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Improvement and Analysis of Paper Properties by Adding Modified Polysaccharides

Dissertation

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submitted by:

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Science is always wrong. It never solves a problem without creating ten more.

– George Bernard Shaw

*Dành tặng Ba Mẹ
cùng tình yêu và lòng biết ơn vô hạn
To my Mom and to the memory of my Dad
with love and eternal appreciation*

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List of Acronyms

°SR	Schopper Riegler degree
OAc	<i>O</i> -acetyl
2- <i>O</i> -MeFucp	2- <i>O</i> -methylfucopyranosyl
2- <i>O</i> -MeXylp	2- <i>O</i> -methylxylopyranosyl
AcefA	3-C-carboxy-5-deoxy-L-xylofuranosyl
AGX	arabinoglucuronoxylan
Apif	apiofuranosyl
Araf	arabinofuranosyl
Araf/Xylp	arabinofuranosyl/ xylopyranosyl
Arap	arabinopyranosyl
AX	arabinoxylan
Be-CatX	beech cationic xylan
Bi-CatX	birch cationic xylan
CatX	cationic xylan
CDI	<i>N, N'</i> -carbonyldiimidazol
CED	cupric-ethylene-diamine
CHPTAC	(3-chloro-2-hydroxypropyl)trimethylammonium chloride
DAX	dialdehyde xylan
DE	degree of esterification
Dhap	3-deoxy- <i>D-lyxo</i> -heptulopyranosylaric acid
DHPTAC	2,3-dihydroxypropyltrimethylammonium chloride
DMF	<i>N, N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
DS	degree of substitution
EDTA	ethylene diamine tetraacetic acid
EPTAC	2,3-epoxypropyltrimethylammonium chloride
FT	Fourier transform
FT-IR	Fourier transform infrared

LIST OF ACRONYMS

Fucp	fucopyranosyl
Galp	galactopyranosyl
GalpA	galactopyranosyluronic acid
GAX	glucurunoarabinoxylan
GlcP	glucurunopyranosyl
GlcP A	glucopyranosyluronic acid
GP	glucose pentaacetate
GX	glucuronoxylan
HG	homogalacturonan
HH	hydroxylammonium hydrochloride
HPLC	high performance liquid chromatography
IAP	Fraunhofer Institute for Applied Polymer Research
IHP	Institute of Wood and Paper Technology
IPHC	Institute of Plant and Wood Chemistry
Kdop	3-deoxy-D- <i>manno</i> -octulopyranosyloic acid
LC	liquid chromatography
MeGA	4- <i>O</i> -methyl glucuronic acid
MS	mass spectrometry
OS	oat spelt
OS-CatX	oat spelt cationic xylan
OS-DAX	oat spelt dialdehyde xylan
OSX	oat spelt xylan
RCF	relative centrifugal force
RG-I	rhamnogalacturonan-I
RG-II	rhamnogalacturonan-II
Rhap	rhamnopyranosyl
RT	room temperature
SD	standard deviation
SEC	size exclusion chromatography
SG	substituted galacturonans
TBAI	tetrabutylammonium iodide
TFA	tri-fluoroacetic acid
TUD	Technische Universität Dresden
W-CatX	wheat cationic xylan
W-DAX	wheat dialdehyde xylan
WAX	wheat arabinoxylan
Xylp	xylopyranosyl

List of Glossaries

∅	diameter
<i>et al.</i>	Latin abbreviation for <i>et alli</i> , means “and others”, “and co-workers”
abs.	absolute
approx.	approximately
aq.	aqueous solution
ca.	Latin abbreviation for <i>circa</i> , means “around”, “about”, “approximately”
e.g.	Latin abbreviation for <i>exempli gratia</i> , means “for example”, “for instance”
i.e.	Latin abbreviation for <i>id est</i> , means “that is”
M _w	molecular weight
p.a.	per analysis
rpm	revolutions per minute
v/v	volume concentration, expressed in volume of solute per volume of solution
w/w	mass concentration, expressed in weight of solute per weight of solution

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Abstract

Polysaccharides are now popularly used in paper technology since they are able to improve mechanical properties of the paper. Xylan and pectin are two natural polymers that have a wide range of applications nowadays. These two polysaccharides can be used in their native forms as well as derivatives. In this study, xylan and pectin were modified to obtain cationic derivatives before adding into the paper for enhancement. The work was motivated by the fact that xylan and pectin share the same negative surface properties as cellulose. This leads to a slight repulsion effect between them once they are close. Their cationic derivatives, therefore, are believed to strengthen the paper due to electrostatic interactions between two oppositely charged objects beside hydrogen bonds. To fulfill the purposes, oat spelt xylan and apple pectin were chemically modified using different modification methods, including oxidation, amidation, and a combination of these two methods. A quantitative method to determine the adsorbed amount and relative retention capacity of xylan onto paper using high performance liquid chromatography was also developed.

The results show that oat spelt and apple pectin as well as their modified derivatives can improve the paper mechanical properties. The oat spelt xylan significantly enhances the tensile strength, however, this contribution has a saturation level in correlation with the relative retention capacity of the xylan onto cellulose. The best retention capacity is achieved when 5% of xylan is added. This finding provides a practical approach to the application of xylan as a paper additive in consideration of economic issues. Although the application of apple pectin and its amidated derivatives in paper is rather rare, some interesting points have been discovered in this work. Among the apple pectin samples used, only the ones with low degree of esterification give a slight enhancement in tensile index at 5% dosage. The results also suggest that the dosage of the pectin added into the paper should not exceed 5% due to its viscous property at high concentrations.

Introduction

Hemicellulose, together with cellulose and pectin are three main components which build up the primary cell wall of plants. In which, cellulose and hemicellulose are major substances in paper making. Paper is basically made from cellulose or holocelluloses by forming a network among fibers and fibers fragments via hydrogen bonds. Mechanical properties of the paper depend on many factors including properties of the fibers themselves as well as supporting components used. There are many types of additives that can be used in order to strengthen the paper as well as to reduce cost, including synthetic materials and natural materials. Applications of the natural materials, especially plant polysaccharides in paper are drawing much attention and becoming more popular since these materials possess many advantages over the synthetic materials. The plant-based materials can be used in their original forms or as modified derivatives.

Xylan, the most abundant part of hemicellulose, has great advantages to modify cellulose fibers due to its natural affinity to cellulose [17, 61, 65, 124]. The presence of xyans on cellulose fiber surfaces has enhanced the strength properties of the fiber network by increasing the bonding ability among fibers. Many authors have pointed to the importance of preserving xylan during the pulping process because xylan–cellulose interaction results in a final paper structure that possesses good tensile properties, wettability and displays acceptable physical properties [41, 65, 124]. When adding birch xylan into pulp, it was believed to make the beating process of the pulp easier and resulted in high tensile strength as well as stiffness of the paper product [18]. Despite of the difference in structure, substitution patterns, and degree of substitutions, xylan from non-wood materials is also able to improve the paper strength properties. Köknke *et al.* showed an evidence of tensile strength improvement of kraft softwood pulp when it was modified with a water-soluble glucuronoarabinoxylan from barley husks [65]. It is believed that the ability of xylan to improve paper strength is a result of the retention phenomenon. This is explained by co-crystallization of xylan segments with cellulose and the formation of strong xylan–cellulose hydrogen bonds.

Besides native polysaccharides, cationic derivatives have been also used widely in

paper technology. The wet and dry strength of paper were improved significantly when using cationic xylan from birch [63, 115, 117] or from oat spelt [21]. Depending on modification method, cationic xylylans with different molecular weight, degree of substitution, and total surface charges can be derived. The electrostatic interaction is believed to be one of the most important factors influencing the adsorption of cationic xylan onto cellulose, and therefore mechanical properties of the paper product [117].

In this work, the role of two polysaccharides, namely oat spelt xylan and apple pectin in paper strength properties was analyzed by using them as a paper additive. The two studied substances were originated from agricultural residues. The xylan was extracted from oat spelt, a by-product of oat flake manufacturing. Oat spelt is a promising material for xylan isolation. On the one hand, it can be collected in a large quantity since it constitutes up to 25% of total grain of the mill [135]. On the other hand, it contains a high amount of hemicellulose (22 – 39%, in which xylan is the most abundant part) and with relatively low content of lignin (18 – 24%) [110]. The pectin was derived from apple pomace, which is a residue from a juice production line. About 10 – 15% of pectin on a dry matter basis is found in apple pomace [131]. In addition, utilization of agricultural residues does not only have meanings in science but also mitigates the amount of waste disposed into the environment. Oat spelt xylan and apple pectin were modified by oxidation and/ or amidation before adding into pulp slurry during a paper making process at laboratory scale. The mechanical properties of paper, including tensile, tear, and burst index, were tested in order to evaluate the influence of the modification process on the paper strength.

The work aims to investigate the influences of using polysaccharides extracted from agricultural wastes, namely oat spelt xylan and apple pectin and their cationic derivatives on the paper mechanical properties. The work was motivated by the fact that xylan and pectin share the same negative features on their surface like cellulose. When adding the cationic derivatives of these polysaccharides into cellulose, the fibers network will be strengthened due to electrostatic interactions between oppositely charged molecules. This research comprises the following contents:

- Investigation the application and influences of using xylan from different sources in paper strength.
- Developing a method to modify oat spelt xylan and apple pectin to produce their positively charged derivatives for paper application.
- Applying the polysaccharides and their cationic derivatives into paper, analyzing their interaction with cellulose fibers, as well as evaluating their influence on paper strength through mechanical properties of the papers.

- Comparing the effectiveness of the developed method with that of the well-known one, which uses cationizing reagents.
- Comparing the modification ability of oat spelt xylan with xylan samples isolated from other sources.
- Developing a new quantitative method to determine the adsorbed amount of xylan and its relative retention capacity on paper.

The remainder of the dissertation is organized into four chapters. Chapter 1 reviews recent research relating to the current topic. In chapter 2, the used materials and methods in the project were introduced. The experimental results are presented and discussed in chapter 3. Finally, chapter 4 summarizes the findings of this work and draws to conclusions.

1

Literature review

The plant cell wall is constructed by several layers, namely middle lamella (ML), primary wall (P), secondary wall, and a warty layer (W). The secondary wall contains an outer layer (S1), a middle layer (S2), and an inner layer (S3). The structure and chemical compositions of these layers are different from one to another [128]. A simplified structure of a woody cell and some main components of the primary wall are displayed in figure 1.1.

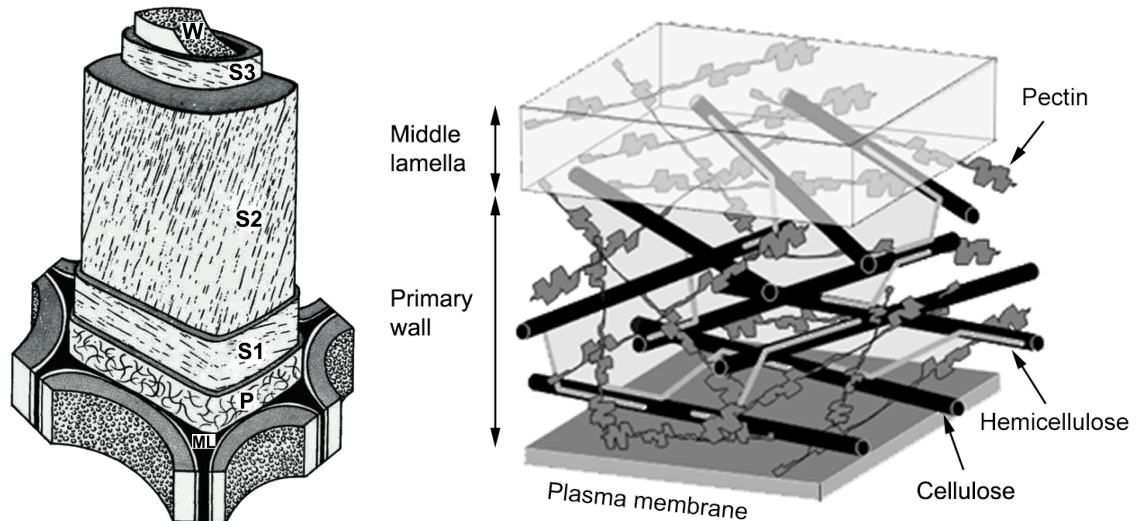


Figure 1.1: *Basic structure of a woody cell, showing the middle lamella (ML), the primary wall (P), the outer (S1), the middle (S2), and inner (S3) layer of the secondary wall, and the warty layer (W) (image sources: [128] and <http://www.ccruc.uga.edu>)*

Growing plant cells are surrounded by polysaccharide-rich primary walls, which are built up from three main polysaccharides, namely hemicelluloses, cellulose, and pectin besides lignin. Polysaccharides can be divided into two major classes: homopolysaccharides and heteropolysaccharides. Hemicellulose and pectin are subsumed in the class of

heteropolysaccharides while cellulose is classified as a homopolysaccharide [74]. This section summarizes briefly the three main polysaccharides present in the primary cell wall, which are also the three main substances used in this work.

1.1 Cellulose

Cellulose is a homopolysaccharide, mainly composed of β -D-glucopyranose units via β -(1, 4)-glycosidic bonds (figure 1.2). Cellulose molecules are linear and have a strong tendency to form intra- and intermolecular hydrogen bonds.

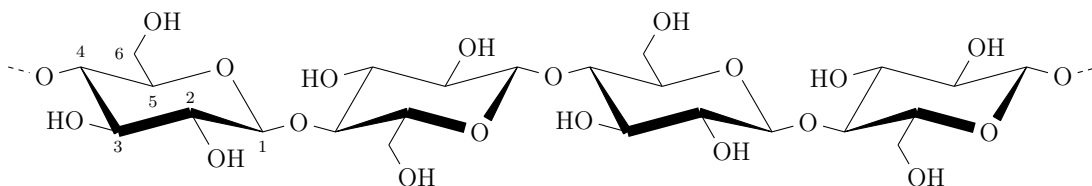


Figure 1.2: *Structure of cellulose*

In paper, cellulose contributes mainly to the formation of the paper. Hydrogen bonds created among hydroxyl groups on its surface build a network of fibers. There are two types of hydrogen bonds within each cellulose chain as shown in figure 1.3.

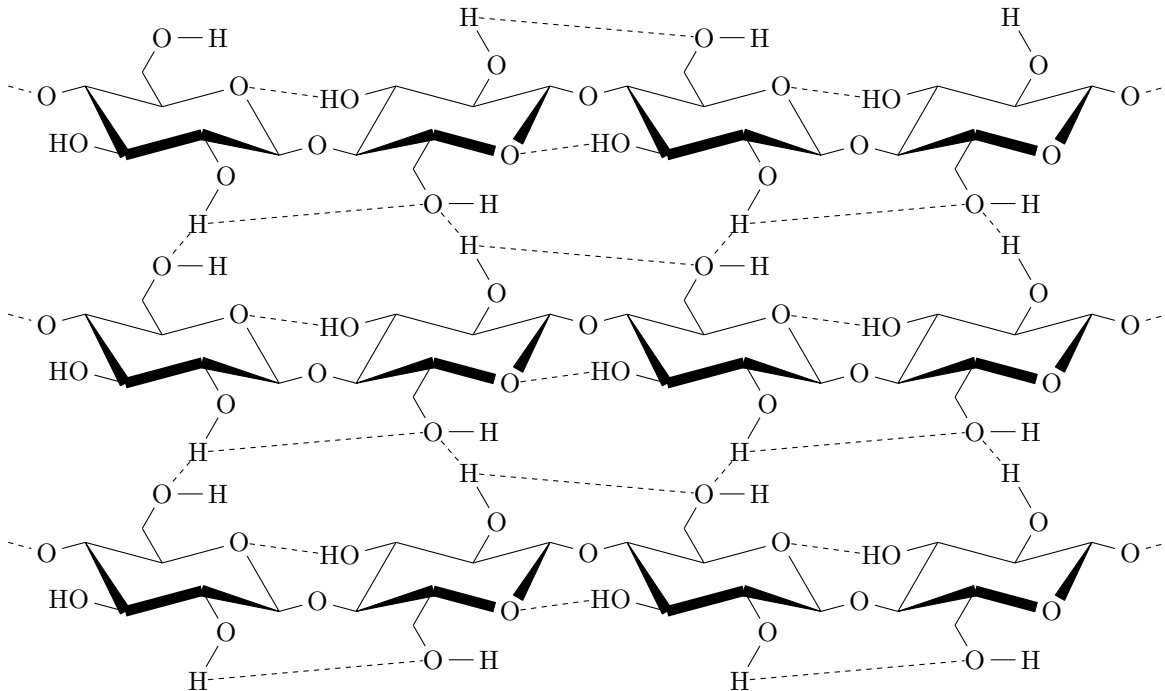


Figure 1.3: *Intra- and intermolecular hydrogen bonds between cellulose chains*

One bonding type is from the OH attached to the C6 of one glucose residue to the OH attached to the C2 of the adjacent glucose. The second bonding type is from the

OH attached to the C3 of one glucose ring to the ring oxygen [128].

In fact, additives are used in order to obtain some specified properties of the finished paper. The application of polysaccharides beside the traditional additives, starch, is getting more popular. In this work, cellulose is used in form of bleached kraft softwood pulp for studying the adsorption of other polysaccharides on it.

1.2 Hemicellulose and xylan

In contrast to cellulose, which is a homopolysaccharide, hemicelluloses represent heteropolysaccharides with much lower molecular weight than that of cellulose. The monomeric components of hemicellulose consist of D-glucose, D-xylose, D-mannose, L-arabinose, a small amount of L-rhamnose in addition to D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, and D-galacturonic acid [128]. The contents of these substances vary in considerably depending on their natural sources.

Among different components of hemicelluloses, xylan is known as the most abundant constituent. This compound is not only present in wood but also in almost all kinds of non-wood plant such as grasses, cereals, and herbs.

1.2.1 Classification and structure of xylan

General structure of xylan

Xylan constitutes the most abundant part of hemicelluloses found in the cell walls of plants and grasses and is the second most common biopolymer in the plant kingdom [23]. This natural polymer with many promising properties has been studied for many years [23, 122, 134]. Like other polysaccharides, xylan exists as both homopolysaccharide and heteropolysaccharide. The homopolysaccharide of xylan, which comprises only xylose units, is difficult to isolate. Normally, xylan contains two to four different kinds of sugar monomers. Xylan structure is variable, ranging from the linear β -(1, 4) linked polyxylose chains to highly branched heteropolysaccharides, where the prefix ‘hetero’ denotes the presence of sugars other than D-xylose. The structure of xylans varies from plant to plant and even in one single plant. Ebringerová [22] has given a clear summary about xylans and classified them into the following types :

- Homoxylans: present in some seaweeds such as in red and green algae and hardly found in higher plants. As the name says, these xylans are linear polysaccharides composed of D-xylopyranosyl ($Xylp$) residues. These monomers are linked together by β -(1, 3)-linkages (X_3) and/ or β -(1, 4) linkages (X_4) and/or mixed β -(1, 3), (1, 4) linkages (X_m) as shown in figure 1.4.

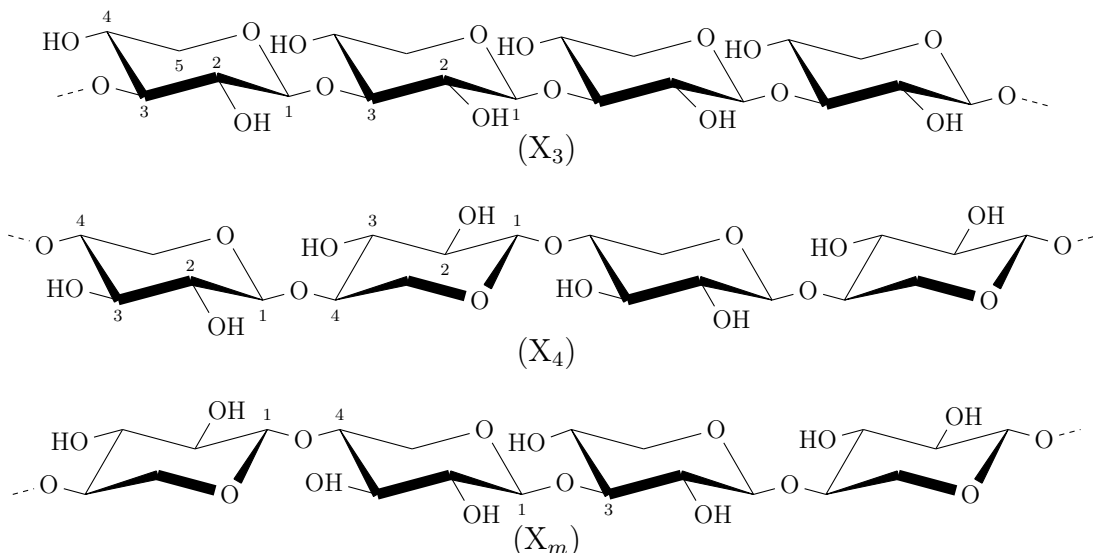


Figure 1.4: Structure of homoxylan chain with β -(1, 3) linkages (X_3), β -(1, 4) linkages (X_4), and mixed β -(1, 3), (1, 4) linkages (X_m) [22]

- Glucuronoxylans (GXs): D-glucurono-D-xylan type is the main hemicellulose component of the secondary cell wall of woody tissues in dicots. The main backbone is composed of β -(1, 4)-Xylp monomers. It is attached with single side chains of β -D-glucopyranosyluronic acid (Glc pA) and/ or its 4-*O*-methyl derivative attached at position O2 of the Xylp monomer units (figure 1.5). The 4-*O*-methyl derivative contributes more than 90% of the hemicellulose component in hardwoods and herbal plants of the temperate zone. The ratio between 4-*O*-methyl glucuronic acid (MeGA) side chain and Xylp varies from 1 : 4 to 1 : 16 depending on the plant parts as well as the plant species.

