose modification. However, due to the complex structure and diversity of building blocks in hemicelluloses, full acetylation in ILs proved to be a challenge. Several publications discuss efficient esterification or optimization of highly substituted hemicellulose esters, but fully esterified hemicelluloses in a preserved form have not been published.[76, 83, 92, 93, 95, 117-119] Paper III presents two IL systems to acetylate rye AX and spruce AGX that maintain the high molecular weight of the hemicelluloses.

Table 7 Summary of different experimental set-ups tested to achieve full acetylation of hemicelluloses in ionic liquids

Ionic Liquid[a]	**Reagent**	**Reaction Temp. (°C)**	**Time**	**IR**[b]	**Product Color**	**Solubility in $CHCl_3$**[c]
-	Ac_2O*	50	20 h	partial	yellowish	-
[emim][OAc]	Ac_2O[d]	50	16 h	high	dark sand	-
	AcCl[d]	50	2 h	n.a.	dark	-
	AcCl[x]	50	5 min	full	dark brown	-
[amim]Cl	Ac_2O[d]	50	16 h	full	brick red	-
	Ac_2O	50	4 h	partial	white	-
	AcCl[x]	50	5 min	full	orange	-
[emim][Me_2PO_4]	Ac_2O[d]	50	16 h	high	brown/red	-
	AcCl[x]	50	5 min	full	white	+
	Ac_2O[x]	50	5 min	partial	white	-
[DBNH][OAc]	Ac_2O[x,y]	50	5 min	full	white	+
	Ac_2O[z]	50	5 min	partial	white	-

*ionic liquid-free acetylation: 1.5 % AX suspended in pyridine, then Ac_2O added (Molar ratio of pyridine to Ac2O was 4:3); [a] 2% rye AX was dissolved in IL on 80 ºC; [b] acetylation degree based on IR spectra; [c] + soluble; - not soluble; [d] additional pyridine (1:1 molar ratio compared to reagent); [x] the methods are described in detail in the methods section; [y] also performed with 5 % rye AX in the IL; [z] the DBN:Ac2O molar ratio was 5:4

The products were first evaluated by FT-IR. The studied xylans have about 2 OH groups per anhydro-sugar unit. These OH groups appear on an IR spectrum between 3000 and 3500cm^{-1} as a characteristic broad signal. In the case of full acetylation, all hydroxyl groups are replaced by acetyl groups, causing the hydroxyl peak to disappear and three distinct new signals to appear on the spectrum. A characteristic signal at 1740 cm^{-1} indicates the carbonyl group (C=O) in ester bonds, a signal around 1380 cm^{-1} is the stretch for the methyl group (CH3) in the acetyl group and a strong signal around 1215-1250 cm^{-1} results from the acetate stretch (CC(=O)-O). See an example in Figure 22.

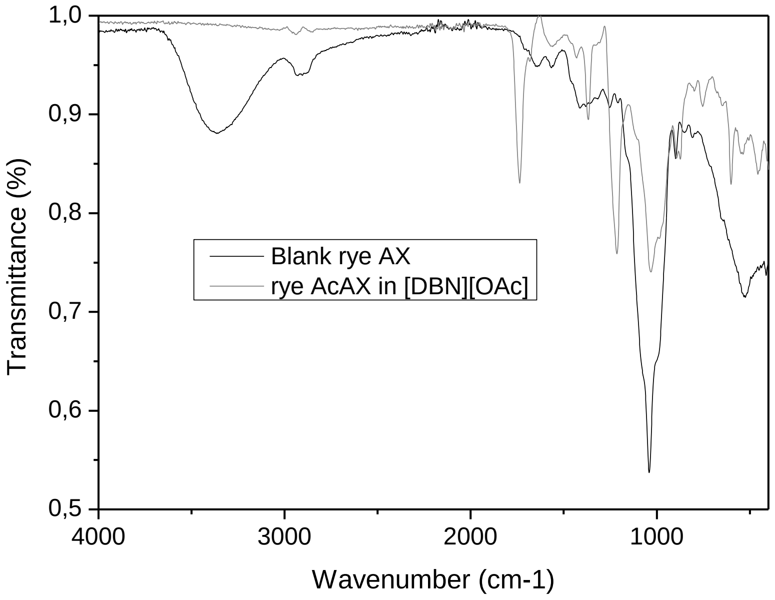

Figure 22 Native and the AcAX that was made in [DBN][OAc]

In addition to full acetylation, it is critical that the product is soluble in chloroform for further processing and has an appealing appearance in terms of being a white/colorless material. Solubility in chloroform indicates a high degree of acetylation, since a lower DS of acetylation enhances water solubility by acting as a spacer between the polymer molecules. At higher DS of acetylation, the lack of OH groups will result in the loss of water solubility and result in an increase in solubility in (aprotic and) non-polar solvents, such as $CHCl_3$.

The spruce AGX acetylated in [emim][Me_2PO_4], for example, was not soluble in chloroform but only in DMSO with heating, possibly indicating an incomplete substitution of OH groups with acetyl groups or cross-linking of the carboxyl groups.[120] Coloration of the product was an issue in some IL systems, resulting in abrown or reddish heavy powder, often not dissolving in chloroform despite the lack of the OH peak in the IR. These products were not evaluated further.