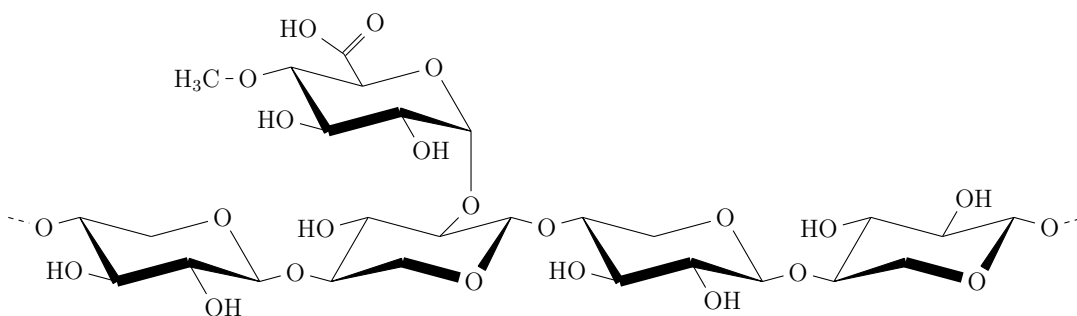


Figure 1.5: Structure of 4-*O*-methyl-*D*-glucurono-*D*-xylan [22]

- Arabinoglucuronoxylans (AGXs): the minor hemicellulose component found in temperate softwoods and some kinds of tropical hardwood. This type of xylan is similar to GX but contains single α -L-arabinofuranosyl (Araf) residues at the O3 positions of the monomer (figure 1.6). The content of MeGA in AGX is higher than that in GX from hardwood.

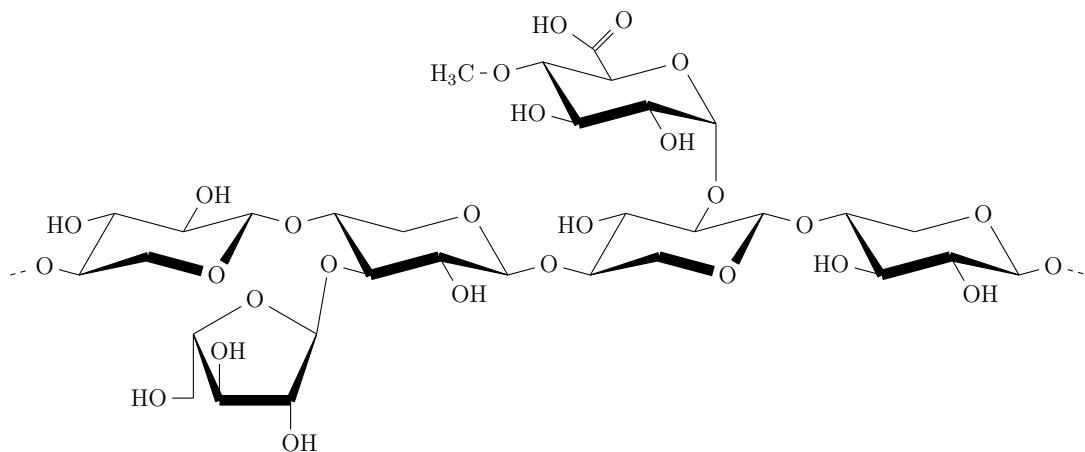


Figure 1.6: Structure of *(L-arabino-) 4-O-methyl-D-glucurono-D xylan* [22]

- Arabinoxylans (AXs): L-arabino-D-xylans are the typical hemicellulose components in starchy endosperm, which can vary from 0.15% in rice endosperm to approx. 13% in whole grain flour from barley and rye, and up to 30% in wheat bran. The β -(1, 4)-xylopyranose backbone of AXs is attached with α -L-Araf at positions O2 or O3 and/ or at both positions O2 and O3 of the same D-Xylp unit (figure 1.7). The former is called monosubstitution while the latter is called disubstitution. Due to this feature, AXs have a very large structural variety and therefore differ in their solubility as well. In some Araf residues of the AX, O5 positions are esterified, mainly with ferulic acid or *p*-coumaric acid.

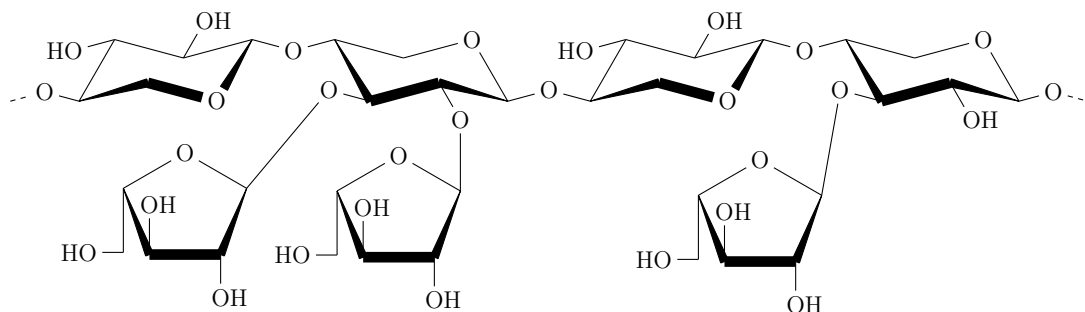


Figure 1.7: Structures of water-soluble *L-arabino-D xylan* [22]

- Glucurunoarabinoxylans (GAXs): D-glucurono-L-arabino-D-xylans are important hemicellulose in grasses and cereals. In contrast to AGXs, they are composed of Xylp residues, which are disubstituted with Araf, MeGA residues and partly *O*-acetylated. The ratio among MeGA, Xylp, and Araf varies in a range of 3 – 9 : 10 : 1–10.

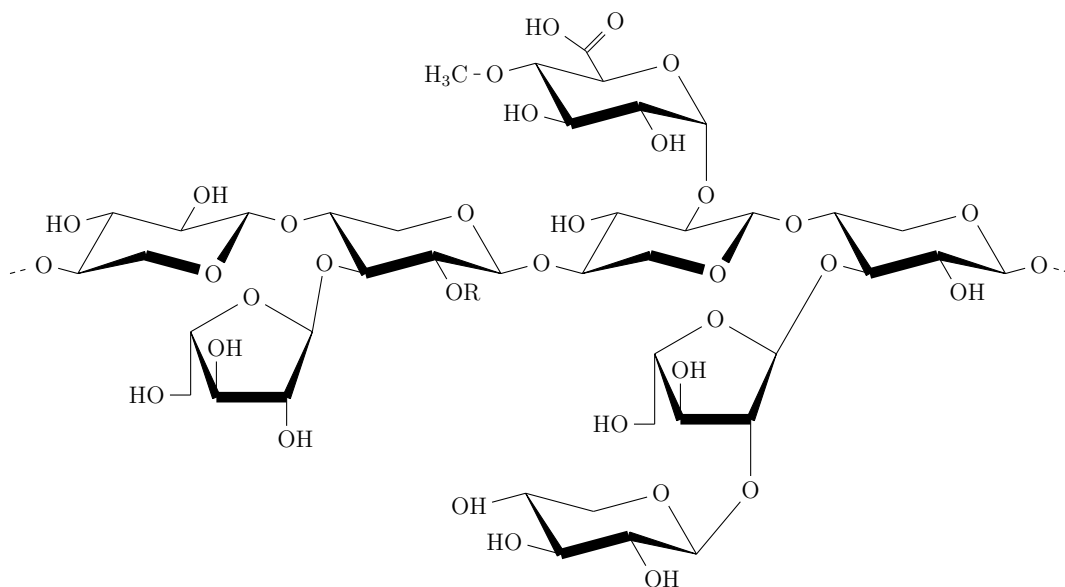


Figure 1.8: *Structure of water-soluble (D-glucurono)-L-arabino-D-xylan; R: H or α -L-Araf [22]*

- Heteroxylans: these complex structural xylans are present in cereal brans, seeds, and gum exudates. Their β -(1, 4)-xylopyranan backbone is substituted frequently with a variety of single and oligosaccharide side chains, which results in highly viscous solutions.

Hardwood xylan

The predominant xylan from hardwood is *O*-acetyl 4-*O*-methylglucurono- β -D-xylan (sometimes called glucuronoxylan). Depending on the species, the content of this xylan varies in the range of 15 – 30% of dry wood. At room temperature, the xylan is stable in alkaline solution [137]. Around 70 – 80% of the xylose residues are attached with acetyl groups at O2 and more frequently at O3. Approximately seven acetyl groups are detected per ten xylose units [137]. However, the acetyl groups are easily cleaved by alkaline [128]. The 4-*O*-methyl- α -D-glucuronic acid side chain appears every ten xylose units [128]. It is noticed that the linkages among xylose units are easily hydrolyzed by acid, whereas the bonds between the uronic acid groups and xylose are very stable. The degree of polymerization of the xylan is found to be in the range of 40 – 200, commonly 180 [11].

Softwood xylan

Softwood contains about 5 – 10% of arabinoglucuronoxylan, which has a backbone composed of (1, 4)-linked β -D-xylopyranose residues. Every ten xylose units, two 4-*O*-methyl- α -glucuronic acid groups attached at O2 positions and (1, 3)- α -L-arabinofuranose groups are found [128]. The xylan has a degree of polymerization of 100 – 120 and lacks of acetyl groups as mentioned by Biermann [11].

Xylan from annual plants

Xylans extracted from non-wood materials such as grasses and cereals consist of linear chains as mentioned above with α -L-arabinofuranosyl residues attached at O2 and/ or O3 positions; α -D-glucuronopyranosyl or its 4-*O*-methyl derivative at O2 positions (figure 1.9) [8, 13, 14, 123]. Furthermore, a small amount of ester-linked coumaric acid is found to be linked to some arabinosyl residues [46, 114, 125, 139]. The content of galactose and rhamnose is very low with a small amount of less than 2%.

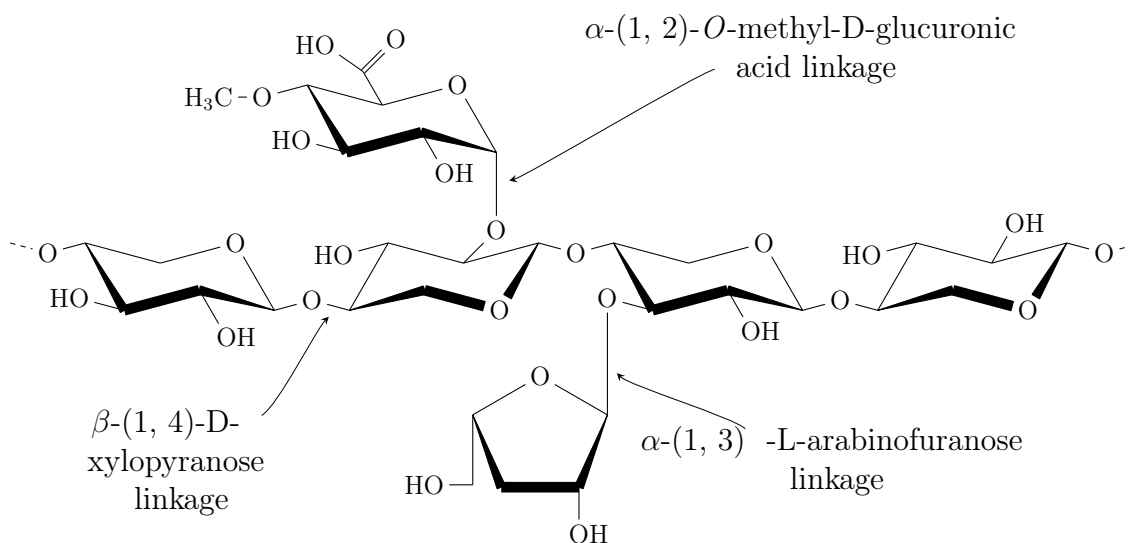


Figure 1.9: Structure of xylan from annual plants [8, 43]

1.2.2 Some important properties of xylan

Solution properties

An important characteristic of xylan, which affects its ability to adsorb onto cellulose fibers is its solubility in water and other solvents. On the one hand, although almost xylans are substituted with side chains and contain different sugars, most of them are poorly soluble in water but fully dissolve in alkaline environment [66, 140]. On the other hand, among a number of solvents, dimethyl sulfoxide (DMSO) is most efficient. When investigating the solution properties of birch xylan in the DMSO system, Lebel and Goring discovered that the solubility of alkaline extracted xylan was strongly dependent on the ratio between DMSO and water [70]. According to their findings, the solubility reached the maximum level when 12% (w/w) of water was added into DMSO. This feature was confirmed later by Saake and co-workers [111]. They indicated that the solubility of xylan was not related to its molecular mass but to the substituents and substituent patterns. Samples with higher degree of substitution resulted in higher solubility in an aqueous system. In addition, all of the

substituents, including acetyl groups, lignin-carbohydrates, and phenolic substances play an important role in this matter. This affirmation is emphasized again in a work of Köhnke *et al.* [64]. Their findings showed that the solubility and the hydrodynamic properties of arabinoxylan were determined not only by the degree of substitution (DS) but also by the substitution pattern. In another work of Yang *et al.*, the solubility of xylan from corncobs was able to be improved by a pretreatment with dilute acid and followed by a dry-steaming process [142]. Recently, the role of DS in the solubility of xylan was once again confirmed by Fundador and partners [34].

Degradation of xylan in alkaline environment

Since the extraction of xylan requires an alkaline solution as a major step, it is necessary to know the behavior of xylan in a basic environment to have a better understanding of the processes during the isolation.

A research conducted by Aspinal *et al.* [5] showed that the degradation of rye flour arabinoxylan in an alkaline solution under normal extraction conditions only occurred slowly at the reducing end-groups. The reaction of arabinoxylan from rye flour and barley husks in alkaline also caused a reduction of about 20% in molecular mass. However, it processed slowly for a long period of time (20 – 25 days). Later, Johansson and co-workers again specified that β -elimination occurred easily at the reducing xylose units causing isomerization and removal of these groups [60]. This was in agreement with the findings of Aspinal's team when they found out that in the presence of alkaline, the β -(1, 4) linkages were degraded to form xyloisosaccharinic acid as a major product [6]. However, Whistler *et al.* indicated that xylan oligosaccharides were readily degraded by lime water at 25 °C and formed a new C5 saccharinic acid [140].

1.2.3 Oat spelt xylan

Oat spelt – a potential source for xylan isolation

Oat spelt (OS) is a by-product of oat flake manufacturing. It is the outer part of the grain, which needs to be removed before processing. A simplified structure of an oat grain is shown in figure 1.10. OS has been used as livestock feed and for fuel production. However, due to the European legislation, it becomes more and more difficult to deal with this residue in this way nowadays [101]. OS is easy to purchase at a reasonably moderate price. Furthermore, this material can be collected in a large quantity since it constitutes up to 25% of the total weight of the grains in the production line [135]. With a high content of hemicellulose (22 – 39% of arabinoxylan depending on the origin of the OS) and relatively low content of lignin (18.5 – 22.9%) [110], OS can be

considered as a potential source for xylan extraction.

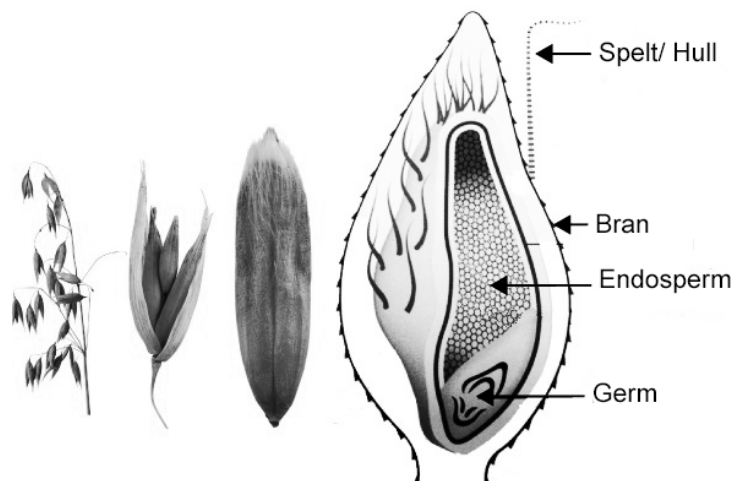


Figure 1.10: *Oat grain structure* (image source: <http://www.friendoatwell.ch>)

As compared to woody materials, the extractability of xylan from non-wood materials, including annual plants is much easier and gives higher extraction yield. This is due to the lower content and difference in structure of lignin as well as that of cell wall. The conditions for isolation, therefore, will be milder. However, when using hulls or husks as starting materials for xylan isolation, the procedures are more complicated since these materials contain proteins, phenolics, and other soluble polysaccharides. The isolation process, therefore, requires several steps to purify the product [23]. The most popular and effective method to extract xylan is to include an alkaline extraction as the main step, in which sodium hydroxide or aqueous ammonia are mainly used. According to Saake *et al.* [110], oat spelt xylan (OSX) with high purity and high yield can be obtained when carrying out the extraction with 5% NaOH solution at 90 °C.

OSX consists of *Araf* residues attached as branches to xylopyranosyl polymeric backbone. The *Xylp* units are either mono-substituted at O2 or O3 positions or di-substituted at both O2 and O3 positions. Only 1.8 – 4.3% of MeGA is present in alkaline extracted OSX as indicated by ¹H-NMR analysis [103]. Furthermore, the ratio among xylose, arabinose and MeGA varies from plant to plant, species to species. A ratio of 7.6 : 1 : 0.03 was indicated by Saake *et al.* [110]. The molecular weight of xylan in general and that of xylan from annual plants in particular is much lower than that of cellulose. The molecular mass of xylans depends not only on source of extraction but also on the determination method and the sample preparation procedure [101]. The molecular weight of OSX is also very different depending on the xylan source and the growing conditions, including climate, soil, and nutrients. An average molecular mass of 22000 g/mol of isolated OSX was reported by Saake *et al.* [110]. While Hettrich *et al.* [43] could isolate OSX with the molecular weight of 79000 g/mol.

1.2.4 Chemical modification of xylan

Chemical modification is a promising way to discover hidden properties of xylan in order to enable it for a variety of applications. Figure 1.11 presents some reaction paths for preparation of xylan derivatives via etherification in aqueous system or esterification in *N, N*-dimethylformamide (DMF)/ LiCl DMSO [94]. Cationic xylans can be obtained through quaternization using hydroxypropyltrimethylammonium either in aqueous sodium hydroxide or DMSO, *N, N'*-carbonyldiimidazol (CDI) (figure 1.11, path 2 and 4). In addition, anionic xylans can be derived by the introduction of carboxymethyl groups (figure 1.11, path 1 and 6). Moreover, the esterification with methyl halides can result in water-soluble products, which is necessary for the characterization of the DS and substitution patterns [92].

In a work of Ebringerová, beech xylan was alkylated using *p*-carboxybenzyl bromide under alkaline conditions. Carboxylated xylan derivatives with degrees of substitution of 0.05 – 0.25 were obtained. The derivatives were believed to be applicable to the preparation of antitumor, antiviral, antibacterial agents, paper additives, detergents, photosensitive materials, adhesives, and flocculants [26]. Among various means of modification, carboxymethylation is known as one of the most versatile methods providing an access to bio-based materials with valuable properties [39, 93]. Carboxymethylated birch xylan was synthesized by Petzold *et al.* using sodium monochloroacetate with presence of sodium hydroxide [93]. Later, Vega *et al.* conducted a study on several ionic xylan derivatives, including xylan sulfate, carboxymethyl xylan, and xylan-4-*[N, N, N]*-trimethylammonium butyrate chloride. These xylans derivatives were considered as polyelectrolytes, which were said to be able to modify fiber surfaces [138]. For different purposes, ionic derivatives like cationic and anionic carboxymethyl xylans are commonly produced via etherification and esterification [12, 40, 93]. Cationic xylans were commonly prepared via quaternization using 3-chloro, 2-hydroxypropyltrimethylammonium chloride or 2, 3-epoxypropyltrimethylammonium chloride in aqueous sodium hydroxide medium [12, 25, 94, 100, 106, 116].

Besides organic chemicals, inorganic reagents are also able to modify xylan. The modification method might, therefore, be cheaper and easier to conduct. In some cases, even the treatment of waste is not necessary. The following sections present two methods, which are researched in this work, namely oxidation, amidation and a combination of these two, which is called two-step method.

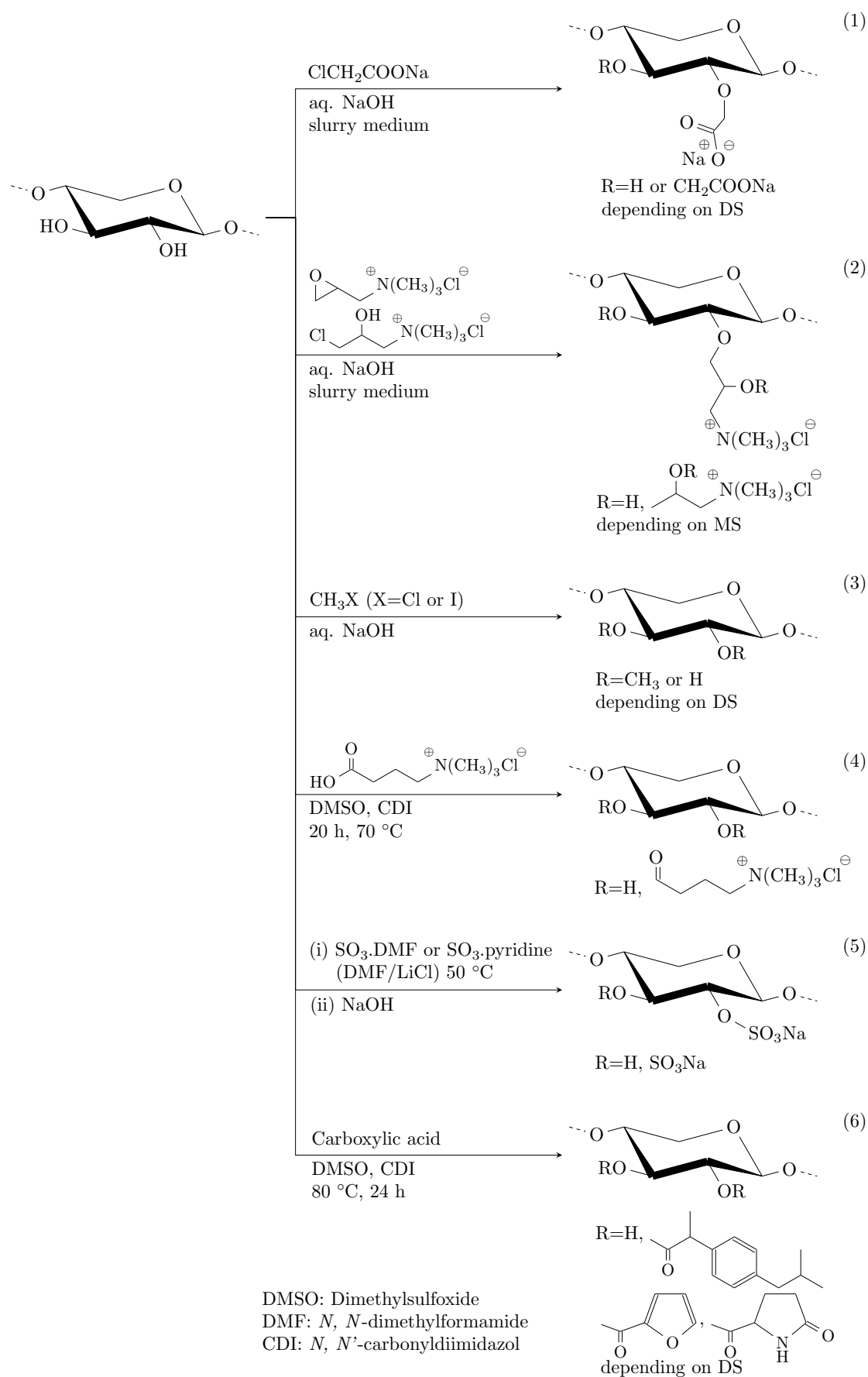


Figure 1.11: Reactions for preparation of some xylan derivatives via etherification or esterification [94]

Oxidation

Polysaccharides can be oxidized with different oxidants to form dialdehyde, dicarboxylic substances. For oxidation of vicinal diols to occur, it is required that the hydroxyl groups are oriented in equatorial – equatorial (E-E) or axial – equatorial (A-E) positions (figure 1.12). Vicinal OH groups in a rigid axial – axial (A) position cannot react because the intermediate complex formation can not take place [68]. Therefore, the oxidation reaction of xylan can only occur at C2 – C3 linkages and/ or C3 – C4 linkages of each xylose unit.

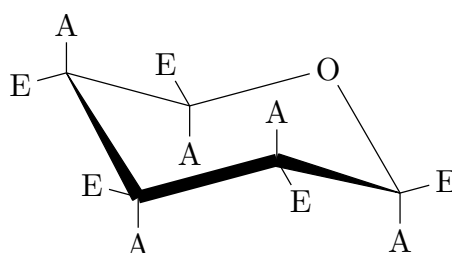


Figure 1.12: *Distribution of axial (A) and equatorial (E) positions in a xylose unit*

Sodium metaperiodate, hydrogen peroxide, and ozone are a few of specific agents, which can selectively attack and cleave at the C2 – C3 and/ or C3 – C4 bonds of the xylopyranose rings of xylan. However, this interaction depends on several factors, including the type of sugar units, type of linkages among these units, degree of polymerization, degree of substitution, and even the frequency of branching [15]. Possible products of the oxidation reaction of xylose units and other monosaccharides at different positions are expected as shown in figure 1.13.

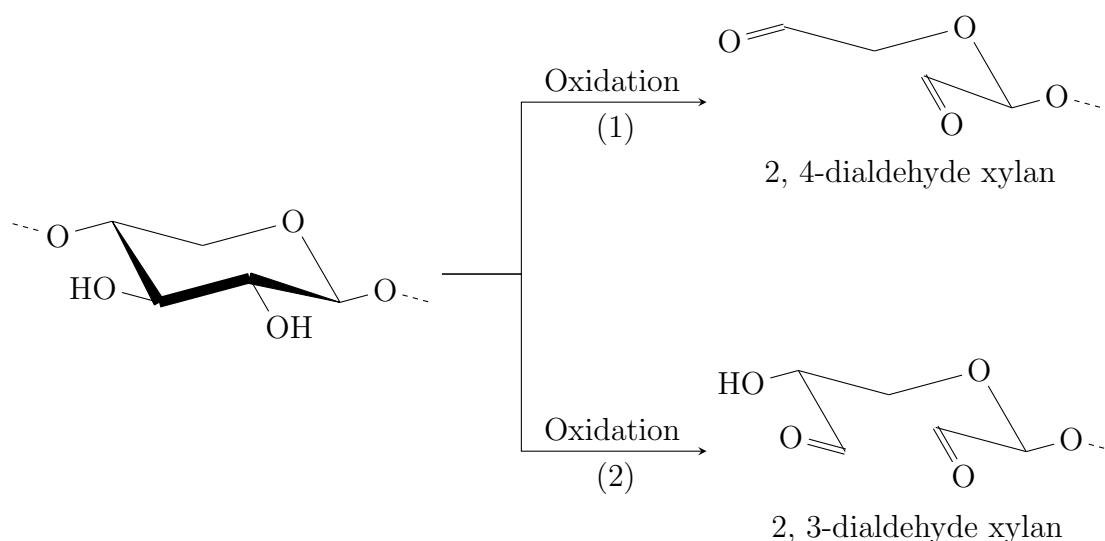


Figure 1.13: *Overall scheme for general oxidation of xylan illustrated by β -D-xylopyranose residues: (1) Terminal residue, double oxidation at O2, O3 and O3, O4 positions, respectively; (2) β -(1, 4)-linked residues where cleavage occurs at C2 – C3 bonds [15]*

Sodium *m*-periodate is a mild oxidant, which has been used widely in glycol cleavage reaction of carbohydrates. Several studies were conducted on the periodation of different polysaccharides, for instance, cellulose [15], xylan from oat straw [6], water-soluble arabinoxylan from rye grain [3], water-soluble arabinoxylan from wheat [20], and 4-*O*-methylglucuronoxylans from beech wood [16]. Regarding the periodation, Painter and Larsen [89] indicated that 6-membered rings hemiacetal was produced as an intermediate product. This compound was formed between the aldehyde group of an oxidized xylose residue and the nearest hydroxyl group of an unoxidized xylose residue.

Hydrogen peroxide is a moderately strong oxidizing reagent, which acts as an oxidant in a redox reaction. Hydrogen peroxide together with catalysts were early used to break down the cell walls of polysaccharides resulting in depolymerization of the polysaccharides [79]. Furthermore, the chemical was applied to oxidize cellulose [36], and starch [90], and more commonly as a bleaching agent in paper industry and in xylan isolation [42, 43, 110]. In this work, the reagent was aimed to break the xylopyranose rings of xylan and form dialdehyde derivatives. The principle of the oxidation reaction using hydrogen peroxide is based on the free hydroxyl radical mechanism. In aqueous environment, especially with presence of catalysts, hydrogen peroxide is degraded to form hydroxyl radicals, which have a very high activation energy. Metal ions were used in the reaction between xylan and hydrogen peroxide as a catalyst to activate functional groups in the modified xylan [90].

The third oxidizing agent used is **ozone**, O_3 . Ozone is made in the upper atmosphere layer by collision of an oxygen atom and an oxygen molecule. It is also created in the lower atmosphere layer by the reaction between oxygen and oxides of nitrogen formed in photochemical smog. Its polarity makes it fairly reactive. The three oxygen atoms are arranged symmetrically with an angle of $116^\circ 49'$ and equal oxygen–oxygen bond length of 0.128 nm. The resonance structures of ozone are illustrated in figure 1.14.

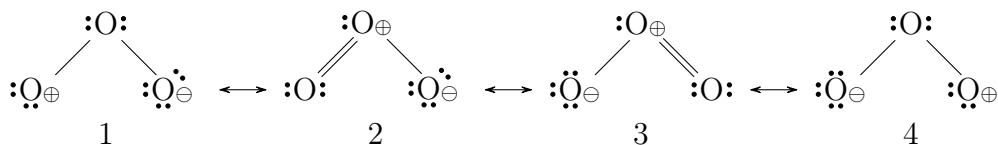


Figure 1.14: *Resonance structures of ozone* [86]

Ozone is also a diamagnetic substance with a low dipole moment of 0.53 D and a low magnetic susceptibility of 2.00×10^{-9} cgs units. Ozone reacts with both inorganic and organic substances. In the reaction with organic compounds, ozone acts as an electrophile, selectively attacking on high electron density positions in the organic

substances [86]. In liquid phase, ozone is decomposed through a complex radical chain mechanism, producing free hydroxyl radicals like in the case of hydrogen peroxide.

In paper industry, ozone has been used mostly in bleaching by reacting with lignin. However, it is found that ozone reacts not only with lignin but also with hemicelluloses, which results in the formation of carbonyl groups. By controlling the reaction conditions, such as concentration, generation method and air flow rate, polysaccharides can be modified to form aldehyde derivatives without decreasing their chain length too much.

Amidation

As mentioned in section 1.2.4, the oxidation process aimed to activate the xylan structure for amidation in the next step. The two possible oxidized xylans react with ammonia in isopropanol. Expected products are depicted in figure 1.15.

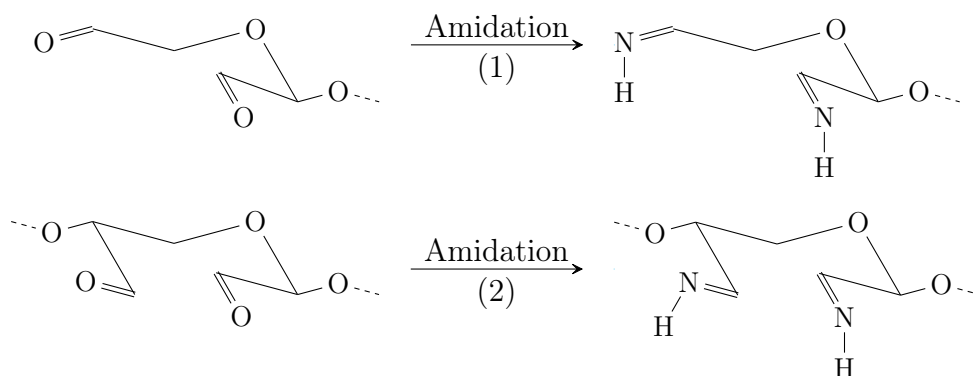


Figure 1.15: Possible reactions between oxidized xylans and ammonia solution

1.3 Pectin

Pectins are the most complex group of polysaccharides in the plant kingdom. Pectin is found in the primary cell walls of grass, algae, fern, lycopod, and bryophyte (figure 1.1). The content of pectin varies from plant to plant, and from species to species. Approx. 35% of pectin is present in the primary cell walls of dicotyledenous and non-graminaceous monocotylenous plants, 5 – 10% in grasses, and up to 5% in woody tissue [2, 80].

In nature, pectin plays a very important role in plant growth, morphology, plant defense, signaling, pollen tube growth, seed hydration, and fruit development. In fact, pectin has been used mainly in food and cosmetic industries as a gelling agent, thickening agent and stabilizer. Pectin is also widely applied in medicine and bio-medicine since it supports human health [80].

Citrus fruits (oranges, lemons, grapefruits) and apples are main sources of pectin. Commercial pectin is mostly extracted from citrus peels and apple pomace, residues from fruit processing and beverage manufacturers by hot acidified water. Apple pomace contains 10 – 15% of pectin on a dry matter basis, while citrus peel consists higher content of pectin with 20 – 30% [131]. Amongst citrus peels, lime contains the highest amount of pectin with probably the highest molecular weight. The content of pectin in oranges is very variable, depending on source and variety [76]. Other sources for pectin are sugar beet waste from sugar production, sunflower heads, and mango waste [131].

1.3.1 Classification and structure of pectin

Pectins are a group of complex polysaccharides, whose structures have not been yet specified clearly. Depending on environmental conditions, sources and geography, the structure and chemical compositions of pectin may differ. In general, pectin is mostly composed of approx. 70% of (1, 4)-linked α -D-galactopyranosyluronic acid (GalpA) and attached with many side chains. Types of side chain and their undetermined locations in the molecule make pectin an interesting object for studying.

To date, there are three primary plant cell wall pectic polysaccharides that have been isolated and characterized, namely homogalacturonan, rhamnogalacturonan-I, classified as linear pectins and substituted galacturonan referred as rhamnogalacturonan-II [84].

Homogalacturonan

Homogalacturonan (HG) constitutes the major part of the pectic family with 70%. It is a linear polymer, which is composed of 65% (1, 4)-linked α -D-GalpA residues. Some (perhaps most) of the carboxylic groups are methyl esterified at C6. Depending on the plants' origin, it can also be partially *O*-acetylated at O2 or O3 [107], and may contain other potential crosslinking esters of uncertain structure [80]. The structure of HG is presented in figure 1.16 [107].

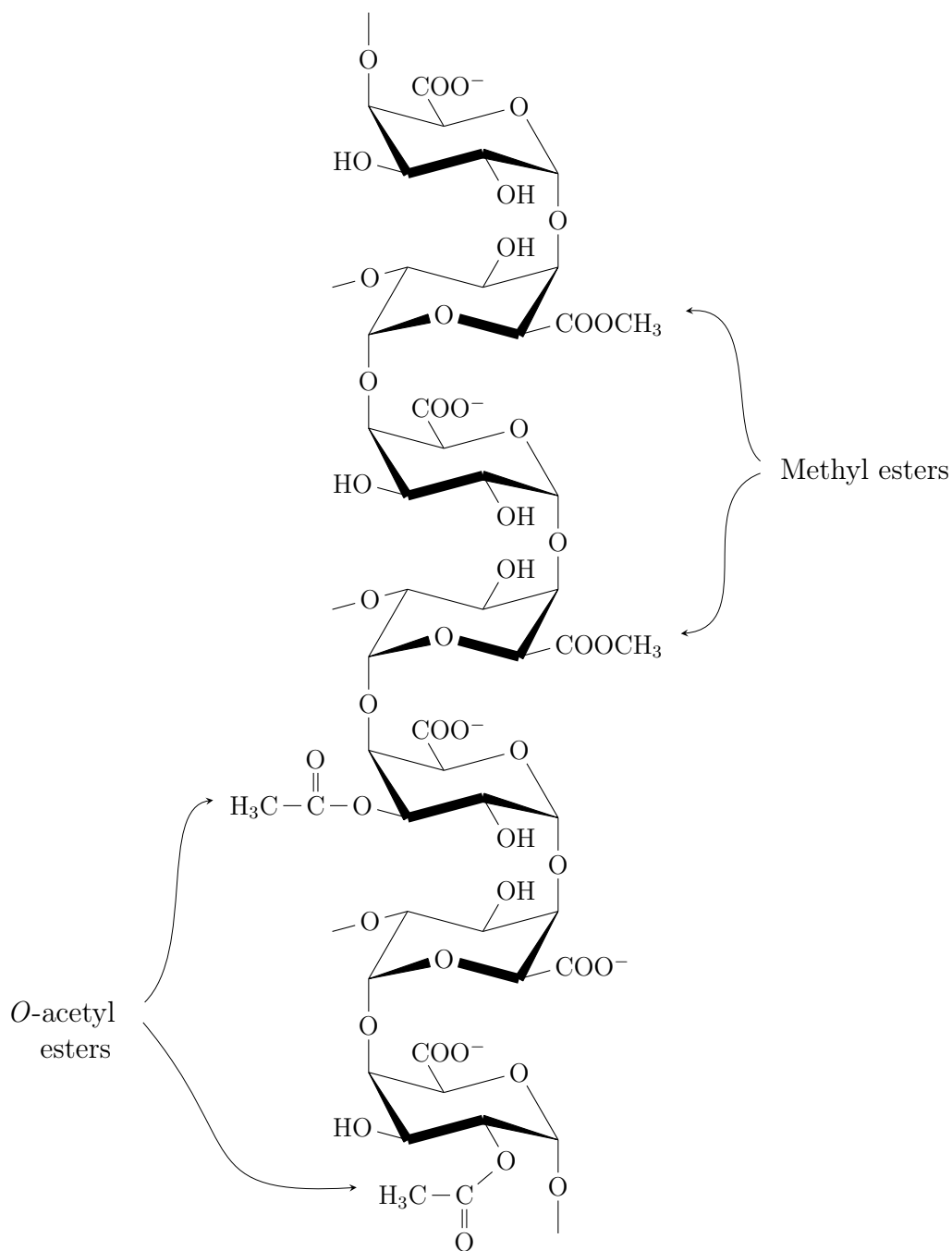


Figure 1.16: *The primary linear structure of homogalacturonan composed of (1, 4)-linked α -D-GalpA residues, partially methyl esterified at C6 and/ or O-acetylated at O2, O3 [107]*

Rhamnogalacturonan-I

Rhamnogalacturonan-I (RG-I) represents 20 – 35% of the pectin family. Its backbone is structured of alternating disaccharides [$(1, 4)$ - α -D-GalpA-(1, 2)- α -L-rhamnopyranosyl (Rhap)-(1,)] in which about 20 – 80% of the Rhap residues are substituted with neutral and/ or acidic oligosaccharides at C4 [84]. The oligosaccharides contain linear and

branched α -L-Araf and β -D-galactopyranosyl (Galp) residues.

Around 2% of the GalpA residues of the backbone are found to have β -D-GlcpA and 4-O-methyl β -D-GlcpA substituted at C3 positions. O-acetylated substitution is often detected in the backbone GalpA of many plants. The basic scheme for RG-I is illustrated in figure 1.17 [83].