H1-NMR was performed on the products that fulfilled all three of the above mentioned criteria (full acetylation according to IR, white color, solubility in $CHCl_3$). The DS of acetylation was calculated by comparing the integrals of the signals of the acetyl groups (at 2ppm) and those of all carbohydrate signals (3.0-5.6 ppm).[55, 64],[II] Following equation (1), the DS can be calculated for acetylated AX and, following equation (2), for acetylated AGX:

(1) DS= (integral of acetyl group protons/3)/(integral of carbohydrate ring protons/6)

(2) DS= (integral of acetyl group protons/3)/(integral of carbohydrate ring protons/6.3)

Based on these formula, both rye AX and spruce AGX acetylated in [DBNH][OAc], showed a full DS, for rye AX in [emim][Me_2PO_4], practically complete acetylation was also achieved. The calculated percents correspond to DS values of 2, 2 and 1.96, respectively. The maximum DS for AX is 2 and for AGX 1.9.

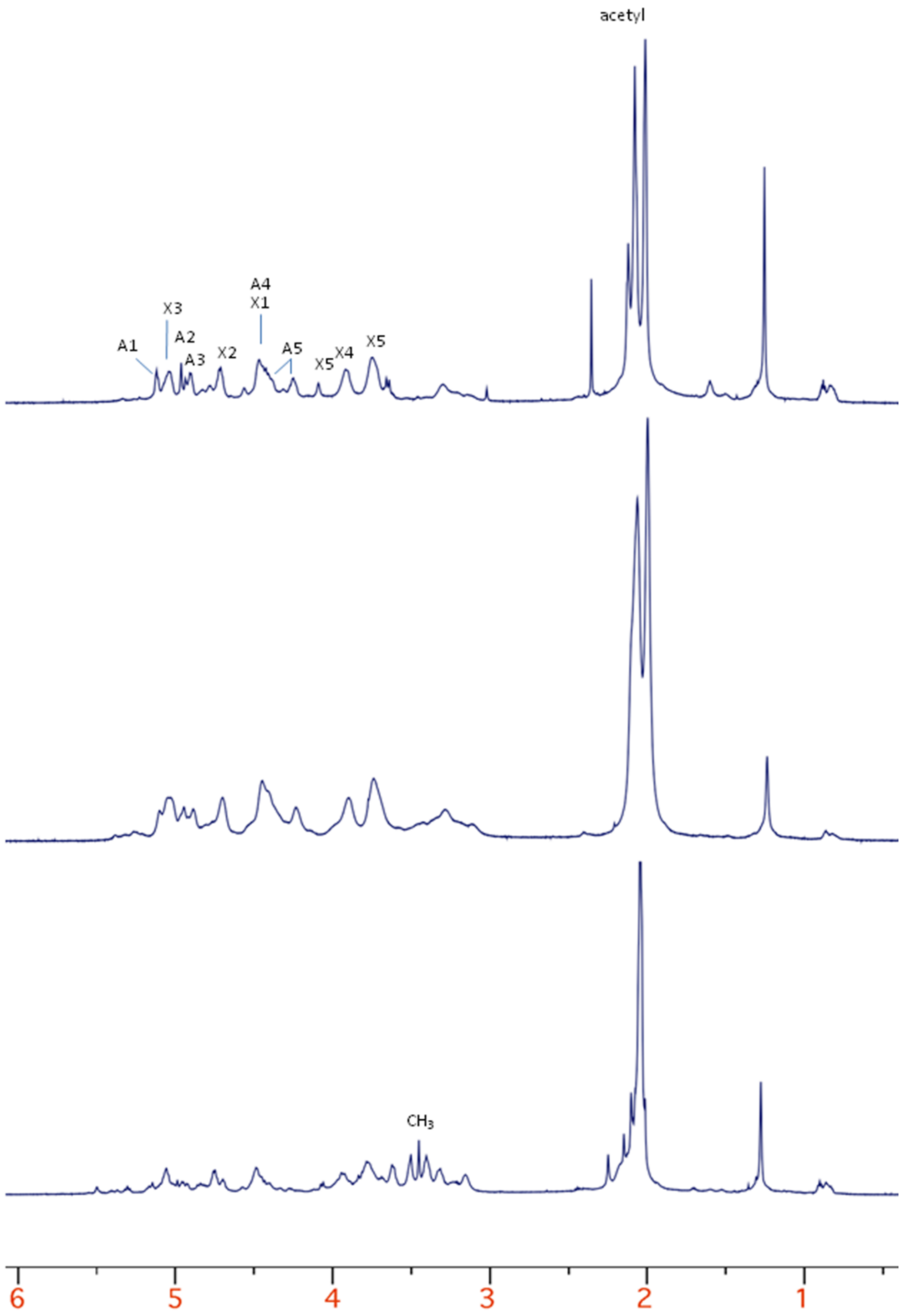

Figure 23 1H NMR Spectra in CDCl3 of acetylated rye AX in [emim][Me2PO4], [DBNH][OAc] and acetylated spruce AGX (from top to bottom)

The greatest achievement of this work was to show the acetylation of hemicelluloses in ILs without a significant molecular weight degradation, which was supported by a water and DMSO based SEC for the starting and derivatized materials, respectively (Table 8).