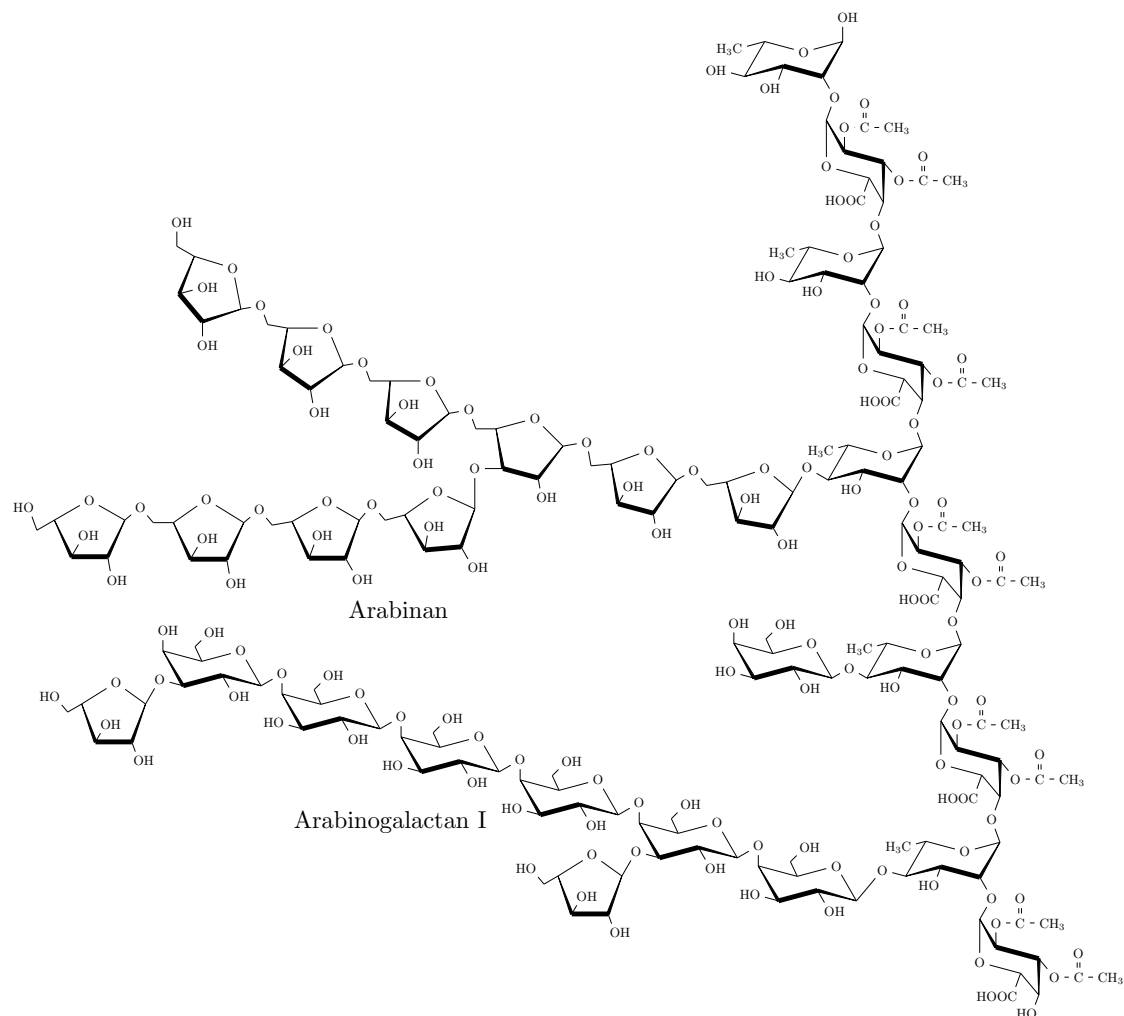


Figure 1.17: Basic scheme for rhamnogalacturonan-I with a backbone of [4)- α -D-GalpA-(1, 2)- α -L-Rhap-(1,] [83]

Substituted galacturonans

Substituted galacturonans (SG), the most complex pectic substance, constitutes up to 10% of pectin (figure 1.18). Their backbones are composed of at least 8 α -D-GalpA residues linked together via (1, 4)-linkages. There are 4 side chains with 12 different types of sugar and over 20 different linkages are attached to O2 and/ or O3 positions [80]. Although O-acetylate substitutions are observed at some of the glycosyl residues of the polysaccharides, their exact locations have not been determined [84].

Terminologies and concepts

Methyl esterification is common in natural pectins, while acetylation is rarer. The degree of esterification (DE) is defined as percentage of the C6 carboxylate groups that are methyl esterified [120]. The DE strongly influences the solubility, gel forming ability, conditions required for gelation, gelling temperature, and gel properties of the preparation [33]. Pectins are classified as high-methyl-ester (HM pectins) if their DE is greater than 50%. Otherwise, they are classified as low-methyl-ester (LM) pectins [120]. Besides that, the terminologies like high-methoxyl pectins [9], or methoxylated [35] are still commonly used. Amidated pectin is evaluated through its degree of amidation, which is the percentage of C6 position of the galacturonic acid groups that are converted to amide groups [120].

1.3.2 Apple pectin

Apple pomace, a by-product from beverage manufacturers, is known as one of the most popular sources for pectin extraction. It contains the peel, core, seed, calyx, stem, and soft tissue of apples during juice production. This residue constitutes approx. 25 – 30% of the total weight of the fresh fruit. Besides using as animal feed, most of it ($\sim 80\%$) is buried or burnt. However, apple pomace is a potential source of polysaccharides, especially pectin. The utilization of apple pomace for pectin extraction meets both the economical and environmental criteria.

Fishman and Jen [33] successfully elucidated the fine structure of apple pectin. According to their study, apple pectin consisted of 'hairy' regions and 'smooth' ones. Of these two regions, the former had a backbone of rhamnogalacturonan, which was attached with arabinogalactan side chains and xylogalacturonan. The latter is referred to homogalacturonan with a DE of 70 – 80%. As mentioned in an earlier work [19], in native pectin, the methyl esterification was found more popular than the acetylation. In apple pectin, acetyl groups were present generally in the 'hairy' regions, while only a small amount was found in the homogalacturonan parts. Furthermore, the methoxyl groups are distributed randomly over the backbone of the pectin.

1.3.3 Chemical modification of pectin

By chemical modification of pectin, many new products can be produced upon industrial importance and purposes. As gelling agents in food industry, pectins were modified to obtain amidated pectins and amidated pectic acid as alternatives to low-methoxyl pectins [104], amidated apple pectin [67], or *N*-alkyl pectinamides [126]. Alkylation using *p*-carboxybenzyl bromide in aqueous alkali and benzyl bromide in the DMSO/

tetrabutylammonium iodide (TBAI) catalyst system were used by Morris *et al.* to derive citrus pectin [81]. Furthermore, low-esterified derivatives with decreased content of methoxyl groups and non-uronic acids from apple pectin were prepared by acid and ammonia modification [69]. For some medical applications, drug delivery, pectins were also chemically modified with acetyl chloride and phenyl acetyl chloride [10].

For paper application in this work, apple pectin was amidated with aqueous ammonia. As mentioned by May *et al.*, pectin could be reacted with ammonia, usually by a heterogeneous reaction in the presence of alcohol. This reaction resulted in amidated pectin, which contained acid amide groups in addition to acid and ester groups [76]. A mechanism including two parallel reactions, namely ammonolysis and hydrolysis proposed by Kratchanov *et al.* [67] is illustrated in figure 1.19.

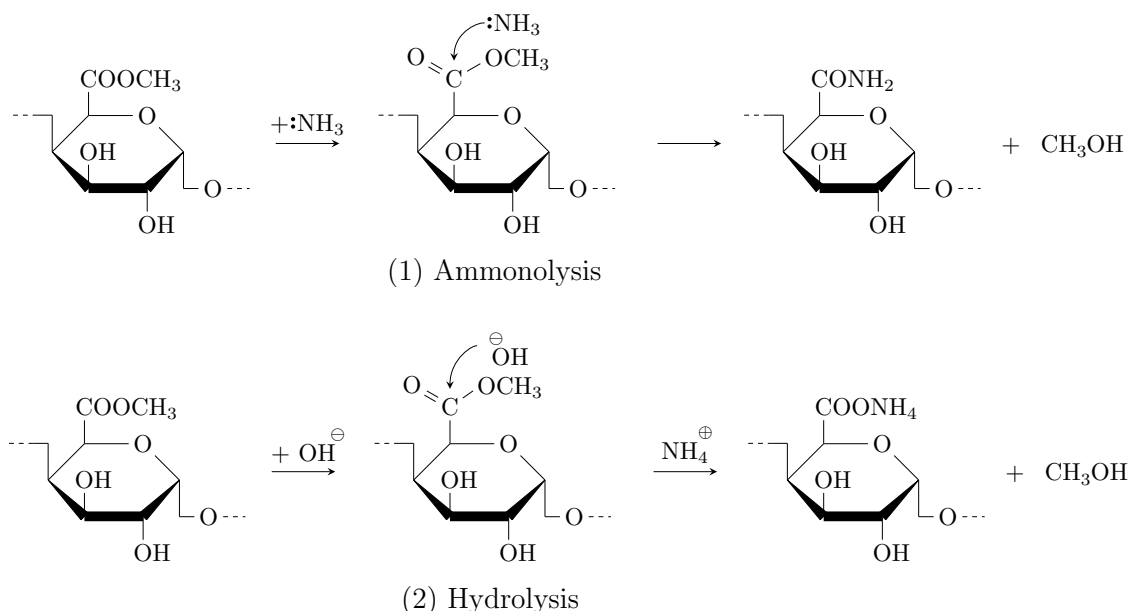


Figure 1.19: *Proposed mechanism for reactions between apple pectin and ammonia in isopropanol [67]*

As also specified by Kratchanov [67], in the presence of alcohol, the extent of ammonolysis and hydrolysis depended on kind and concentration of the used alcohol. With alcohol this rate was much faster than without. They also indicated that the extent of these two reactions of the ester groups were influenced remarkably by the medium and the balance of the reaction equilibrium between ammonia and water:

$$\text{NH}_3 + \text{H}_2\text{O} \rightleftharpoons \text{NH}_4^+ + \text{OH}^-$$

1.4 Application of polysaccharides in paper

1.4.1 Paper formation and mechanical properties

Paper is a fiber network, which is built up from individual fibers, fillers, additives, and other components. This network is formed based on the hydrogen bonds between OH groups on the surface of the cellulose fibers. During the water drainage and drying stages of the paper making process, the hydrogen bonds are created, which contribute significantly to the formation of the final paper. A very simple structure of paper is emulated in figure 1.20.

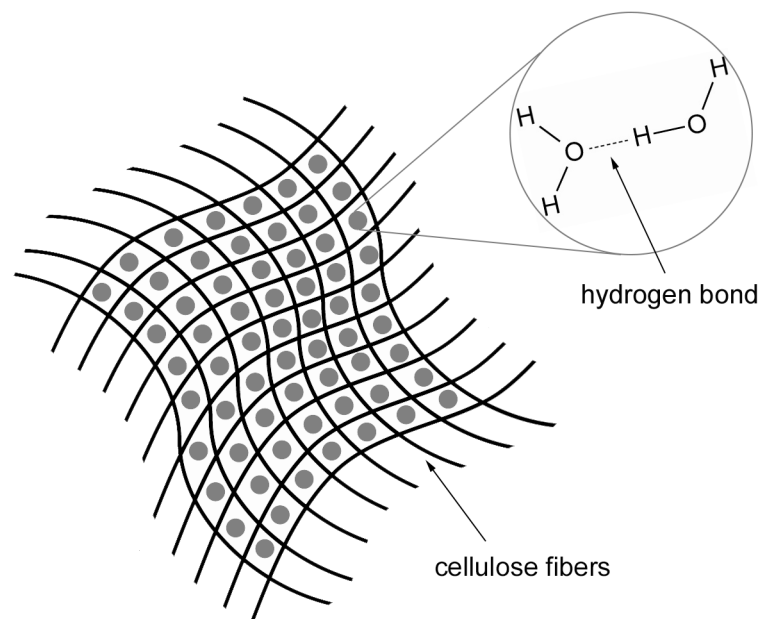


Figure 1.20: *Basic structure of paper based on hydrogen bonds among cellulose chains*

The properties of the components and the interactions among them decide the properties of the paper. Depending on the type of paper, there are different requirements for testing. In this project, tensile, tear, and burst strength of the paper were tested and discussed. This section presents briefly the definition and some characteristics of these properties according to Levlin *et al.* [71].

Tensile strength

Tensile strength is a force required to produce a rupture in a strip of paper or paperboard, expressed in kN/m. Tensile strength depends on fiber strength but primarily on the degree of fibers bonding. Therefore it is commonly used to characterize the capability of bonding among fibers. An increase in the loading rate results in an increase of the tensile strength, whereas moisture content of the paper reduces the tensile strength but increases elongation. In addition, tensile strength is greatly dependent

on directionality of the paper. It is usually measured in MD (machine direction) and CD (cross direction). The property, therefore, can be used to detect fiber orientation. Moreover, tensile strength can be used as a potential indicator of resistance to web breaking during printing or converting. It is noted that when using a paper making machine at a laboratory scale, the paper is formed in a round shape and thus has no MD or CD.

Tensile index is the tensile strength in N/m divided by the basis weight in g/m². The tensile index is expressed in Nm/g. The standard procedure to measure tensile index of paper is described in ISO 1924-2:2008 [48].

Tearing resistance (strength)

Tearing strength is the force required to continue the tearing of paper from an initial cut of a single sheet or a pad of sheets. This parameter depends highly on fiber orientation of the sheet. As a result, there will be machine direction tearing resistance and cross direction tearing resistance corresponding to the cut in the machine direction and cross direction, respectively.

Tearing resistance indicates the behavior of paper in various end use situations, such as evaluating web runnability, controlling the quality of newsprint, and characterizing the toughness of packaging papers where the ability to absorb shocks is essential. Fiber length and inter-fiber bonding are both important factors in tearing strength. The fact that longer fibers improve tear strength is well known. The explanation is straight forward; longer fibers tend to distribute the stress over more fibers and more bonds, while short fibers allow the stress to be concentrated in a smaller area.

Tear index is defined as tearing strength divided by basis weight. The unit of tear index is mN m²/g. The standard procedure to measure tear index is described in ISO 1974:2012 [49].

Bursting strength

Bursting strength is the maximum pressure that the paper can tolerate before rupture and expressed in kPa. The pressure is applied perpendicular to the plane of the test pieces. This parameter is important for bag paper. The procedure for determining bursting strength is described in ISO 2758:2014 [50].

Burst strength depends on basis weight of paper and somehow relates mathematically and physically to the tensile strength and elongation of the paper. To normalize the bursting strength for various types of paper, burst index is usually used. It is defined as bursting strength divided by basis weight. The unit of burst index is kPa m²/g.

Major factors influencing mechanical properties of paper

The mechanical properties of paper depend on many factors, including source of pulp and beating process. **Woods** used for pulping can be divided into two major sources, hardwoods and softwoods. Depending on environment where the trees grow as well as the parts of the tree from which the material is derived, the fiber properties can be different. Softwood fibers are longer and stronger resulting in stronger paper compared to hardwood fibers. However, hardwood pulps are easy to bleach since they contain less lignin content than softwoods. In fact, hardwood and softwood pulps are often mixed together to utilize the advantages from each type for producing desired papers [59].

Besides the source for pulping, **milling** (so-called beating or refining) process plays a very important role in fiber properties. Refining is a mechanical treatment or modification of pulp fibers to optimize their properties for papermaking [11, 75]. The refining process can affect the fibers in many aspects as specified by Page [88]:

- cutting or shortening of the fibers;
- fine production and the complete removal of parts of the fiber wall, creating debris in suspension;
- external fibrillation, the partial removal of the fiber wall, leaving it still attached or swelling;
- curling or straightening the fibers;
- inducing or removing nodes, kinks, slip planes, micro-compressions in the cell wall;
- dissolving or leaching out colloidal material into the external liquor;
- redistribution of hemicellulose from the interior to the exterior of the fiber;
- abrasion of the surface at the molecular level to produce a more gelatinous surface.

After beating, fibers are flattened, become more flexible, and reveal more surface area for bonding resulting in an increase of fiber-to-fiber bonding strength [11, 75]. Strength properties of paper, which are dependent on the fiber-to-fiber bonding like tensile and burst strength will increase with pulp refining. On the contrary, individual fibers are also weakened and becoming shorter due to cutting action during refining [11]. Tear strength usually decreases with refining since this parameter highly depends on individual fiber strength. After a certain point, the role of fiber-to-fiber bonding is switched to the role of individual fiber strength. This leads to the fact that refining

beyond this point begins to decrease other strength properties than tear strength. The refining process is evaluated through refining degree of the milled pulp expressed as Schopper Riegler degree ($^{\circ}\text{SR}$).

1.4.2 Polysaccharides as paper additive

Additives are used widely in paper technology for many purposes [11]. For a long time, fillers like calcium carbonate, titanium dioxide, or clay have been used to improve the opacity and brightness of the paper. Regarding the dry strength of the papers, many synthetic and natural compounds have been used. Among the well-known dry strength additives, polyacrylamides and their ionic derivatives are most popularly used. These chemicals are sometimes used to allow increased use of lower quality pulps such as secondary or hardwood fibers. Due to the presence of many components in the paper, retention aids are necessary to improve the retention of all the materials on the paper machine wire. High molecular weight polymers like cationic and anionic polyacrylamide and cationic starch are applied widely as retention agents. Besides them, polyethylenimine and poly-diallyldimethylammonium chloride (DADMAC) referred to high charge density reagents are other choices.

It is well-known that wood fibers carry negative charges on their surface. As the result, they repel other fibers or any like charged objects. Whereas, the fiber surface is attracted to oppositely charged particles or polymers [11, 117]. Besides a number of synthetic chemicals as mentioned previously, natural polymers like plant-based polysaccharides are of interest since these materials are sustainable, renewable, and recyclable.

In this work, we used xylan and pectin extracted from agricultural residues and modified them to obtain their cationic derivatives for paper application. This section briefly presents the application of these two polysaccharides.

Application of xylan and its derivatives in paper

The applications of hemicelluloses in general and xylan in particular have been studied for decades. Hemicellulose as well as xylan are known as potential bio-polymers, which can be used in their natural or modified forms in various areas including food and non-food applications [23]. Hemicelluloses are used mainly for production of sugars, useful chemicals, and biofuels. Some examples of xylan applications are found in production of wound dressing, drug carrier as well as in food technology, such as food additives.

Besides applications in food and medicine as mentioned above, the utilization of

hemicelluloses as an additive in paper is also relatively common since they are able to enhance paper strength. In particular, the use of xylan in paper technology is also drawing much attention. A lot of research has shown that xylan from different sources, especially from wood materials can contribute significantly to enhance paper properties. Han *et al.* [38] discovered that when adding birch xylan into eucalyptus kraft pulp, tensile, burst, and tear index were all improved. The used xylan also contributed significantly to the pulp beatability. The positive effect of xylan on paper strength properties was also proved by Schönberg *et al.* [115]. In their study, they investigated the addition of xylan onto spruce kraft pulp and found that the sorption of xylan obviously influenced the tensile index of the hand sheets. The adsorbed xylan also affected the swelling of fibers, which in turn increased the density of the fiber network.

Xylans from non-wood materials, including agricultural residues, are also fascinating objects for studying. Addition of arabinoxylan from corn cob and oat spelt into spruce kraft pulp was investigated by Ramírez *et al.* [103]. When the xylans were added into the pulp before beating, they affected the beating degree of the pulp most effectively. Their results indicated that tensile and burst index of the papers with xylan added were improved impressively over the whole range of the refining degrees of the pulp. However, the tear index was decreased with the increase of the beating degree of the pulp.

Xylans were also used in its modified forms for improving paper properties. Many previous studies have demonstrated that cationic derivatives of xylan were able to enhance the paper strength. Quaternized xylan from beechwood was prepared using 3-chloro-2-hydroxypropyltrimethylammonium chloride and applied as the retention aid in papermaking [25]. Cationic birch xylan modified with 2-hydroxypropyltrimethylammoniumchloride was added into spruce sulfite pulp and birch kraft pulp in order to evaluate its effect on the pulp properties [117]. Their results suggested that the cationic xylan could improve significantly the tensile and burst index with both types of pulp. The electrostatic interaction was believed to be the most important factor influencing the adsorption of the cationic xylan. In another work of Deutschle and co-workers [21], cationic xylans from oat spelts and wheat were added into bleached eucalyptus kraft pulp. They found that the addition of the cationic xylans brought a remarkable enhancement in tensile, burst, and tear index, which was compatible to cationic starch except for tear index.

Adsorption mechanism of xylan onto cellulose fibers. Regarding the xylan assembly onto cellulose surfaces, Linder *et al.* [73] proposed a clear hypothesis, which is illustrated in figure 1.21.

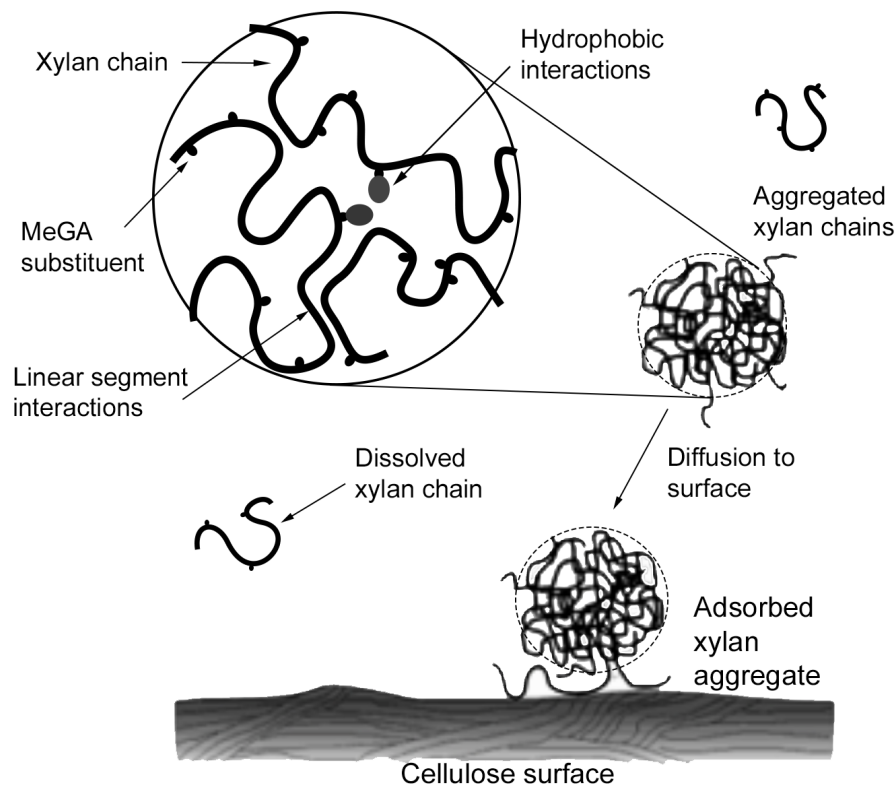


Figure 1.21: *Mechanism for xylan aggregation in solution and interaction with cellulose surfaces [73]*

In their study, 4-*O*-methyl glucuronoxylan isolated from birch was adsorbed onto bacterial cellulose. According to their results, the main part of xylan existed in aggregate structure although it could also exist as dissolved single molecules. These two structures of the xylan were able to diffuse and adsorb onto cellulose surfaces. However, the aggregate parts contributed mainly to the adsorbed xylyns. On the one hand, the aggregation could be favored by interactions between unsubstituted, linear chains, as well as the hydrophobic reactions, which occurred due to a small amount of aromatic substituents. On the other hand, the presence of negatively charged 4-*O*-methyl glucuronic acid (MeGA) side chains increased the repulsion among xylan molecules and supported the dissolution.

In summary, based on this scheme, each fragment of xylan was first attracted by the cellulose surface, adsorbed on the surface, gradually unfolded and finally set in a stable location, orientation and conformation. During the interaction, cellulose and xylan were subjected to constant conformation fluctuation.

Factors influencing the adsorption of xylan on cellulose fibers. Several studies were conducted to discover how the structure of xylan affected its adsorption onto cellulosic materials. The arabinofuranosyl (Araf) substituents were proved to have a

clear impact on the adsorption of xylan onto bacterial cellulose surfaces by Kabel and partners [61]. Their findings showed that xylans with lower degree of *Araf* substitution like OSX resulted in higher amount of adsorbed xylan on the bacterial cellulose.

Adsorption was known to depend on the molecular structure of xylan, especially when it contained ionic acid groups as indicated by Linder *et al.* [73]. According to this work, xylans with low content of ionic acid groups produced the highest adsorption, particularly at 125 °C.

In another work, Paananen *et al.* suggested that the driving force for adsorption of xylan on cellulose was a combination of two factors. The first one was the inherent entropy increase, which was associated with the release of solvent molecules when polymers were adsorbed. The other was the weak Van der Waals attraction rather than the formation of hydrogen bonds [87]. Concerning the driving force, Köhnke *et al.* also agreed that the structure of xylan definitely had an effect on adsorption characteristics. According to their results, a decrease in the arabinofuranosyl/ xylopyranosyl (*Araf*/*Xylp*) ratio caused an increase in the amount of adsorbed xylan on the microcrystalline cellulose. This made the driving force for adsorption stronger and easier for approaching the xylan molecules [64]. Research on cationic xylan for paper application started in 1991 by Antal *et al.* They used 2-hydroxypropyltrimethylammonium xylan gained by treating aspen wood powder with 1-chloro-2-hydroxypropyltrimethylammonium. Their results showed that the cationic xylan improved tensile and burst index but influenced the tear index insignificantly. In further studies, Antal *et al.* found that retention behavior was mostly related to the surface charge of the polymer and independent on the xylan source [4]. In the same year, Kataja-aho *et al.* used cationized xylan from birch in pulp and tested its influences on dry and wet strength of fine paper. They published that cationized birch xylan had a positive effect on the tensile index, but not with values as high as when cationized starch was used [63]. Application of cationized xylan from birch in paper was also studied by Schwikal's team. In their project, cationized birch xylans with different degrees of substitution were added into birch kraft pulp and spruce sulfite pulp as paper making additives. The trend in tensile and burst indices they found was similar to the results of Kataja-aho as mentioned previously. [63,117]

Non-wood materials, including agricultural residues have also been utilized recently as sources of hemicelluloses. A study on absorption of arabinoxyylan from corn cob onto bleached and unbleached softwood kraft pulp by Ramírez *et al.* showed significant improvements in tensile and burst index [103]. In the same year, 2008, Köhnke and co-workers also published their discovery about the influence of barley husk arabinoxyylan on the properties of cellulose fibers [65]. Their results showed that the adsorption of the used xylan enabled the ability to produce market pulps with increasing tensile

strength and beatability.

Applications of pectin

The most popular and well-known use of pectin is in food industry due to its ability in gelling, thickening, emulsifying and stabilizing. Pectin is used as a gelling agent in jelly, and jams [121]; as a stabilizer in UHT-treated drinkable yogurt [76]. For some soft drinks, pectin can be used to improve the texture of the product. In water ice, pectin is used to control ice crystal size [76].

Pectin is also used widely in medical industry. It is traditionally applied in diarrhea treatment as well as to maintain the viscosity of some syrups. Pectin can also be used in the development of drug delivery systems [78]. Another recent application is its use as hydrophilic filler in some self-adhesive colostomy flanges, and in wound dusting powders and ulcer dressings.

As a polysaccharide, pectin might also have potential to be applied in paper although this application is not popular at present. Due to its high viscosity, pectin should be modified before using. Depending on the source, pectin has different properties which will require corresponding conditions to modify it. In this project, apple pectin was modified with ammonia solution before applying it into paper. Like xylan and cellulose, pectin is also a slightly negative molecule due to its high content of carboxylic groups. By modification, pectin can be converted into cationic derivatives before adding it into paper. However, there are very few publications that discuss the issue of pectin application in paper.

2

Experimental

2.1 Materials

2.1.1 Polysaccharides

Cellulose

An elementary chlorine-free (ECF) bleached softwood Kraft pulp purchased from Zellstoff Stendal GmbH was used for paper making process. The pulp was received in form of pulp sheets with dry content of about 95%.

Pectins

Apple pectins with different DE were purchased from Herbstreith & Fox KG (Neuenbürg, Germany). The used pectins are listed in table 2.1.

Table 2.1: *List of used apple pectins*

<i>Product name</i>	<i>Code</i>	<i>Properties</i>
Classic AU 910	AP1	light beige powder pH: 4.8 ± 0.3
Herbapekt SF 50-A-LV (instant pectin)	AP2	light beige powder almost no viscosity pH: 3.6 ± 0.3
Classic AU 202	AP3	light beige powder pH: 2.8 ± 0.3

Xylans

Oat spelt xylan samples and some cationic xylan derivatives were received from Dr. Kay Hettrich (Fraunhofer Institute for Applied Polymer Research (IAP), Potsdam-Golm, Germany). Wheat arabinoxylan was purchased from Jäckering Mühlen- u. Nahrungsmittelwerke GmbH (Hamm, Germany). List of xylans used in the project is described in table 2.2.

Table 2.2: *List of used xylans*

<i>Sample</i>	<i>Source</i>	<i>DS</i>	<i>Description</i>
PST7	oat spelt xylan	-	Wolff Technikum
09-Xyl-47	oat spelt xylan	-	Extraction IAP
09-Xyl-01	oat spelt cationic xylan	0.14	modified with EPTAC
09-Xyl-14	oat spelt cationic xylan	0.26	modified with EPTAC
09-Xyl-15	oat spelt cationic xylan	0.09	modified with CHPTAC
09-Xyl-21	beech cationic xylan	0.15	modified with CHPTAC
09-Xyl-24	beech cationic xylan	0.08	modified with EPTAC
09-Xyl-23	birch cationic xylan	0.15	modified with CHPTAC
09-Xyl-25	birch cationic xylan	0.09	modified with EPTAC
12-Xyl-14	oat spelt xylan	-	Extraction IAP
13-Xyl-01	oat spelt xylan	-	Extraction IAP
13-Xyl-02/1	oat spelt xylan	-	Extraction IAP
13-Xyl-03	oat spelt xylan	-	Extraction IAP
13-Xyl-04	oat spelt xylan	-	Extraction IAP
13-Xyl-05	oat spelt xylan	-	Extraction IAP
14-Xyl-01	oat spelt xylan	-	Extraction IAP
14-Xyl-04	oat spelt xylan	-	Extraction IAP
15-Xyl-01	oat spelt xylan	-	Extraction IAP
15-Xyl-04	oat spelt xylan	-	Extraction IAP
WAX	wheat arabinoxylan	-	Jäckering Mühlen- u. Nahrungsmittelwerke GmbH

2.1.2 Chemicals

Used chemicals, mostly from Germany are listed in table 2.3.

Table 2.3: *List of used chemicals*

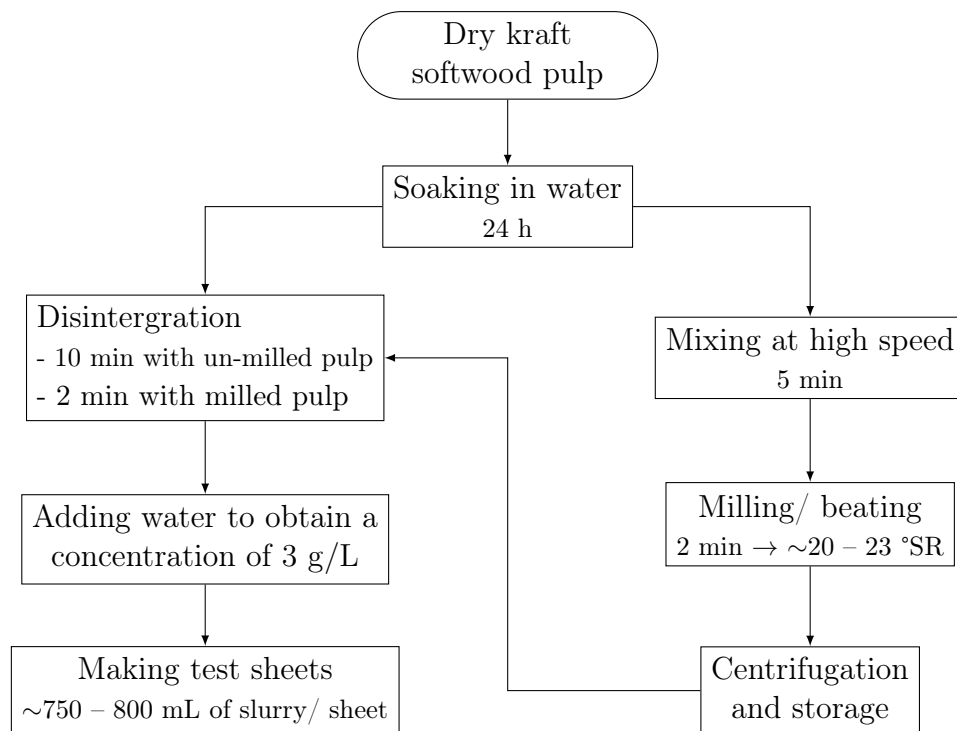
<i>Chemical</i>	<i>Supplier</i>
2,3-epoxypropyltrimethylammonium chloride, $\geq 90\%$, technical grade	Fluka Chemie GmbH
(3-chloro-2-hydroxypropyl)trimethylammonium chloride, $\geq 60\%$ aq.	Fluka Chemie GmbH
Acetic acid, 100%, p.a.	Carl Roth GmbH+Co KG
Ammonia, $>25\%$, p.a.	Carl Roth GmbH+Co KG
D(-)-Arabinose, $\geq 99\%$ (HPLC)	Fluka Chemie AG
Chlorine dioxide	Zellstoff- und Papierfabrik Rosenthal GmbH, Mercer
Copper sulfate pentahydrate, p.a.	VEB Jenapharm
Cupric-ethylene-diamine, 3 N aq.	VWR Chemicals
D(+)-galacturonic acid monohydrate, $\geq 97\%$	Fluka Sigma Aldrich
EDTA, $\geq 99\%$, p.a.	Carl Roth GmbH+Co KG
Ethylene glycol, $\geq 99.5\%$, p.a.	Carl Roth GmbH+Co KG
Eriochromschwarz-T	VEB Berlin
Ferulic acid, $\geq 98\%$	Merck-Schuchardt
Ethanol, $\geq 96\%$	Berkel Unternehmung GmbH+Co KG
Ethanol, ca. 70%	inventory
D(+)-Galactose, $\geq 99\%$ (HPLC) for microbiology	Fluka Chemie AG
D-Glucose, $\geq 99.5\%$	Sigma Aldrich
Glucose pentaacetate, $\geq 98\%$	Merck-Schuchardt
Hydrochloric acid, $\geq 32\%$, p.a.	Carl Roth GmbH+Co KG
Hydrochloric acid, 32%, technical grade	Carl Roth GmbH+Co KG
Hydrogen peroxide, 35% aq., p.a.	Carl Roth GmbH+Co KG
Hydroxylammonium hydrochloride, $\geq 99.5\%$, p.a.	Carl Roth GmbH+Co KG
Iron (III) chloride, purified	Carl Roth GmbH+Co KG

Table 2.3: *List of used chemicals (cont.)*

<i>Chemical</i>	<i>Supplier</i>
Iso-propanol, $\geq 99\%$, technical grade	Berkel Unternehmung GmbH+Co KG
Iso-propanol, 80%, v/v	inventory
D(+)-Mannose, $\geq 99\%$ (HPLC), biochemistry grade	BioChemie Bernd Belger
Methanol, $\geq 99\%$	Merck KGaA
Methanol, 60%, v/v	inventory
Methyl orange	inventory
Murexid indicator	VEB Spezialchemie Leipzig
Oxygen	AIR LIQUIDE Deutschland GmbH
Perchloric acid, 70% aq.	Carl Roth GmbH+Co KG
Potassium bromide, spectroscopy grade	Merck KGaA
Potassium iodide, $\geq 99.5\%$, p.a.	Carl Roth GmbH+Co KG
Silver nitrate, ca. 1% aq.	inventory
Sodium acetate trihydrate, $\geq 99\%$	Carl Roth GmbH+Co KG
Sodium hydroxide, 0.1 N aq.	Carl Roth GmbH+Co KG
Sodium hydroxide, $\geq 99\%$, granulate	Carl Roth GmbH+Co KG
Sodium <i>m</i> -periodate, $\geq 99\%$, p.a.	Sigma Aldrich
Sodium thiosulfat, 0.1 N aq.	Carl Roth GmbH+Co KG
Starch, ca. 1% aq.	inventory
Sulfuric acid, $\geq 95\%$	VWR Chemicals
Trifluoroacetic acid, $\geq 99\%$, p.a.	Merck KGaA
Xylose, microbiology grade	BioChemie Bernd Belger
Zinc sulfate heptahydrate, $\geq 99.5\%$, p.a.	Carl Roth GmbH+Co KG

2.2 Pulp preparation

In order to determine the effect of the milling process on sheet formation and paper properties, un-milled and milled pulp slurry were prepared following the process described in figure 2.1 according to ISO 5269-1:2005 standard [52]. Tap water was used in all cases to bring the experiments as close as possible to the industrial conditions.

Figure 2.1: *Pulp preparation process*

2.2.1 Preparation of un-milled pulp

A calculated amount of pulp (to make a pulp slurry of 3 g/L concentration) was soaked in tap water for 12 – 24 h to make it swollen completely. The mixture was then disintegrated in a high speed disintegrator for 10 min (rotation speed was set at 30 000 rpm). Mixed pulp was transferred to a 10-L tank and filled up with tap water. Each test sheet was made from about 800 – 850 mL of the pulp slurry. Finished sheet should weigh approx. 2.4 g (equivalent to the basis weight of 80 g/m²). The volume of slurry for each sheet was adjusted after making the first one.

2.2.2 Preparation of milled pulp

At the first step, the pulp was prepared using the same process as for the un-milled pulp. Swollen pulp was mixed carefully in a lab-scale disk refiner equipped with 2 plates: the top plate is stationary and the plate under that rotates. Some important parameters of the refiner are as follows:

- plates: \varnothing 200 mm, Raffinator type D, Defibrator (Stockholm, Sweden)
- knife angle: 60°
- knife width: 5 – 10 mm (from inside to outside)
- groove between knives: 4 mm

The milling time and consumed energy were controlled to obtain the desired °SR of pulp. Milled pulp was then centrifuged to remove water and estimated its dry content before storing in a refrigerator for further use.

2.2.3 Determination of Schopper Riegler degree

The procedure was carried out according to ISO 5267-1:1999 standard [51]. 2 g (abs. dry) of pulp was taken and dispersed in one liter of tap water. The suspension was poured in the fill chamber, which was closed by the conical nipple. The conical nipple was lifted pneumatically after pushing the start handle, and the suspension discharges. The fibers were retained while the filtrate drains. The drainage time depends on the composition of the fiber suspension. The filtrate flows into the measuring beaker through the lateral outlet. There the freeness is shown in °SR.

The Schopper Riegler degree (°SR) describes the amount of drained suspension and is the degree of the drainage properties of the pulp, which has been diluted in water. One °SR corresponds to 10 ml of water. Since water hardness and temperature are significantly influencing the results, it is very important to ensure that the measurement is always performed with the same water hardness and at 20 °C.

2.3 Paper making process

Paper sheets were made with a paper making machine at laboratory scale following the ISO 5269-2:2004 [53]. In each experiment, 4 – 5 replicates resulting in corresponding number of sheets were carried out. This process was done at the Institute of Wood and Paper Technology (IHP), Technische Universität Dresden (TUD).

2.4 Paper properties test

Finished paper sheets were kept in a climate-controlled room according to ISO 187:1990 [47] for moisture balancing before testing their properties. Major parameters with corresponding testing standards are listed in table 2.4. All of the paper sheets were made and tested with the equipment at the IHP, TUD, Germany.

Table 2.4: *Paper properties and testing standards*

<i>No.</i>	<i>Parameter</i>	<i>Unit</i>	<i>Standard</i>	<i>Reference</i>
1	Basis weight	g	ISO 536:2012	[56]
2	Thickness	μm	ISO 534:2011	[54]
3	Tensile index	$\text{mN m}^2/\text{g}$	ISO 1924-2:2008	[48]
4	Tear index	Nm/g	ISO 1974:2012	[49]
5	Burst index	$\text{kPa m}^2/\text{g}$	ISO 2758:2014	[50]

2.5 Xylan extraction from oat spelt

This experiment was conducted at the IAP, Potsdam-Golm, Germany. The isolation was performed according to the well-known procedure published by Puls *et al.* [101]. Oat spelts were received from Peter Kölln KGaA Elmshorn. The extraction was carried out in a 5 L ploughshare mixer designed by Lödige. 373.3 g of oat spelt was dispersed in 2.5 L of sodium hydroxide solution (NaOH, 5%, w/w). The dispersion was heated during moderate mixing at 90 °C for 90 min.

OSX was precipitated from the extract by adding 5.4 L of methanol (60%, v/v) and separated by centrifugation using a Cryofuge 6000i centrifuge (Heraeus Sepatech, Germany; relative centrifugal force (RCF) = 4096, 4000 rpm, 45 min). Subsequently the precipitate was treated with 9.97 g of hydrogen peroxide solution (35%, v/v) and 2.32 g of NaOH at 90 °C for 2 h in 1.2 L of the methanol/ water mixture. The mixture was then again cooled down and centrifuged.

The product was isolated and washed three times with 2 L of the methanol/ water mixture and twice with 2 L of pure methanol. The washing process was carried out by vacuum filtration using a G3 filter. Approx. 65 g of xylan were obtained after drying in a vacuum dryer at 40 °C.

2.6 Chlorine dioxide bleaching process applied for xylan

Approx. 1 g (abs. dry) of xylan was placed in a cylinder flask. Active chlorine dioxide solution (10 g/L, pH \sim 2.4, 60 mL) was added subsequently. The reaction flask was placed in a water bath set at 70 °C for 2 h with stirring. After the reaction time, the xylan was separated from the liquid part by using 0.45 μm cellulose acetate membrane

filter, \varnothing 47 mm (Carl Roth GmbH Co. KG). The product was then washed with distilled water for several times before recording its spectra with an Fourier transform (FT)-Raman spectrometer.

2.7 Cationization of xylan using etherifying agents

Two etherifying agents, 2,3-epoxypropyltrimethylammonium chloride (EPTAC) and (3-chloro-2-hydroxypropyl)trimethylammonium chloride (CHPTAC), were used to cationize oat spelt xylan. These substances are known as etherifying or cationizing reagents, which are widely used in polymer modification. The work was done at the Fraunhofer Institute for Applied Polymer Research (IAP), Potsdam, Germany.

2.7.1 Cationization of xylan with 2,3-epoxypropyltrimethylammonium chloride

2,3-epoxypropyltrimethylammonium chloride is so-called glycidyltrimethylammonium chloride and commonly referred to as epoxide or QUAB[®]151 as a trade name. The structure of EPTAC is given as in figure 2.2.

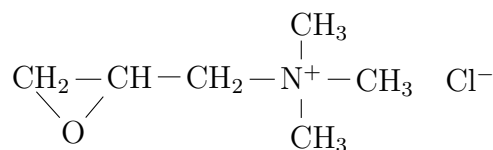


Figure 2.2: *Chemical formula of 2,3-epoxypropyltrimethylammonium chloride*

EPTAC is unstable in air, since the epoxy group is quickly decomposed and forms 2,3-dihydroxypropyltrimethylammonium chloride (DHPTAC) as shown in figure 2.3.

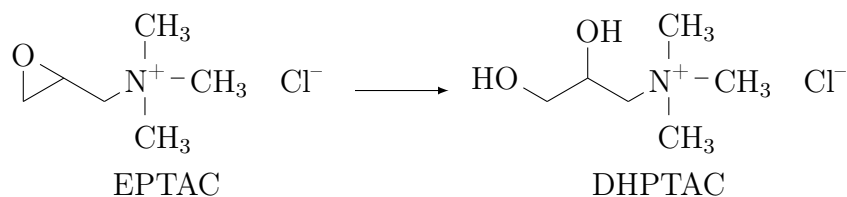


Figure 2.3: *Decomposition of 2,3-epoxypropyltrimethylammonium chloride in air*

When using EPTAC, it is only needed in a catalytic amount (1 mole of EPTAC requires 0.1 mol of NaOH), which leads to a very thick reaction mixture. The stirring process, therefore, can be difficult. The ratio in mole among xylan, EPTAC, and NaOH was 1 : 1 : 1. Chemicals were calculated for 10 g (abs. dry) of xylan, which was equal to 0.0758 mol.