Table 8 DP of native xylan before acetylation and DP of acetylated xylans

Type of xylan	**^{a} native DPn**	**IL system used**	**^{b}DPn after acetylation**
rye AX	1140	[emim][Me2PO4]	n.a.
		[DBNH][OAc]	1199
spruce AGX	186	[emim][Me2PO4]	139
		[DBNH][OAc]	121

The success of these experimental setups is eventually believed to lie in the cosolvent system used or, in other words, the complete solubility of the starting material, the intermediate and the product. The addition of cosolvent during the reaction is crucial when hydrophobizing polysaccharides to resolubilize the partially substituted polymers, thus allowing for full derivatization according to Gericke et al. [90] It has also been shown that, in certain reaction systems, if cellulose is functionalized with hydrophobic functionalities, the product typically precipitates at a certain DS value and a variation in the dosage of reagents typically does not change the DS.[121] Precipitation in the solvent depends on the solubility of the polymer at a specific DS. If a co-solvent is added to those mixtures during the reactions, it is typical that one can drive the DS values to nearly 100 %.[90] These phenomena have been described with solvatochromic parameters, which can serve as a base for careful assumptions for the xylan-IL systems. It is clear that only strong hydrogen bond acceptors (β>0.8) ILs are able to dissolve cellulose. Table 9 presents the solvatocromic parameters of the ILs used in our study or ILs similar to them.

Table 9 Solvatochromic parameters of ILs and of co-solvents used in IL systems

Ionic liquid		**Solvatochromic parameters[#]**			**Reference**	
Cation	**Anion**	**E_T^N**	**α**	**β**	**π***	
$[emim]^+$	$[Me_2PO_4]^-$	n.a.	0.51	1.0	1.06	[88]
$[DBNH]^+$	$[CO_2Et]^-$	-	0.64	1.11	1.04	[122]
Molecular Solvents						
Pyridine		0.302	0.0	0.64	0.87	[123]
$CHCl_3$		0.259	0.2	0.10	0.58	[123]

[#]E_T^N: normalized empirical polarity; α: hydrogen bond donor ability; β: hydrogen bond acceptors ability; π: dipolarity/polarizability.*

Compounds with high normalized empirical polarity (ETN) > 0.3 are miscible with cellulose-IL solutions. As a guide, co-solvents with low hydrogen bond donor acidity ($\alpha < 0.5$) and high basicity ($\beta > 0.4$) can be added without causing precipitation.[89] Pyridine satisfies these criteria and is assumed to be a suitable co-solvent in the IL-hemicellulose system used (Table 7). For $CHCl_3$, the normalized empirical polarity (ETN) is close to the suggested value and the hydrogen bond donor acidity (α) value is in the suitable region.

The DBN system had a lower viscosity, which aids an easier homogenization of the reaction system. Beyond this, the byproduct of this reaction was more IL, which further diluted the system, ensuring even lower viscosities.

To highlight the potentials and relevance of conducting xylan modifications in IL systems, it is important to touch upon the literature on selective dissolution and fractionation of lignocellulosics in ILs.[108, 124, 125] Eventually, the possibility to combine fractionation and modification of xylans into a single system could decrease the number of processing steps and costs. A combined fractionation and derivatization step is also more likely to be implemented in the design of refinery concepts.

Acetylation with enzymes

Esterification of xylans with the aid of enzymes was another environmentally benign approach based on reasons listed in the introduction. Rye AX films were surface acetylated and stearated catalyzed by cutinases and lipases in a water-free reaction environment. Working in a non aqueous reaction system with these enzymes promote the esterification, compared to hydrolysis, which takes place in aqueous reaction mixtures. The vinyl acetate (VAc) and vinyl stearate (VSt) were the reagent and the non aqueous reaction media at the same time. Three lipases and the cutinase were tested with both reagents for surface acylation of AX films with conclusive results.

ESCA (XPS) was the primary tool used to characterize the surface composition of the blank and surface treated samples. The atomic concentration table showed the presence of nitrogen[IV] in the native rye, which is possibly from residual protein amino acids[22], which are known to migrate to the surface of polysaccharide films.[126, 127] This also gives an explanation to the presence of the C1 and C4 peaks in the native unmodified blank AX films spectrum, since these can be attributed to the alkyl chains and peptide bonds in proteins, respectively. The oxygen to carbon ratio is 0.57 instead of the theoretical 0.8, which also indicates the presence of non-polysaccharide components in the sample. Nevertheless, after surface esterification, the C4/C3 ratio increase will indicate new carbonyl groups from the ester bond on the surface, and the relative increase of the C1 peak compared to the other peaks will indicate the new, non-protein originated C-C bonds from the alkyl chains of the acetyl and stearate groups. This is clearly represented in Figure 24, where a native, untreated AX film´s surface is compared to the sample surface acetylated by cutinase from *Fusarium solani pisi*.

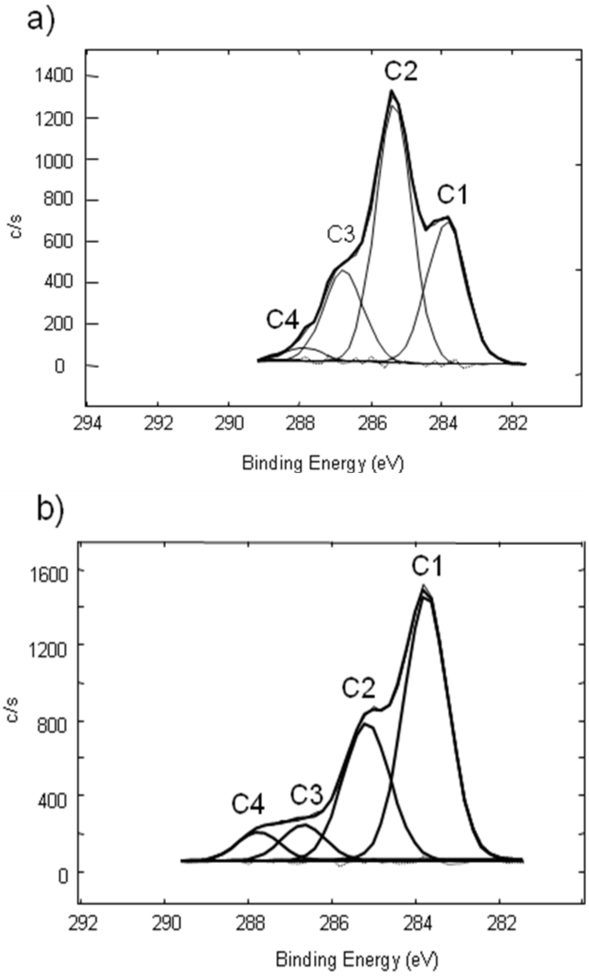

Figure 24 High resolution ESCA spectra of a) native rye AX and b) surface acetylated with cutinase

The major findings of this experiment series are summarized in Table 10. It is clear that the cutinase is more active in acetylation than stearation, and the lipases show activity in stearation only. No acetylation was found to be catalyzed by lipases compared to the blank film (VAc blank). These reaction specificities reflect the same behavior as is known for the hydrolysis reaction of these enzymes. The lipases have specificity towards fatty acids with a longer alkyl chain and cutinases having less preference and acting on fatty acids with shorter alkyl chains as well, but are active on a variety of fatty acid substrates. Cutinases also display the unique characteristic of being active regardless of the presence of an interface.[128, 129]

Table 10 a) Comparison of lipase and cutinase activity on vinyl acetate as substrate; b) Comparison of lipase and cutinase activity on vinyl stearate as substrate; based on the C4/C3 ratio from ESCA (where the C4/C3 for the untreated blank is 0.13)

a) **Enzyme**	**enzyme origin**	**Acetylation 3 days**	**Acetylation 7 days**
lipase from *Mucor javanicus*	(Sigma) L8906	0,13	0,23
	(Sigma) L1754	0,12	0,23
lipase from *Candida rugosa*			
lipase from *Rhizopus oryzae*	(Sigma) 86012	0,22	0,22
cutinase from *Fusarium solani pisi*	research enzyme	**0,80**	**0,80**
Respective treated VAc **blank**	none	0,21	0,26

b) **Enzyme**	**enzyme origin**	**Stearation 3 days**	**Stearation 7 days**
Lipase from *Mucor javanicus*	(Sigma) L1754	**0,22**	**0,34**
lipase from *Candida rugosa*	(Sigma) 86012	**0,18**	**0,34**
lipase from *Rhizopus oryzae*	(Sigma) L9031	**0,32**	**0,30**
cutinase from *Fusarium solani pisi*	research enzyme	**0,21**	n.a.
Respective treated VSt **blank**	none	0,12	0,14

The samples were further analyzed with FT-IR, where newly formed ester bonds and the alkyl chains of the alkyl groups from the acetyl and stearate groups were detected at their respective wavenumbers.

The relevance of this study lies in the changing of the hydrophilicity of the outermost layer of the surface of the AX films. The advancing contact angle against water at 1 second presented a clear increase for the successfully enzymatically surface acylated samples compared to their treated respective blanks. The samples were dissolved after a certain time under the droplet. Static contact angle measurements after a longer time would therefore not have given informative results on the surface hidrophilicity of the samples due to the eventual dissolution of the whole films under the droplet (Figure 25).

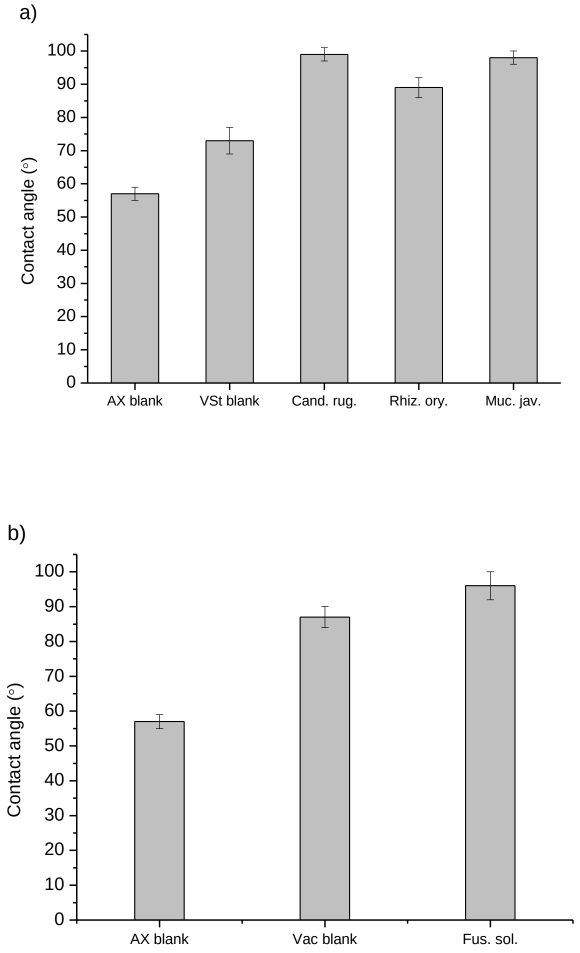

Figure 25 Contact angle against water: a) AX films' surface stearated with lipases and b) AX film's surface acetylated with cutinase

With time, the contact angle decreased significantly for all samples, and the films eventually dissolved under the droplet. This might be due to the acyl substitution achieved was a very thin monolayer, and the water induced molecular rearrangement of the surface.[130] The bulk of the film is still rich in hydroxyl groups, which are obviously more hydrophilic. Due to the molecular mobility of the surface, the more hydrophilic hydroxyl groups can turn towards the surface, come into contact with the aqueous layer and become hydrated, thus slowly allowing water to penetrate the whole film. However, the surface acylated films have a great potential in improved surface adhesion to more hydrophobic, water barrier films.