Sodium hydroxide (0.3 g) was placed into a glass bottle. Distilled water was added

until the net weight of the mixture reached 24 g. Xylan (10 g abs. dry) was added subsequently and the mixture was mixed well until homogeneous before 0.0758 mol of EPTAC (11.4 g) was added. The reaction was conducted at 40 °C, for 4 h. After that, the bottle was cooled down to room temperature before precipitating with 250 mL of ethanol. The sample was washed with ethanol (80%, v/v) for 3 times by vacuum filtration using a G3 filter. When the filtrate was free of chloride (tested with silver nitrate solution), the product was washed one more time with pure ethanol. Clean product was dried in a vacuum dryer at 40 °C and 7 mbar.

2.7.2 Cationization of xylan with (3-chloro-2-hydroxypropyl) trimethylammonium chloride

(3-chloro-2-hydroxypropyl)trimethylammonium chloride is known as QUAB[®]188 as its trade name and commonly referred to as chlorohydrin. The structure of this substance is shown in figure 2.4.

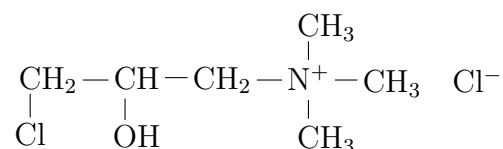


Figure 2.4: *Chemical formula of (3-chloro-2-hydroxypropyl)trimethylammonium chloride*

Unlike EPTAC, CHPTAC is more stable and usually used at much higher amount (1 mol of CHPTAC needs 1.2 mol of NaOH). The reaction mixture, therefore, will contain more liquid, which makes the stirring process much easier. In this experiment, CHPTAC was used with two different amounts resulting in different DS of the xylan. For that purpose, two different ratios among xylan, NaOH, and CHPTAC namely 1 : 1 : 1 and 1 : 1 : 0.5, were conducted. Chemicals were calculated for 7 g of xylan, which was equal to 0.053 mol.

The following substances with specific amounts were added in succession to a glass bottle:

- 0.0625 mol (2.5 g) of sodium hydroxide
- an amount of distilled water until the net weight of the bottle reached 50 g
- 7 g of xylan; the mixture was mixed well until homogeneous
- 0.053 mol (9.964 g) of CHPTAC

The reaction was conducted at 40 °C, for 4 h. After that, the bottle was cooled down to room temperature before precipitating with 250 mL of ethanol (96%). The sample

was washed by vacuum filtration using a G4 filter and ethanol (80%, v/v) for 3 times. When the filtrate was free of chloride (using silver nitrate solution for testing) the product was washed one more time with ethanol (96%). The clean product was dried in a vacuum dryer at 40 °C.

2.8 Chemical modification of xylan using different reagents

In this section, OSX was oxidized with three different oxidants and followed by an amidation. The products after oxidation and amidation were dialdehyde xylan (DAX) and cationic xylan (CatX), respectively.

2.8.1 Oxidation

Modification of xylan can produce several chemical compounds, which have potential applications in medicine, food, and paper technology [23]. This oxidation is considered as an important step to activate the xylan structure for further modification. Sodium metaperiodate, hydrogen peroxide, and ozone, which are able to modify the five-carbon ring structures, were selected. The reactions with these chemicals will break C2 – C3 and/ or C3 – C4 linkages of the xylose residues and produce aldehyde groups at those positions.

In all cases, the oxidation was evaluated through the aldehyde content and intrinsic viscosity of the oxidized product following the analytical methods described in section 2.10.

Oxidation with sodium metaperiodate

The periodate oxidation of xylan was performed with 0.1 M sodium *m*-periodate solution, at room temperature, in the dark with continuously stirring for different periods of time ranging from 15 min to 2 h. After the reaction, excess periodate was distorted with 2 mL of ethylene glycol [118,133]. Products, in gel form, were washed by centrifugation using a Biofuge 15 R (Heraeus Sepatech, Germany; RCF = 3960, 5300 rpm, for 45 – 60 min). Distilled water was replaced each time until the conductivity of the filtrate was below 1.00 $\mu\text{S}/\text{cm}$. The oxidized products were dried in a freeze dryer. The molecular weight of xylan after the oxidation was estimated through its intrinsic viscosity, which was measured with a Ubbelohde viscometer. Furthermore, aldehyde content was determined in order to evaluate the oxidation effectiveness following the procedure described in section 2.10.1.

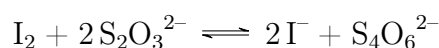
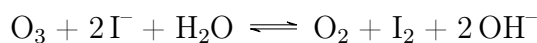
Oxidation with hydrogen peroxide

A solution of 30% hydrogen peroxide was used in this reaction. The expected concentration was obtained by mixing concentrated hydrogen peroxide and distilled water in a calculated volume ratio.

Xylan (3 g abs. dry) was dispersed in a small amount of distilled water and stirred for 10 – 15 min. Calculated volumes of hydrogen peroxide (30%, v/v) were added to obtain the desired concentration of the agent. The reaction was carried out in a three-neck round bottom flask with a slowly bubbling steady air stream. The volume was kept constant by connecting with a reflux condenser. After the reaction time, the mixture was washed several times with distilled water by centrifugation using a Biofuge 15R centrifuge (Heraeus Sepatech, Germany, RCF = 3960). The product was then dried in a freeze dryer.

Oxidation with ozone

Pure xylan was dispersed in water for 5 – 10 min and filled up with water to make a solution of around 3% (w/w). The mixture was then bubbled with a stable air stream containing ozone. Ozone was produced with a Sander Labor ozonisorator using oxygen supplied by AIR LIQUIDE Deutschland GmbH. Speed of the air stream and current intensity were controlled at 60 g/min and 0.3 A, respectively, to reach a concentration of 2 – 3 g O₃/ m³. Excess ozone was determined by the potassium iodide (KI) wet-chemistry method. The principle of this method is based on the reaction between the iodide ion and the ozone to form iodine. The liberated iodine is titrated by standardized sodium thiosulfate using starch as an indicator. The mass of reacted ozone and excess ozone from the oxidation reaction is determined based on a theoretical ozone/ iodine stoichiometry of 1.0 as shown in the following reactions:



The reaction between xylan and ozone was carried out for specific periods of time. After that, the oxidized xylans were washed several times with distilled water by centrifugation using the Biofuge 15R (Heraeus Sepatech, Germany; RCF = 3960; 5300 rpm) before being dried in a freeze dryer. Yield, viscosity, and aldehyde content of the products were analyzed to evaluate the effectiveness of the oxidation process.

2.8.2 Amidation

The amidation was conducted using aqueous ammonia in isopropanol according to the procedure published by Kratchanov *et al.* [67]. Ammonia molecules are known to have a strong tendency to donate their lone pairs of electron of nitrogen to other molecules. Thus, they act like a strong Lewis base in reactions with carboxyl and carbonyl substances, including arabinoxylan, which contains glucuronic acid residues, or dialdehyde xylan.

Procedure

The sample (15 g abs. dry) was weighed into an Erlenmeyer flask. 100 mL of 4 M ammonia in isopropanol (prepared from 25% ammonia solution using 80% (v/v) isopropanol as solvent) was then added. The flask was closed properly with a stopper and allowed to stand for a certain period of time with occasional shaking.

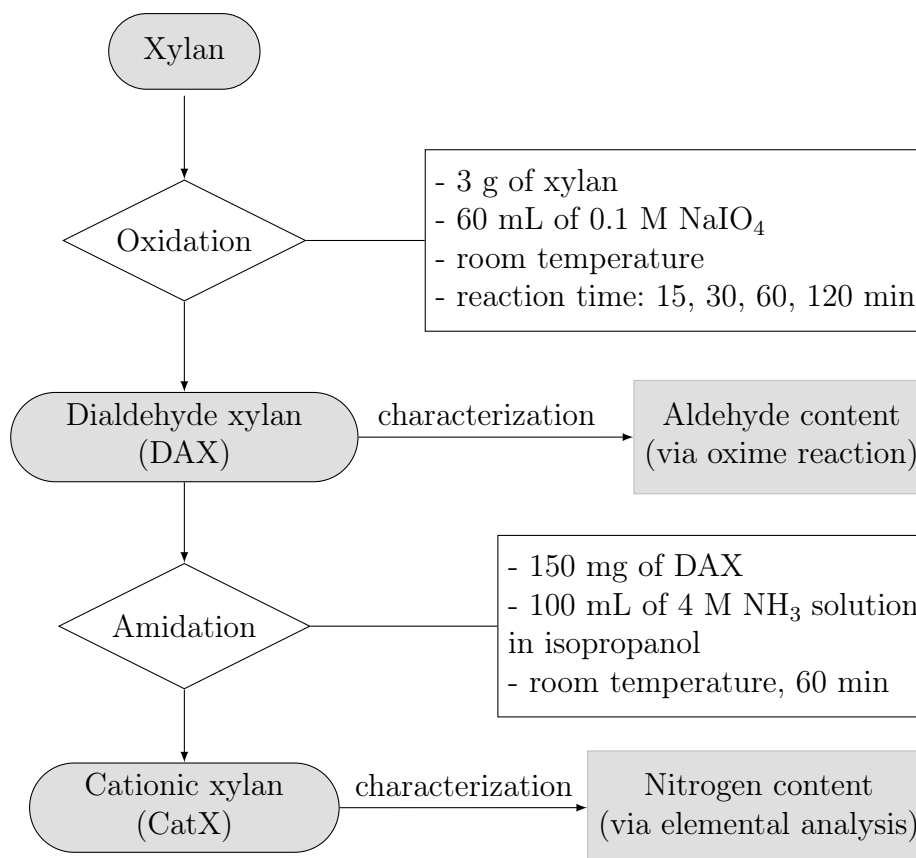
After the required reaction time, the mixture was quickly filtered through a G3 filter (not more than 3 min). The sample was then washed with 100 mL of cold (0 °C) EtOH-HCl by vacuum filtration and then again soaked for 3 min in 100 mL of this mixture. The sample was finally washed with 70% ethanol until the filtrate was free of chloride (tested with silver nitrate solution (AgNO_3)). The product was air dried at 30 °C over night before conducting any further analysis.

Preparation of specific chemicals

- *Hydrochloric-ethanol (EtOH-HCl)*: produced by preparing 5% hydrochloric acid solution using 70% ethanol as solvent. This solution was kept at 0°C before using.

2.8.3 Two-step modification

Two-step modification consisting of an oxidation and an amidation process was applied for xylan. The OSX/ wheat arabinoxylan (WAX) was first oxidized with sodium metaperiodate and followed by the amidation. The modification procedure is illustrated in figure 2.5. Aldehyde and nitrogen content were analytically characterized for the intermediate product, which is dialdehyde xylan, and the final product, which is cationic derivative, respectively.

Figure 2.5: *Two-step modification of xylan*

2.9 Chemical modification of pectin

Due to the high content of acidic groups in the structure of pectin, only the amidation process was needed for the pectin modification instead of the two-step method as applied for xylan. The same procedure as described in section 2.8.2 was conducted.

2.10 Analytical methods applied for xylan

Different parameters of modified xylan were determined using analytical methods. The most important factors are as follows:

- *Aldehyde content via oxime reaction:* This method was used for determining the aldehyde content of the oxidized xylan. This parameter was used to evaluate the oxidation process.
- *Nitrogen content by elemental analysis:* This test was necessary for amidated xylans and oxime compounds. In the former, the nitrogen content was determined to judge the effectiveness of the amidation process. The latter was to determine indirectly the aldehyde content via the oxime method.

- *Intrinsic viscosity*: This measurement aimed to estimate changes in molecular weight in correlation with the aldehyde content after the oxidation.

2.10.1 Determination of aldehyde content

There are several methods to determine carbonyl content as summarized previously by Potthast *et al.* [99]. However, the determination of oxidized groups in xylan, such as aldehyde and carboxylic, has several inherent difficulties as mentioned by Rohrling *et al.* [108]. In this project, we followed the method presented by Sirvio *et al.* [127]. The principle of this method is based on oxime reaction between aldehyde groups and hydroxylammonium hydrochloride (HH), which is depicted in figure 2.6.

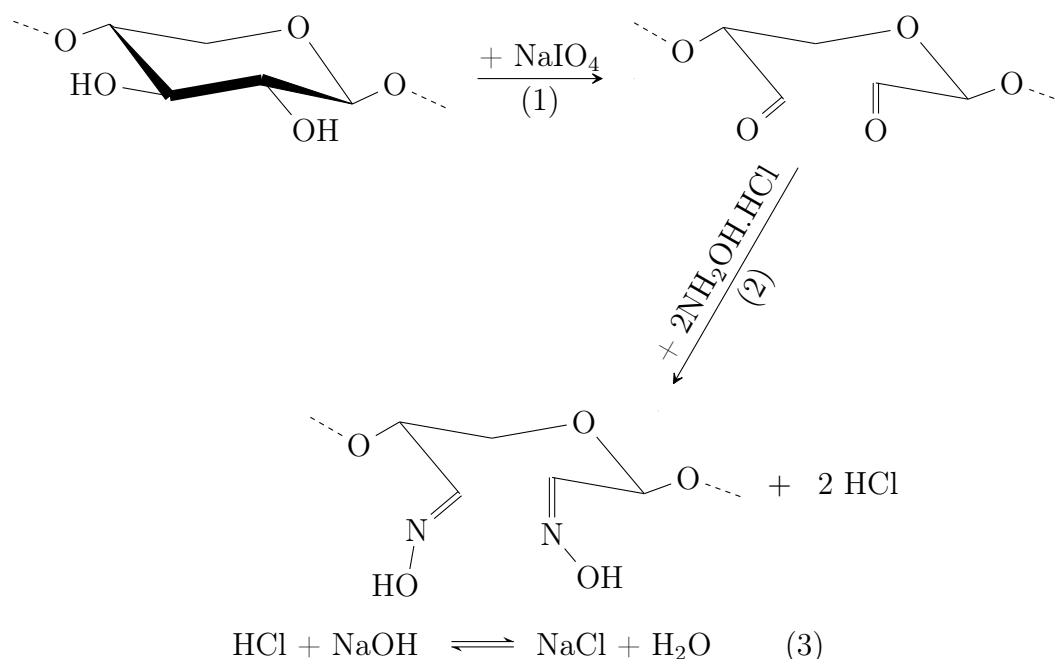


Figure 2.6: (1) Oxidation reaction between xylan and periodate; (2) Oxime reaction between 2, 3-dialdehyde xylan and hydroxylamine hydrochloride, modified from [127]; (3) Hydrochloric acid titration

Hydroxylammonium hydrochloride (1.39 g abs. dry) was dissolved in 100 mL of 0.1 M acetate buffer (pH 4.5) in an Erlenmeyer flask. Aldehyde xylan (0.1 g abs. dry) was added subsequently. The mixture was kept at room temperature for 48 h with stirring. After the required time, the product was filtered and washed with 600 mL of distilled water by filtration before being dried in a freeze dryer. Nitrogen content of the oxime derivative of aldehyde xylan was determined by elemental analysis.

Preparation of 0.1 M acetate buffer (pH 4.5): 13.61 g of sodium acetate was placed in a 1-L volumetric flask and dissolved with a bit of distilled water. 7.5 mL of glacial acetic acid was subsequently added. Finally, water was added to the volumetric flask's calibration mark [127].

Calculation

The aldehyde content was calculated via nitrogen content of the oxime compound according to the following equation:

$$A = \frac{M_o \times 10x}{M_N \times M_x} \quad (2.1)$$

Where:

- A: aldehyde content, mol/g or mmol/g
- x: nitrogen content (measured by elemental analysis), %
- M_o : molecular mass of oxime product, g/mol; $M_o = 160$ g/mol
- M_x : molecular mass of one xylose unit, g/mol; $M_x = 130$ g/mol
- M_N : molecular mass of nitrogen, g/mol; $M_N = 14$ g/mol

2.10.2 Determination of nitrogen content

The elemental analysis was conducted with a CN-Analyse Vario EL-Elementar System. To have reliable results, it is necessary to purify samples before measuring. Dialysis technique may need to carry out for the samples.

2.10.3 Determination of intrinsic viscosity

The intrinsic viscosity of oxidized xylan was measured with a method in cupric–ethylene–diamine (CED) based on the international standard ISO 5351/1-1981 [55], which is applied for determining the limiting viscosity number of dilute cellulose solution. The principle of this method is based on the times of efflux of the diluted solvent and solution of sample through a capillary-tube viscometer at a specified concentration at 25 °C.

A weighed amount of sample (300 ± 0.5 mg) was placed into a dissolving flask. It is a polyethylene flask with screw cap and rubber gasket. Distilled water (25 mL) was added with a pipette, together with some pieces of copper (around 10 pieces). It is noted that the dissolving flask is so constructed that the remaining air can be expelled when the flask is filled with 50 mL of test solution. With a pipette, another 25 mL of CED solution was added and all the remaining air was expelled. The flask was closed properly and shaken well in a shaking device for 2 h. After that, the flask was immersed in a constant-temperature bath set at 25 ± 0.1 °C. A blank sample was prepared in the same way but without the test sample added.

The blank sample and the samples were measured in the same way. By suction, a portion of the solvent was drawn into a capillary tube viscometer (Ubbelohde viscometer) and

allowed the fluid to drain. The timing device was started when the solvent reached the upper mark of the viscometer. The efflux time was recorded when the fluid drained to the lower mark.

Calculation

The viscosity ratio is given by the following formula:

$$\frac{\eta}{\eta_o} = \frac{t}{t_o} \quad (2.2)$$

Where:

- t: efflux time of the test solution, s
- t_o: efflux time of the diluted solvent, s
- η: viscosity of diluted solvent
- η_o: viscosity of sample

According to the appendix B of the reference [55], [η]. C at different values of viscosity ratio η/ η_o is known. From that, the viscosity, η, will be determined.

2.11 Analytical methods applied for pectin

2.11.1 Determination of acetyl group content

The content of acetyl groups was determined following the procedure as published by McComb *et al.* [77].

Standard curves

A range of glucose pentaacetate (GP) solutions at different concentrations was prepared from the GP stock solution. In a 25-mL volumetric flask, the following chemicals were accurately pipetted: 1 mL of NaOH, 1 mL of hydroxylammonium hydrochloride solution, 5 mL of each GP solution (5 mL of water was used in case of blank). After 5 min, 5 mL of the diluted perchloric acid was added and mixed thoroughly before filling up to the 25 mL mark with IPC-II solution. After 5 min, the absorbances at 520 nm were recorded with a photometer.

Procedure for sample measurement

Approx. 0.3 – 0.5 g of pectin sample was dispersed in water until it became homogeneous. The pectin dispersion was transferred into a 50-mL volumetric flask and filled with water to the calibration mark.

In a 25-mL volumetric flask, the following chemicals were accurately pipetted: 1 mL of NaOH, 1 mL of hydroxylamine hydrochloride, 5 mL of each GP solution. After 5 min, 5 mL of the diluted perchloric acid was added and mixed thoroughly before filling up to the 25-mL mark with IPC-II solution. After 5 min, the absorbances at 520 nm were recorded with a photometer.

Each sample needed its own blank, which was prepared in a different order. 5 mL of sample was added first, following by 1 mL of NaOH. After 2 – 3 min, 1 mL of HH was added. From this point onwards, the same procedure described above was repeated.

Preparation of specific chemicals

- *Sodium hydroxide (NaOH)*: dissolve 9.4 g of NaOH in 100 mL of distilled water
- *Hydroxylammonium hydrochloride (HH) solution (100 mL)*: dissolve 3.73 g HH in a small amount of water and then fill up to 100 mL
- *Glucose pentaacetate stock solution*: prepare V mL of the solution containing 500 μg per 5 mL of solution

1 unit of GP ($M_W = 390.34$ g/mol) contains 5 acetyl groups ($M_W = 43.04$ g/mol).

The amount of acetyl groups (μg) contained in V mL of GP:

$$m_{\text{Acetyl}} = \frac{500 \times V}{5} \quad [\mu\text{g}] \quad (2.3)$$

The amount of GP need to weigh:

$$m_{\text{GP}} = \frac{500 \times V}{5} \times \frac{390.34}{43.04 \times 5} \quad [\text{g}] \quad (2.4)$$

This stock solution was used to prepare a range of different concentrations of GP solution for building a standard curve.

- *Diluted perchloric acid solution*:

To prepare 100 mL of the solution: 7.04 mL of cold 70% perchloric acid was added into in a 100-mL volumetric flask. The flask was then filled up with cold, pure methanol to the calibration mark.

- *Iron (III) perchlorate solution (IPC-I)*:

To prepare 100 mL of the solution: 1.93 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was placed in a beaker where 5 mL of concentrated hydrochloric acid and 5 mL of 70% perchloric acid were alternately added. The solution was then heated until the liquid part was

mostly evaporated. The mixture was transferred into a 100-mL volumetric flask and filled with water to the mark. This solution was kept in a refrigerator and used within 1 month.