AcAX reinforced with NFC

In my previous work I suggest using AcAX as binder of matrix in composites. This leads the research to the fifth paper, where AcAX films were reinforced with nanofibrillated cellulose from spruce (NFC) to form composite films. The films were cast from a dimethyl formamide (DMF) solution, and no plasticizer was necessary for the formation of flexible, free standing films. The films were prepared with a gradient of NFC content and the thermal, mechanical and water interaction properties of the films were evaluated.

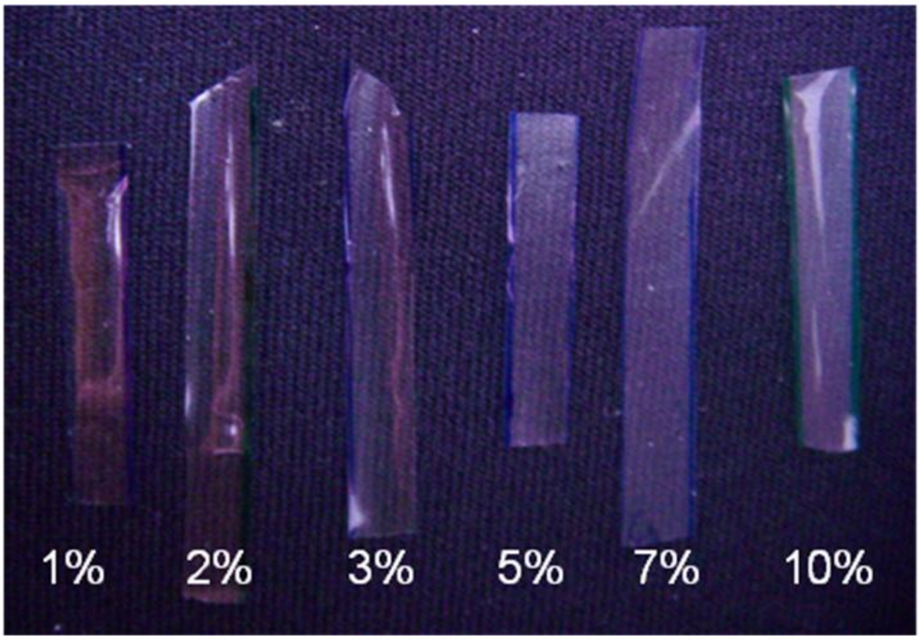

Figure 26 Photograph of NFC-reinforced AcAX films with 1-10 % NFC

No agglomeration was observed by the eye during the preparation of the films, and homogeneous films were obtained, where there was a decreased transparency for films with higher concentrations of NFC.

Measuring the individual surface energies of the composites components can provide useful information on the possible nature of dispersion of NFC in AcAX. The Owens, Wendt, Rabel and Kaelble method was used to determine the surface tensions and thier polar and dispersion components of AcAX, NFC and AX.[131] The calculations were made with the contact angles acquired from five different liquids against AcAX, NFC and AX films (Table 11).

Table 11 Surface tensions of the testing liquids and for AcAX and NFC

material	**Dispersive comp. (mJ/m^2)**	**Polar comp. (mJ/m^2)**	**Total surface tension (mJ/m^2)**	**R^2**
Ethylene glycol*	30.9	16.8	47.7	
Formamide*	39	19	58	
Diiodomethane*	50.8	0	50.8	
Glycerol*	37	26.4	63.4	
Water*	21.8	51	72.8	
AcAX	39.3	9.4	48.8	0.72
NFC	37.1	13	49.9	0.88
AX	35.8	13.8	49.6	0.82

*[132, 133]

The surface tension of a solid material is affected by several factors. Small differences in the surface tensions of materials will result in a low interfacial surface energy, which is a requirement for a high work of adhesion. This adhesion can be observed in composites, for example through the reinforcing effect of the fillers, since the high work of adhesion will facilitate a good stress transfer through the interfaces. A similarity in the surface tension of two components can also contribute to a prevention of agglomeration of the individual components, facilitating good dispersion of the NFC phase in the AcAX solution.

One of the main goals of this work was to improve the mechanical properties of the AcAX films. The films became stiffer with the addition of NFC to the AcAX, and the stiffness further increased with increasing NFC content. Although the strain to failure decreased upon an increasing NFC content, the film with 10 wt% NFC broke at 13% strain, which is quite remarkable in comparison to previously reported work with similar nanocomposites.[114, 134-136] The stress-strain behavior of the composites is summarized in Figure 27. It is clear that the addition of 1% NFC improved the overall strength of the material and, notably, the elongation at break was comparable to the neat AcAX film.

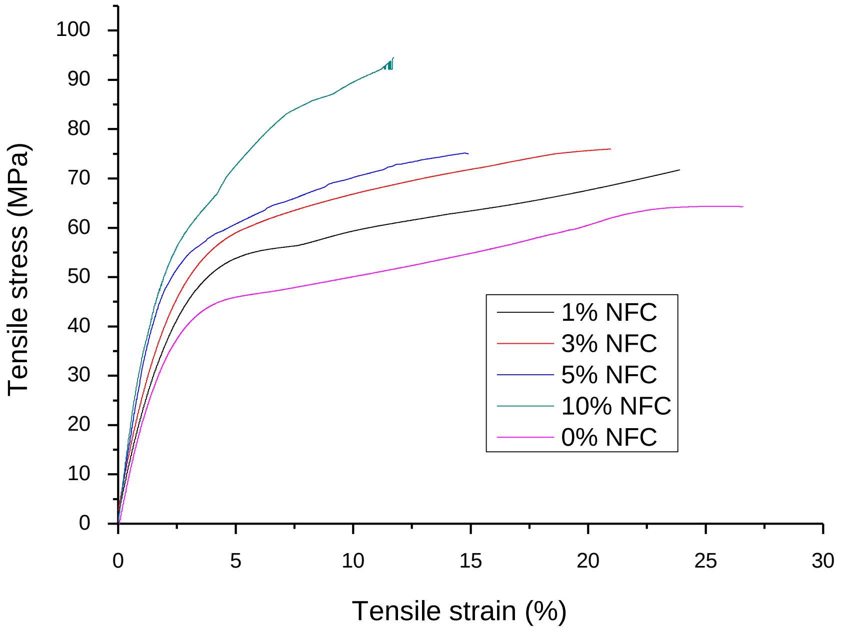

Figure 27 The stress-strain behavior of AcAX/NFC composites with 0-10% NFC

DMA scans were performed on the films in order to determine the dependence of material properties on temperature.

The addition of NFC to AcAX significantly increases the glassy storage modulus of the films from about 3000 MPa to 3900 MPa. It is interesting to note that the NFC not only increases the storage modulus but also delays the onset of the rubbery transition and slows the drop in storage modulus (slope of the curve after transition starts). The films with 5 and 10% NFC were strong enough to exhibit the glass transition as well as melting, while the films with a lower content (1, 2 and 3%) failed during the transition to the rubbery phase. The greater stability of the high NFC content films may be attributed to the formation of a percolating network of the fibrils, which is estimated to happen somewhere between 1 and 5% NFC for similar reinforced systems.[137] According to the percolation theory, there is a critical concentration of entanglement of the rods/fibers. The critical concentration is when there is more than one connection with another rod/fiber in the system. This is when network reinforcing phenomena start to take effect.

The glass transition temperatures (T_g),which were determined by the peak of loss modulus curves, only displayed a dramatic increase in the Tg between the neat and the composite films (from 125°C to 139°C). The subsequent increase in NFC content did not seem to have any significant impact on the T_g and it stayed approximately the same (around 140°C).

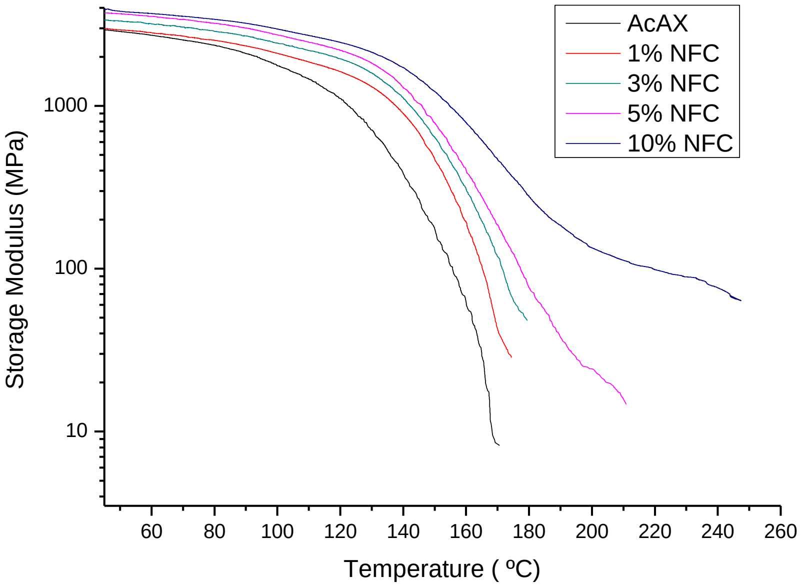

Figure 28 DMTA – Storage modulus of neat and NFC reinforced AcAX films as a function of the temperature

While the AcAX matrix is stable against water, with a moisture uptake of about 6 % in 97% RH, the NFC on the other hand (depending on the preparation methods) can hold up to 25% moisture at the same conditions. Eventually it was concluded that 10% of NFC did not significantly influence the moisture sorption properties of AcAX films. All the films presented a moisture sorption curve shape categorized as type III isotherm according to IUPAC classifications. Type III is described as hydrophobic or low hydrophilic material with weak sorbent–water interactions. This type of isotherm is also what the neat AcAX film presents.[138]