- *Iron (III) perchlorate solution - (IPC-II):*

To prepare 500 mL of the solution: 8.3 g of 70% perchloric acid was added into 60 mL of IPC-I. The mixture was ice cooled before filling with chilled absolute methanol to the desired volume. The solution was prepared and used within one week.

2.11.2 Determination of ferulic acid content

The content of ferulic acid was determined according to Jankovska *et al.* [58].

Ferulic acid stock solution and standard curve

A stock solution of 20 mg/L of ferulic acid was prepared. This solution was then used to make a range of ferulic solutions at different concentrations, ranging from 0 – 20 mg/L. A standard curve was built based on the relationship between absorbance at 345 nm and the known concentration of the solutions.

Procedure

Approx. 0.1 g (abs. dry) of pectin sample weighed precisely with a 4-digit analysis balance was placed in a beaker. The pectin was dispersed in 50 – 60 mL of distilled water with stirring. 5 mL of NaOH was added and slowly stirred at room temperature for 2 h. After that, the mixture was adjusted to pH 10 with 0.5 M HCl before transferring to a 100-mL volumetric flask. Distilled water was filled up to the flask's calibration mark. The sample was taken to 10 mm cuvetts and its absorbance at 345 nm was recorded with a photometer. The concentrations of ferulic acid (in mg/L) of the samples were determined from the standard curve.

The ferulic contents (%) of the samples were calculated according to the following equation:

$$Ferulic\ content = \frac{\beta_{Ferulic}}{\beta_{Sample}} \times 100\% \quad (2.5)$$

where:

$\beta_{Ferulic}$: concentration of ferulic acid in pectin sample, mg/L

β_{Sample} : concentration of the pectin sample, mg/L

2.11.3 Determination of degree of esterification

The degree of esterification of pectin was determined by a titration method according to Tibensky *et al.* (1963) [136]. Approx. 0.5 g (abs. dry) of pectin sample was dispersed in about 80 mL of distilled water under stirring. The dispersion was then transferred into a 100-mL volumetric flask and filled up with water to the flask's calibration mark. 20 mL of this solution was pipetted and placed in each of four beakers. These four beakers were named in turn from 1 to 4.

Beaker 1 and 2: to each beaker, 5 mL of 1 M NaOH solution was added. The beakers were slowly stirred at room temperature for 2 h.

Beaker 3 and 4: with stirring, 5 mL of 1% (w/w) CuSO₄ (prepared from CuSO₄·5H₂O) was added drop-wise. After 15 min, the solution in each beaker was filtered through a cellulose acetate membrane filter. The sample was washed out of Cu²⁺ ions with distilled water by filtration. The presence of Cu²⁺ ion was tested with concentrated ammonia solution (solution turns to blue). Clean sample together with the filter were transferred to an Erlenmeyer flask, where 5 mL of 5% NH₃ was added. When the solution became clear, the filter was rinsed for a few times with distilled water to make sure there was no sample lost before disposing. The clear blue solution now was ready for titration with 0.01 M EDTA with murexid indicator (a mixture of NaCl and Murexid with a ratio of 90/ 1). The color of the solution was changed from blue to yellow or sometimes light green. The volume of EDTA was recorded when the color switched from yellow/ light green to violet.

After 2 h, the pH of the beakers 1 and 2 was adjusted to 5.3 – 5.5 with 10 M and 0.1 M acetic acid. The same procedure applied for beaker 3 and 4 was then processed for these samples.

The degree of esterification was calculated as the following equation:

$$DE = \frac{(m_2 - m_1)}{m_2} \times 100 \quad (2.6)$$

Where:

DE: degree of esterification of pectin, %

m₁: amount of pectin before the de-esterification with Cu²⁺ ion, g

m₂: amount of pectin after the de-esterification with Cu²⁺ ion, g

2.12 Quantitative determination of relative retention capacity of xylan on paper

2.12.1 Definition

In this work, the term 'relative retention capacity' of xylan on paper refers to the percentage of the polysaccharide remained on the paper after the addition.

2.12.2 Principle

To investigate the retention capacity of xylan onto fibers during paper making, papers with different contents of oat spelt xylan and without xylan added were made with four replicates in each case. Tri-fluoroacetic acid (TFA) was used for hydrolysis because it has several advantages in comparison with sulfuric acid.

For this purpose, different amounts of the OSX were added into pulp slurry during the paper making process. The paper sheets with different contents of xylan added were hydrolyzed with TFA under mild conditions. Three groups of sample were conducted: (1) paper sheets without xylan added; (2) paper sheets with different amounts of xylan added; and (3) the xylan itself. Sugar contents regarding xylose, arabinose, mannose and galactose were analyzed by high performance liquid chromatography (HPLC).

The adsorbed amount of xylan was calculated based on the difference in xylose content between paper with xylan addition and the xylan itself. Relation between the adsorbed amount of xylan on paper and actual amount of xylose present in the added xylan represents the relative retention capacity of xylan on paper.

2.12.3 Mild hydrolysis with tri-fluoroacetic acid

This experiment aimed to produce monosaccharides from polysaccharide samples for further sugar analysis by HPLC. The hydrolysis can be carried out using acids or enzymes. For economic reasons, acids are more common, especially sulfuric acid. However, sulfuric acid causes several troubles [82]. First of all, the use of sulfuric acid might destroy sugars and the reaction between monosaccharides and sulfuric acid reduces the yield of the hydrolysis. Second, the process using sulfuric acid requires a lengthy and messy procedure to remove. In comparison to sulfuric acid, TFA has several advantages. On the one hand, the reaction with TFA requires shorter time. On the other hand, TFA is volatile and therefore can be easily removed by evaporation without any traditional neutralization [2, 30].

The hydrolysis was performed according to the work of Fengel *et al.* [30, 31]. 20 mg

of dried paper sample was placed into a 50-mL round bottom flask where 3 mL of concentrated TFA was added subsequently. The mixture was allowed to stand overnight at room temperature. After that, the TFA solution was diluted to 80% by adding 1.108 mL of distilled water. The reaction was conducted at 105 °C for 35 min. Subsequently, the solution was diluted the second time to 30% with 9.233 mL of distilled water and kept at 105 °C for two more hours. After the required time, the flask was cooled down to room temperature. TFA was first removed by using a rotary evaporator at 40 °C. Afterwards, distilled water was replaced to wash the products until they were free of acid. Clean samples were kept in at 4 °C for later analysis by HPLC.

2.12.4 Sugar analysis and calculation of relative retention capacity of xylan on paper

Sugar contents of the hydrolyzed samples, including xylose, arabinose, mannose and galactose were analyzed using an Agilent 1200 HPLC series system, equipped with an liquid chromatography (LC)-mass spectrometry (MS) detector and a ReproGel Pb column. The hydrolyzed sample was filtered through a 0.45 µm polytetrafluoroethylene (PTFE) syringe filter and eluted with distilled water. Glucose content is not mentioned here since it cannot be determined precisely with this method. On the one hand, this sugar cannot be completely depolymerized into monomers using the mild hydrolysis. On the other hand, the origin of the glucose, which can be from hemicellulose or cellulose, cannot be specified. The adsorbed amount of xylan on cellulose was calculated based on the difference in xylose content of the paper with xylan added and the pure paper. The relative retention capacity of the xylan (based on xylose) on paper (A) is a relation between the adsorbed amount of xylan and the actual amount of xylose present in the amount of xylan added into paper. The calculation is shown in the following equation:

$$A = \frac{(a_n - a_o)}{n \times a_x} \times 100 \times 100 \quad (2.7)$$

Where:

- A: relative retention capacity of xylan, %
- n: percentage of added xylan on paper, %
- a_n : xylose content of the paper with n% of xylan added, %
- a_o : xylose content of the paper without addition of xylan, %
- a_x : xylose content of the use xylan, %

This calculation was also applied for arabinose.

2.13 Structural analysis

2.13.1 Fourier transform Raman spectroscopy

FT-Raman spectra of xylan, pectin, and their modified derivatives were recorded using a Bruker Raman spectrometer (MultiRAM III NIR-FT). The laser power was set at 150 mW. For each measurement, 200 – 400 scans were accumulated with a spectral resolution of 4 cm^{-1} in the transmission mode. Obtained spectra were normalized and processed by using Origin Pro software, version 9.0 SR1.

2.13.2 Fourier transform infrared spectroscopy

Infrared spectra were obtained using an Fourier transform infrared (FT-IR) spectrometer (Tensor 27 Bruker) equipped with an RT-DLaTGS detector. The KBr method was used. The spectra were measured in the range from 4000 to 400 cm^{-1} and 16 scans were accumulated at a resolution of 4 cm^{-1} . Obtained spectra were normalized and processed by using Origin Pro software, version 9.0 SR1.

2.13.3 Size-exclusion chromatography

Size exclusion chromatography (SEC) was used to determine the molecular weight of xylan samples. The molecular weight data of the xylan was obtained by SEC using DMSO/ water (9/ 1) and 0.05 M LiBr additive as published by Saake *et al.* [111]. Pullulan standards and glucose were used for calibration. In this work, this measurement was done by Prof. Bodo Saake (University of Hamburg, Germany).

3

Results and discussion

3.1 Investigation of xylan application in paper

In this section, the influence of xylan addition on mechanical properties of paper is investigated. For this purpose, original xylyans and cationic derivatives originating from beech, birch, and oat spelt were added into the paper. The following factors, which considerably influence the paper strength are discussed:

- milling process
- xylan source
- xylan content
- degree of substitution of xylan
- homogenization time of xylan

3.1.1 Influence of milling process

As mentioned earlier, the milling process (so-called beating or refining) plays a very important role in the paper formation and mechanical properties of the final sheets. The pulp with specific refining degree ($^{\circ}\text{SR}$) can be obtained by controlling the beating time and energy consumption of the milling procedure. Due to limitations of the lab-scale milling machine, it is difficult to control these two parameters to achieve the milled pulp with the same refining degree every time. Hence, the process is only allowed to operate in small batches resulting in different refining degrees of the pulp. For each batch of pulp, pure test sheets without any addition were produced and measured their tensile and tear index. Milling curves representing the correlation between the tensile/ tear index and the refining degree of the pulp are shown in figure 3.1.

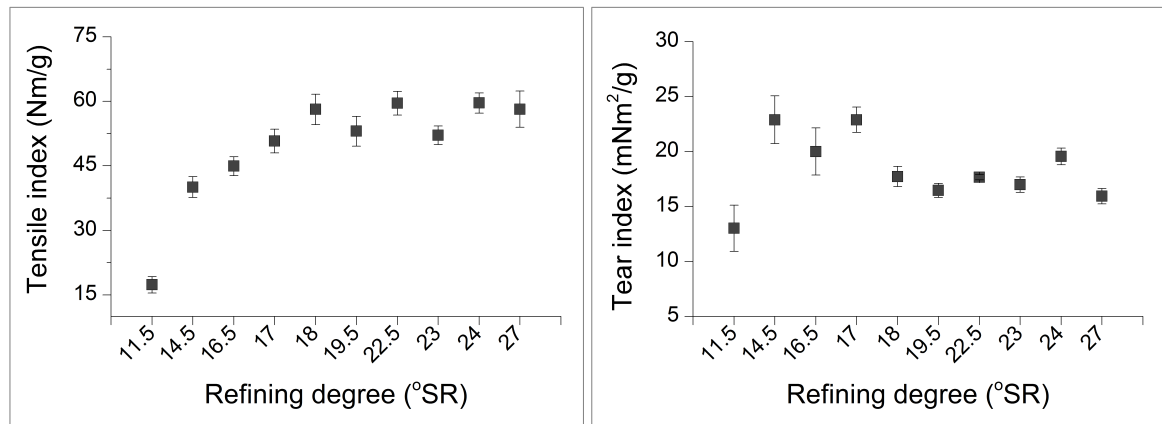


Figure 3.1: *Milling curves built from tensile and tear index of paper without xylan addition*

As can be seen from the graphs, the tensile index tends to increase gradually with increasing refining degree. However, it seems to be steady when the milling point reaches 18 °SR and greater. In case of tear index, the parameter raises for the first milling point and then slowly decreases. The beating process of the pulp is believed to cause this phenomenon as mentioned by Page [88]. According to their work, the beating, on the one hand, results in the reduction in the fiber length leading to the production of fines and fiber fragments in the suspension and therefore favors the external fibrillation. On the other hand, it results in internal changes in the wall structure like internal fibrillation, or swelling property. As a result, the refined fibers are flattened and more flexible, and can create more bonding surface areas. The tensile and bursting strength obviously increase since these parameters strongly depend on the fiber length and fiber bondings. In contrast, the tearing resistance decreases as this property is dependent on individual strength of the fibers [59, 75, 88].

The influence of the refining degree on the tensile, tear, and burst index is also observed when xylan was used as a paper additive. Different xylans and cationic derivatives originating from different sources, were added into the pulp with 2% based on the dry weight of the pulp. These xylans can be classified according to their sources as shown in table 3.1. More information about the samples is available in table 2.2, section 2.1.1. It is noted that the influence of xylan addition is not discussed in this section since the effect of the milling process is greater and more significant.

Table 3.1: *Cationic xylans from different sources added into paper*

<i>Xylan</i>	<i>Code</i>	<i>Full name</i>
09Xyl47	OSX	oat spelt xylan
09Xyl01		
09Xyl12	OS-CatX	oat spelt cationic xylan
09Xyl14		
09Xyl15		
09Xyl21	Be-CatX	beech cationic xylan
09Xyl24		
09Xyl23	Bi-CatX	birch cationic xylan
09Xyl25		

The pulp was prepared according to the procedure described in section 2.2 resulting in the un-milled pulp and milled pulp with the refining degree of 11.5 °SR and 27 °SR, respectively. The tensile, tear, and burst index of the papers with these xylans addition are presented in figure 3.2, 3.3 and 3.4.

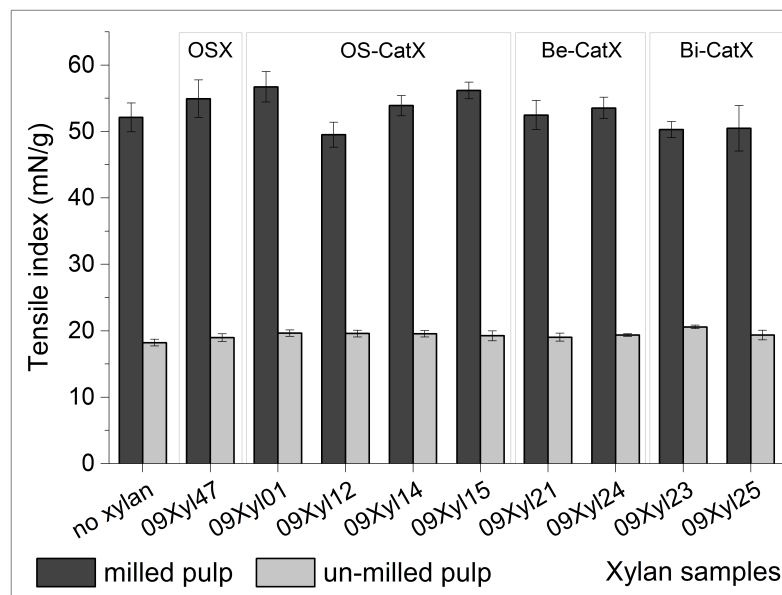


Figure 3.2: *Influence of milling process on tensile index of papers with different xylans addition; oat spelt xylan (OSX), oat spelt cationic xylan (OS-CatX), beech cationic xylan (Be-CatX), birch cationic xylan (Bi-CatX); milled pulp 27 °SR, un-milled pulp 11 °SR; xylan dosage 2 %*

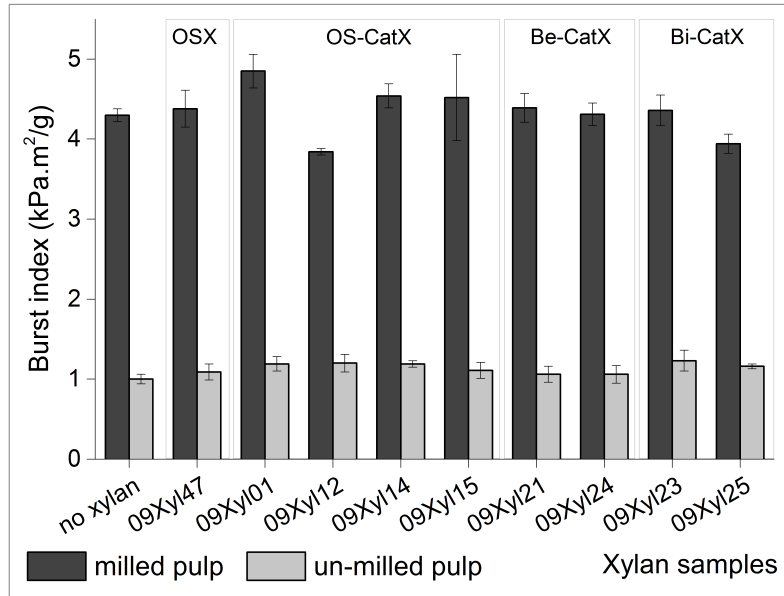


Figure 3.3: Influence of milling process on *burst* index of papers with different xylans addition; oat spelt xylan (OSX), oat spelt cationic xylan (OS-CatX), beech cationic xylan (Be-CatX), birch cationic xylan (Bi-CatX); milled pulp 27 °SR, un-milled pulp 11 °SR; xylan dosage 2 %

As can be seen from figure 3.2 and 3.3, the milled pulp provides the papers with much higher tensile and burst indices. This improvement is much greater than in the tear strength as shown in figure 3.4.

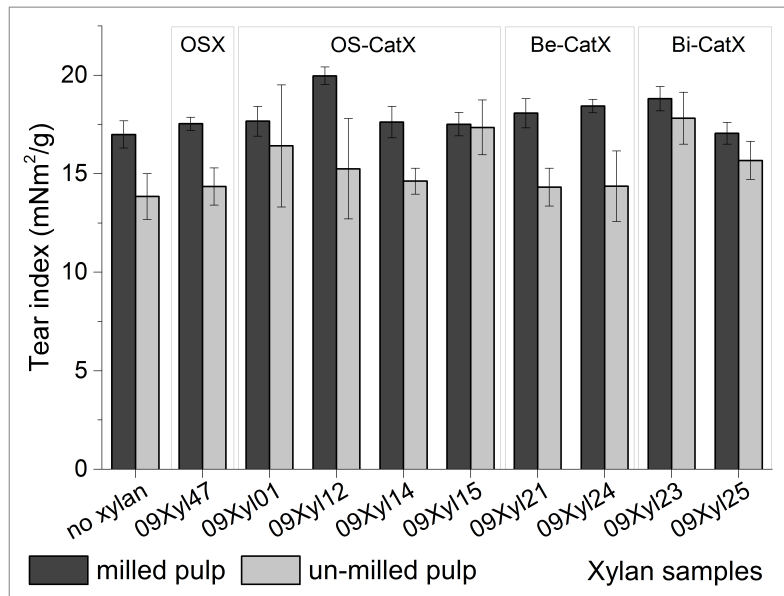


Figure 3.4: Influence of milling process on *tear* index of papers with different xylans addition; oat spelt xylan (OSX), oat spelt cationic xylan (OS-CatX), beech cationic xylan (Be-CatX), birch cationic xylan (Bi-CatX); milled pulp 27 °SR, un-milled pulp 11 °SR; xylan dosage 2 %

From figure 3.4, the milled pulp only gives a small enhancement in the tear index over

the un-milled pulp. This is in agreement with the literature mentioned above [59, 75]. The explanation is straight forward since shorter fibers can make more fiber bondings, which result in the increase of the tensile and burst strength. In case of the tearing strength, the shorter fibers allow the stress to be concentrated on a small area causing the reduction of this parameter.

To summarize, the influence of the beating process on the paper strength properties is obvious when using xylans as paper additives. The milled-pulp with higher °SR (i.e., 27 °SR) provides the papers with stronger mechanical properties, especially the tensile and burst index (about 2.5 times as compared to the un-milled pulp).

3.1.2 Influence of xylan source

Cationic xylans originating from oat spelt, beech, and birch with DS in a range of 0.08 – 0.14 that have been modified either with EPTAC or CHPTAC were selected. Milled pulp of 23 °SR was used with 2% xylan addition based on the dry weight of the pulp. Results in tensile, burst, and tear index of the papers with these xylans addition are illustrated in figure 3.5.