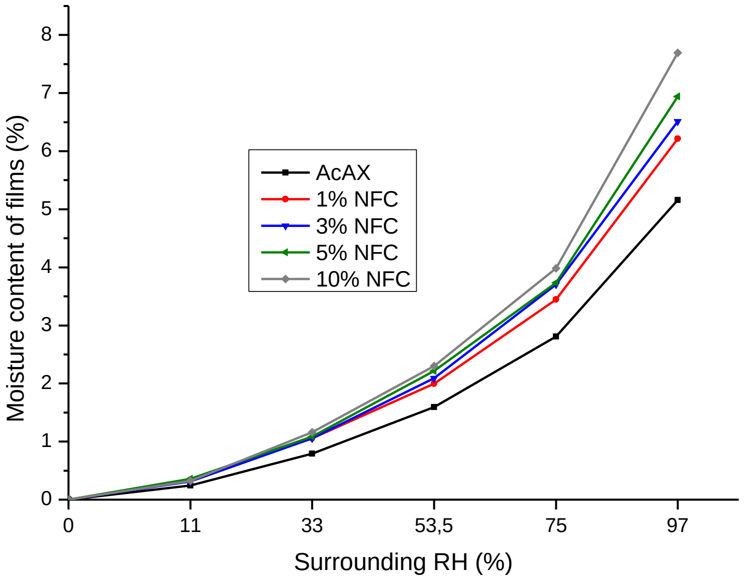

Figure 29 Moisture sorption isotherms of AcAX and AcAX films reinforced with NFC

The water permeability of films was measured since it is also crucial property in packaging industries. The films containing 1 and 3% NFC had a significantly lower average permeability value than the neat film. The permeability of the films with 1, 3 and 5% NFC was also significantly lower than the 10% NFC reinforced film. At low NFC content, fibrils may decrease swelling due to their reinforcement effect and thus decrease permeability. At high NFC content, it is possible that liquid water may diffuse more rapidly along the path of the percolated NFC network, and permeability increases. This speculation with respect to molecular NFC/AcAX interaction is supported by the strong increase in AcAX Tg observed at low NFC content. A similar phenomenon has been observed with MFC reinforced poly(lactic acid) (PLA), polyhydroxybutyrate-co-valerate (PHBV) and polycaprolactones (PCL) films.[139] In the work of Sanchez-Garcia and co-workers, the PHBV and PLC composites with 1, 2, 4 and 5% MFC showed a decrease in water permeability, and the 10% MFC loading had comparable or increased permeability values compared to the neat materials. Thus the behavior of PCL (and PHBV) is in good agreement with our results. Finally, it is interesting to note that the AcAX and its composites have comparable water permeabilities compared to MFC reinforced biocomposites of PHBV and PCL.[139] However, it is important to point out that the water permeability measurements in the study mentioned were carried out with a slightly different experimental set-up than ours. Thus comparing absolute values from this reference with our work is not straightforward, although trends and behaviors are comparable with our work.

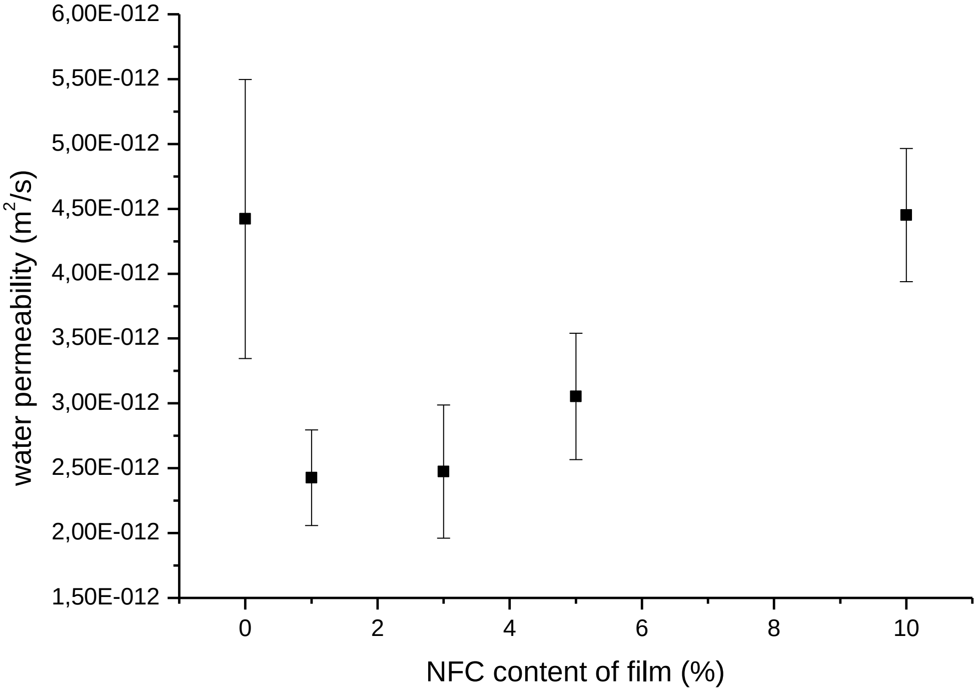

Figure 30 Water permeability of films

Chapter 5

Concluding Remarks and Future Work

This thesis was driven by the vision of the Wallenberg Wood Science Center to convert trees into modern materials and targets a contribution to the development of novel sustainable plastics aiming to replace some of the fossil fuel based plastics.

Each year there is about 200 times more hemicelluloses produced on Earth by nature than plastic produced by man. Still, xylans, which compose most of the hemicelluloses, are not an efficiently utilized valuable and inexpensive source of raw material. Large volumes of xylan exist in the side streams of agricultural industry or forestry and are burnt or never utilized. Xylans have a great potential to replace some fossil fuel based plastics, especially in the market of coatings, binders and packaging. Not only are the xylan films excellent oxygen barriers, but they can also be made thermoplastic by full acetylation. The thermoplasticity and higher thermostability gained from full acetylation of xylans facilitate a range of thermo-processing technologies to be applied in production processes.

In this thesis it has been shown that films produced from fully acetylated arabinoxylans have in several respects similar or superior material properties compared to highly substituted cellulose acetates. The properties in focus were mostly related to thermal and mechanical performance. Films from fully acetylated xylans were not just stronger and had a higher stiffness but also had a remarkably high elongation at break without the addition of plasticizer, whereas most native hemicellulose films are dependent on plasticizers to form free standing films.

Beyond the improved thermal and mechanical performance of the arabinoxylans, stability of the hemicellulose films against water was improved by complete acetylation of the arabinoxylan. The acetylated arabinoxylan films obtained water resistance, thus enabling them to be used in a broader field of applications.

The already good strength and stiffness of the acetylated arabinoxylan were further improved by the incorporation of nanofibrillated cellulose as reinforcement into the films. Despite the lower elongation to break of the composite films, these films still had notably high elongations as compared to other hemicellulose films. The NFC reinforcement also further improved thermal stability of the films, and the low incorporation of NFC decreased the water permeability of the films.

It is important to point out that a relevant advantageous property of a xylan film, the extremely low oxygen permeability, was lost due to the acetylation. This issue may be addressed in the future by developing a multilayer packaging system using native xylan films as the oxygen barrier and acetylated arabinoxylan films as the moisture barrier layer.

As the scientific and industrial trend dictates, green chemistry is promoted for numerous polysaccharide modifications and processes. This challenge was addressed in two papers where a more environmental friendly acetylation was targeted.

It was shown for the first time in a publication of this thesis that xylans were successfully fully acetylated in two different ionic liquid systems in a remarkably short reaction time while maintaining a high molecular weight throughout the modification. Ionic liquids are a novel alternative for a broad range of chemical applications due to their low vapor pressure and a great potential for multi-cycle use. Another emerging and environmentally benign means of acetylation is also presented in this thesis, where enzymes as biocatalysts aided the surface acylation of xylan films in a heterogeneous system. Here, cutinase and lipases were used in an esterification reaction to acetylate the surface of xylan films using vinyl acetate and vinyl stearate as reagent. The cutinase presented a higher activity towards the vinyl acetate as a substrate, but also actively acylated the surface of the xylan films when vinyl stearate was the acylating agent. On the other hand, the lipases were only capable of performing surface stearation of the films. This can give a good basis on selecting and further evaluating the possibilities of enzymatic esterification of polysaccharides. The effects of the chain length of acylating agents on the surface properties were also evaluated, showing the longer alkyl chains to give a more expressed hydrophobicity to the outermost layers of the surfaces.

To be able to propose an optimal field of application for xylan acetates it is practical to map and compare the material properties of similar materials available on the market. Cellulose acetates might be a reasonable group of materials to apply as a guideline for large-scale applications of xylan acetates. The most commonly used cellulose acetates have a DS between 2 and 3, where 3 is the maximum. The properties of cellulose acetates can be tuned by selecting different degrees of substitution. Nevertheless, when comparing with a fully acetylated xylan, it is practical to compare to the rather highly substituted cellulose acetates ($2.5 \leq DS \leq 3$). As many properties are comparable or slightly superior to cellulose acetate, it gives a good indication of possible fields of application. For example, cellulose acetates are widely used in coatings, or as films. The existing industrial production of cellulose triacetate and the well established large-scale film casting technologies might also facilitate an easier scale-up of acetylated xylan film production. However, it is clear, that the industrial scale technologies of cellulose acetate production cannot be directly adapted for industrial scale hemicellulose acetylation. Thermo-processing should also be considered as a solvent-free (but energy demanding) processing route.

Summarizing these material properties, acetylated xylans have a great potential in establishing relevance in the packaging industry, for example as food packaging and wrapping foils or as coatings.

This work is a contribution to the design of novel xylan based materials and their feasible and environmentally friendly production. The thesis intends to bring attention to the outstanding overall properties of acetylated arabinoxylan films compared to other bio-based plastics on the market today. The study also embraces possible upcoming issues of a sustainable industrial production of acetylated xylans by offering new alternative methods for their production. However, these methods need further fine-tuning and more detailed work to achieve a feasible acetylated xylan production.

Future Work

The thesis presented advantageous properties of acetylated xylans, which should be embraced and further improved. Research on other types of reinforcements such as nanocrystalline cellulose or other nano particles or clay platelets should also be investigated. Plasticization of these materials has not been studied in this work, which could harbor a new field of application for these materials. Composites with nanocellulose as main component could be investigated for advanced oxygen barrier properties, where the acetylated xylan is more like a binder. There is also a need to investigate the thermal processability of these materials, which the thesis did not have time to study excessively beyond demonstrators.

In addition to more detailed investigations of material properties and possible processing and applications, the green chemistry reported in this work also pointed out new directions for research. Recently the high molecular weight intact fractionation of wood components was demonstrated in the literature through ionic liquid co-solvent systems. This opens new possibilities for hemicellulose processing and modifications as well. This thesis has now presented the full acetylation of xylans in ionic liquids. The eventual combination of extraction and modification into a one step process could be the key to the commercialization of xylan acetates. There is also a possibility to study the properties of partially separated wood components and the modification of tailored mixtures. Therefore, detailed research should be targeted towards a process where the wood can be dissolved and the hemicelluloses would not require precipitation and redissolution. This would reduce the number of process steps, thus saving energy and reducing costs and production time.

Heterogeneous enzymatic acetylation of xylans was also presented as a part of this thesis. This is a state of the art work, showing possibilities and directions. The next challenge would be to apply the enzymatic esterification of xylans in homogeneous reaction systems. Some enzymes can tolerate and maintain activity in ionic liquids. Therefore this path of enzyme aided acetylation should also be more deeply investigated. However, in this field, close collaboration with protein chemists might be essential for the purification of new enzymes or for the development of engineered enzymes suitable for this task.

Once these novel acetylation methodologies and material modifications become well understood, combining them into industrial scale production could lead to a sustainable future and decreased environmental impact.

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