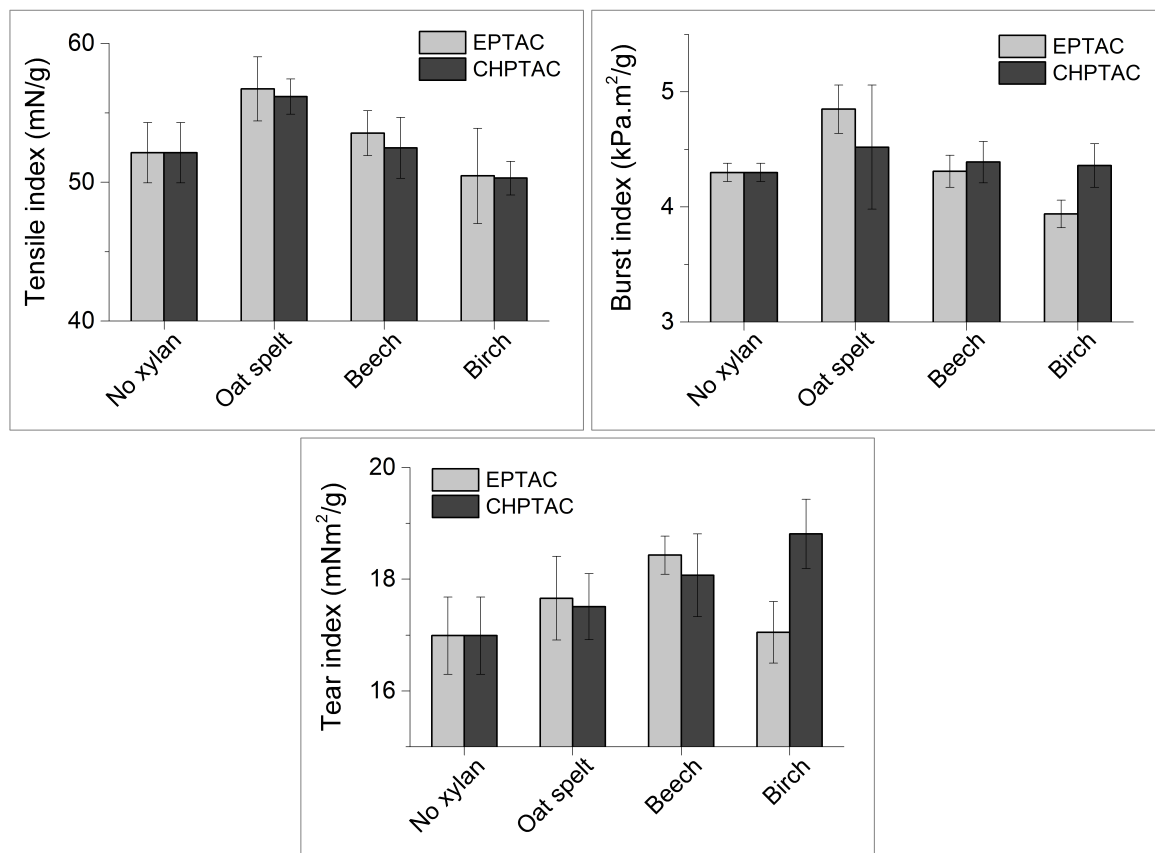


Figure 3.5: Influence of xylan source on mechanical properties of paper; oat spelt cationic xylan (OS-CatX, DS 0.14), beech cationic xylan (Be-CatX, DS 0.08), birch cationic xylan (Bi-CatX, DS 0.09); milled pulp 23 °SR; xylan dosage 2%

When using the OS-CatXs, the tensile and burst index of the papers are remarkably improved, while these properties of the paper with Bi-CatX or Be-CatX addition are just about that of the pure papers. Whereas, the tear index is highest in Be-CatX modified with EPTAC and in Bi-CatX modified with CHPTAC. According to these data, oat spelt seems to be the best source for xylan isolation and modification among the mentioned materials. The difference in structure, including molecular weight and substitution patterns, is believed to be the reason for this finding. As a xylan from cereal grain, OSX is classified as arabinoxylan, which is partly substituted at O2 and/or O3 with α -L-Araf, and α -D-glucuronopyranosyl (Glc p) or its 4-*O*-methyl derivatives at O2 positions. In contrast to cereal xylans, xylans from hardwood, including birch and beech are glucuronoxylans. They contain a high content of MeGA with the xylose-to-MeGA ratio varies from 4 – 16 : 1 [23]. This highly substituted structure of hardwood xylans causes their high solubility as indicated by Saake *et al.* [111]. This leads to the fact that fewer bondings with cellulose fibers are created and therefore results in unfavorable mechanical properties of the final sheets. In addition, since hardwood xylans usually have high degree of substitution, they tend to have fewer reaction sites with cationizing reagents due to the lack of available OH groups for the quaternization. Their cationic derivatives, perhaps, possess lower positive charges leading to the weaker tensile and burst strength. This is in good agreement with the findings of Kabel *et al.*, when they conducted a detailed study about the adsorption of xylans onto bacterial cellulose [61].

To sum up, xylan sources play an important role in the ability of xylan to improve the paper strength properties. Among the used materials, oat spelt might be the best material for xylan isolation and cationization as its cationic derivatives can improve significantly the tensile and burst strength of the paper.

3.1.3 Influence of xylan content

Pure oat spelt xylan (PST-7) and oat spelt cationic xylan (OS-CatX, 09-Xyl-01, DS 0.14) were selected to test the effect of xylan content added in pulp on tensile and tear index of the final sheets. Information about these xylans has been summarized in section 2.1.1. Milled pulp and un-milled pulp with the refining degree of 27 °SR and 11 °SR, respectively, were used. The tensile and tear index of the papers using the milled pulp with different xylan dosages addition are illustrated in figure 3.6.

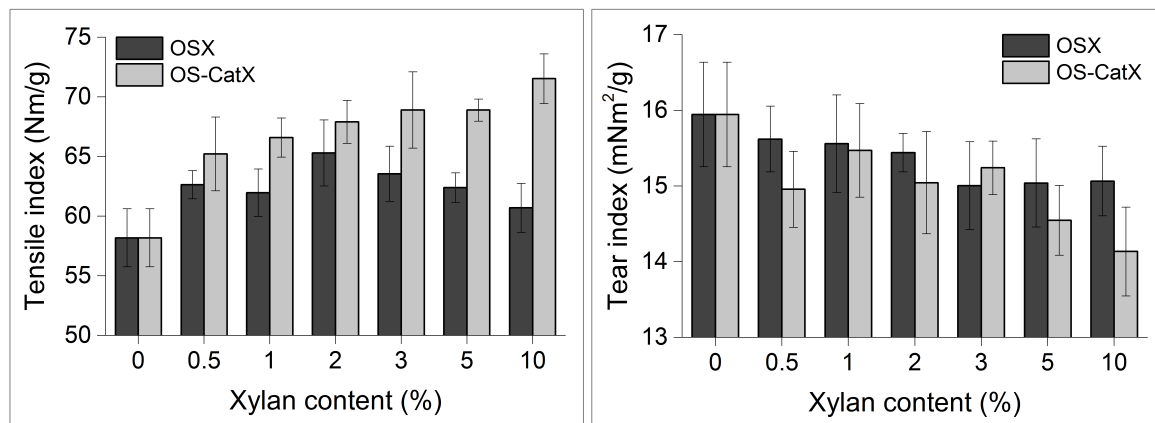


Figure 3.6: Tensile and tear index of papers made from milled pulp (27 °SR) using different dosages of pure oat spelt xylan (OSX) and oat spelt cationic xylan (OS-CatX, DS 0.14)

In general, increasing trends in the tensile index and decreasing trends in the tear index with increasing xylan dosages are observed. In case of using the OS-CatX, the tensile index raises gradually from 58.2 Nm/g (without xylan addition) to 71.5 Nm/g (with 10% xylan addition). However, when using the unmodified OSX, the tensile index reaches its highest value of approx. 65 Nm/g at 2% dosage and then slowly reduces. It is noted that the OS-CatX used in this experiment was cationized using EPTAC. The OS-CatX with positive charges on its surface is assumed to enhance the tensile strength of the paper due to electrostatic interactions [117]. For the papers with the pure OSX addition, hydrogen bonding seems to be the only connection among fibers and the xylan. However, depending on the retention of xylan on cellulose, the number of these bondings might increase gradually until a level, where xylan can no longer connect to the fibers. A saturated status of xylan interaction and the remaining time of xylan in the pulp can influence the xylan retention.

When using the un-milled pulp, there is a considerable increase in both tear and tensile index with the xylan dosages (figure 3.7). These results are compatible with the findings published by Ramírez *et al.* [103]. In their work, OSX and kraft softwood pulp were mixed together in an disintegrator for 20 min. Their results showed that both tensile and tear index increased upon xylan addition.

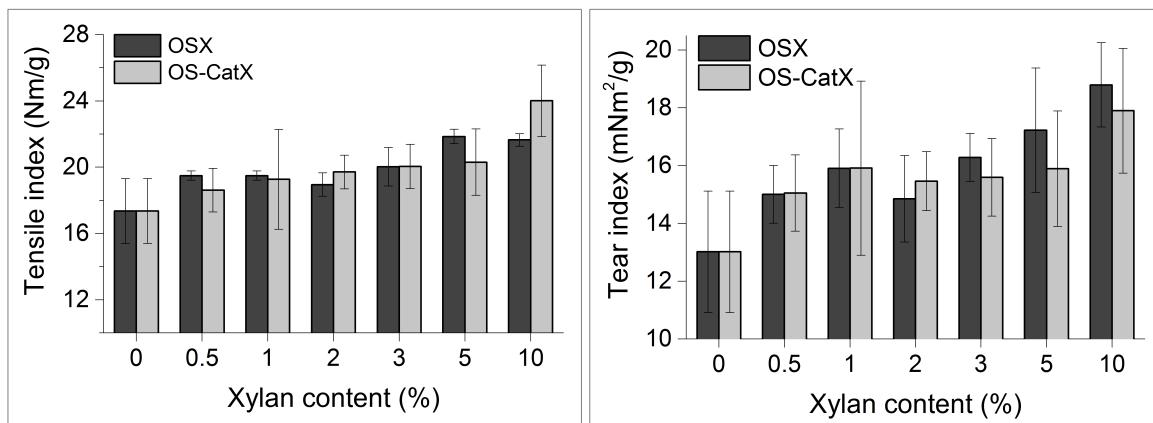


Figure 3.7: *Tensile and tear index of papers made from un-milled pulp (11.5 °SR) using different dosages of pure oat spelt xylan (OSX) and oat spelt cationic xylan (OS-CatX)*

However, there is almost no difference between the OSX and the OS-CatX in both tensile and tear index. This suggests that for un-milled pulp, the ionic property of the modified xylan might not have any advantage over the original form.

In brief, the oat spelt cationic xylan improves the tensile strength significantly while the unmodified oat spelt xylan only increases the tensile strength at the dosages of less than 2% when using the milled pulp. The tear index is not enhanced with both xylan samples. In case of the un-milled pulp, the cationization of the xylan before addition might not be necessary since the both types of xylan can equally improve these parameters.

3.1.4 Influence of degree of substitution of xylan

Substituents and degree of substitution (DS) are believed to influence the solubility of xylan in aqueous media and the distribution of xylan in pulp slurry [34, 111]. In this experiment, the contribution of OS-CatXs with different DS to the paper strength was examined. Three OS-CatX samples with the DS of 0.04, 0.14, and 0.26, respectively were used. The xylans with the DS of 0.04 and 0.14 were OS-CatX modified with EPTAC, whereas xylan with DS of 0.26 was OS-CatX modified with CHPTAC. Although the two cationizing reagents have different structures but they both give the same cationic product due to a conversion in between the two reagents in alkaline environment as discussed later in section 3.2.1. Milled pulp with the refining degree of 24 °SR was used. The tensile and tear index of the papers with xylan addition were compared with the papers using no xylan. The data are depicted in figure 3.8.

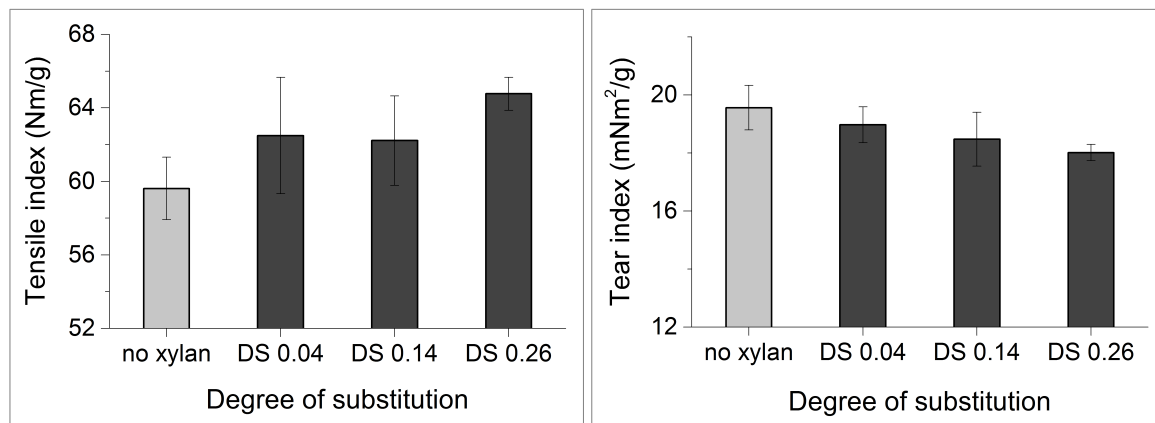


Figure 3.8: *Influence of degree of substitution of cationic xylan on paper properties; milled pulp 24 °SR, xylan dosage 2%*

As can be seen from figure 3.8, the use of OS-CatX with the DS ranging from 0.04 to 0.26 can improve the tensile index significantly. However, the tear index decreases with increasing DS. This phenomenon was also indicated by Schwikal *et al.* [116]. In a work of Deutsch and partners [21], they tested the addition of oat spelt cationic xylan (DS 0.03 – 0.19) into eucalyptus kraft pulp and found that the oat spelt cationic xylan with DS between 0.07 and 0.1 improved the mechanical properties most. A remarkable increase in the tensile index and decrease in the tear index were also observed when increasing the DS of OS-CatX as indicated by Ren *et al.* [105]. These changes in the tensile and tear index are obvious because the xylans with higher DS have higher positive charges on their surface. This characteristics leads to more electrostatic interactions between the cationic xylans and the negative cellulose fibers, which result in higher tensile index.

To sum up, the DS of xylan does have influence on the paper tensile and tear index. Under the certain conditions, the tensile strength increases with the DS, while the tear index decreases.

3.1.5 Influence of homogenization time of xylan

In the scope of this study, homogenization time of xylan is the time that xylan stays in the pulp slurry until the sheet formation phase. This investigation aimed to evaluate whether this parameter makes any impact on the interaction of the xylan with the cellulose fibers. For this purpose, oat spelt cationic xylan (09-Xyl-01, cationized with EPTAC, DS 0.14) was first dispersed in water until homogeneous. The xylan dispersion was then added into the slurry and allowed to stay there for 5, 10, 15, 20, 25 and 30 min in turn before making sheets. The dosage of xylan used in this test was 2% based on the dry weight of the pulp. The milled pulp used in this experiment had the refining degree of 24 °SR. Four to five replicates resulting in corresponding numbers of sheets

were conducted in each case. The correlation between the strength properties of the finished papers and the xylan homogenization time is depicted in figure 3.9.

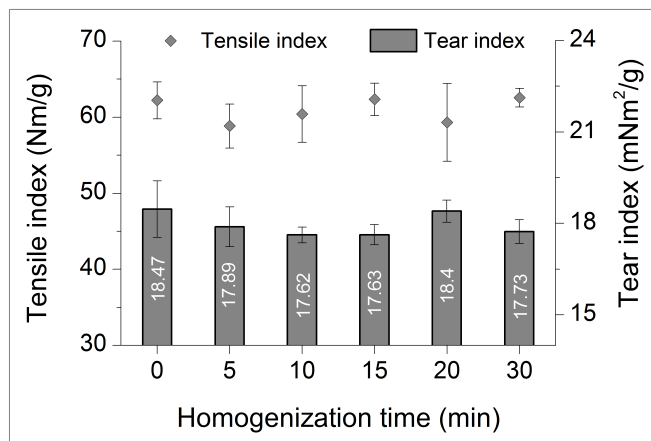


Figure 3.9: Influence of homogenization time of cationic oat spelt xylan ($DS\ 0.14$) on paper strength properties; milled pulp $24\ ^\circ SR$; xylan dosage 2%

Under the used conditions, the homogenization time of xylan in pulp slurry does not have a strong effect on the paper properties. This is in contrast with the publication of Ren *et al.* [105]: In their work, they used cationic hemicellulose originating from sugarcane bagasse (synthesized with EPTAC, $DS\ 0.55$) to add into sulfate kraft pulp. According to their results, after 15 min of homogenization, the breaking length and burst index of the papers reached the highest values and were improved by 16.3% and 23.9%, respectively. Although they did not mention the tensile index, the results are still comparable because tensile and burst index share similar features. There are many possible reasons causing the difference between their results and the results in this work, in which the DS value and the starting materials are the most important factors. The lower DS of the OS-CatX (0.14) than that of sugarcane bagasse xylan (0.55) results in the lower tensile and burst index, which is also mentioned in their work.

In conclusion, when xylan is added after the beating step of the pulp preparation procedure, the tensile and tear index of the finished paper are not influenced by the homogenization time of xylan.

3.2 Chemical modification of xylan

The following chemical modifications of xylans are presented in this section:

- modification of oat spelt xylan and wheat arabinoxylan using cationizing reagents, including EPTAC and CHPTAC. This experiment was conducted at the Fraunhofer Institute for Applied Polymer Research, Potsdam-Golm.

- oxidation of oat spelt xylan with three oxidants
- amidation of oat spelt xylan with ammonia
- two-step modification method

3.2.1 Modification with cationizing reagents

CHPTAC and EPTAC are two popular powerful cationizing reagents, which are widely used in polymer modification. In this experiment, these two chemicals were used to modify OSX (14-Xyl-04) and the WAX following the procedure described in section 2.7. The structure of the two reagents are depicted previously in figure 2.2 and 2.4, section 2.7. Acton [1] mentioned that even when CHPTAC is used as a cationization agent, the final compound that reacts with the xylan is still EPTAC. The final product is, therefore, the same in both cases. CHPTAC is first transformed to EPTAC with an extra equivalent of sodium hydroxide before the cationization reaction can start. The conversion occurs via ring closure reaction of the chlorohydrin group in CHPTAC (figure 3.10). Furthermore, during the cationization process in alkaline solution, the xylan molecules are activated by forming temporary anionic compounds at the hydroxyl groups (figure 3.11 a). The active form of the reagent then can react with the activated xylan as shown in figure 3.11 b.

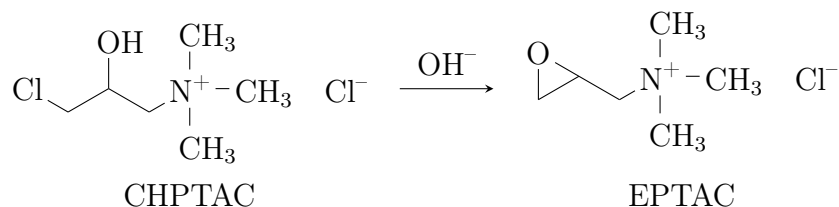


Figure 3.10: Conversion of (3-chloro-2-hydroxypropyl)trimethylammonium chloride (CHPTAC) to 2,3-epoxypropyltrimethylammonium chloride (EPTAC) in alkaline environment [1]

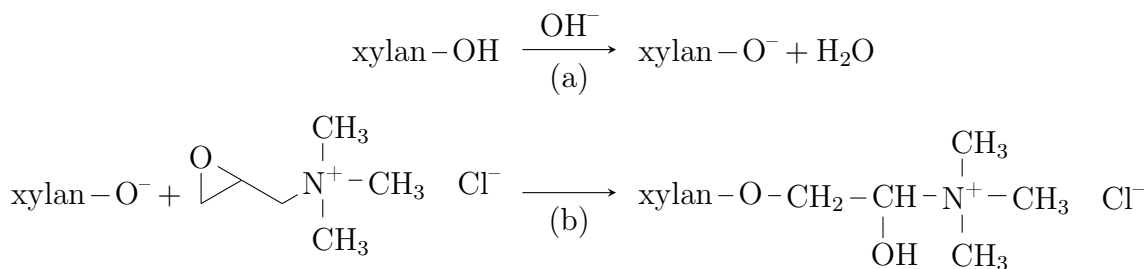


Figure 3.11: Reaction of xylan with 2,3-epoxypropyltrimethylammonium chloride in alkaline environment

When using CHPTAC, two different ratios between the reagent and xylan, 0.5 : 1 and 1 : 1, were used in order to obtain cationic xylan derivatives with different DSs. The same amount of alkaline was used in all cases. The DS of the final products are shown in table 3.2.

Table 3.2: *Degree of substitution of modified xylans using 2,3-epoxypropyltrimethylammonium chloride (EPTAC) and (3-chloro-2-hydroxypropyl)trimethylammonium chloride (CHPTAC)*

<i>Product</i>	<i>Original xylan</i>	<i>Quab</i>	<i>Reagent: Xylan</i>	<i>DS</i>
EPT-WAX-1/1	wheat arabinoxylan	EPTAC	1 : 1	0.27
EPT-OSX-1/1	oat spelt xylan	EPTAC	1 : 1	0.26
CHP-WAX-1/1	wheat arabinoxylan	CHPTAC	1 : 1	0.13
CHP-OSX-1/1	oat spelt xylan	CHPTAC	1 : 1	0.45
CHP-OSX-0.5/1	oat spelt xylan	CHPTAC	0.5 : 1	0.33

As can be seen from the table, the higher CHP-to-OSX ratio results in higher DS with 0.45 compared to 0.33 when using the lower ratio. When using CHPTAC, with the same ratio of 1 : 1, the derivative of the OSX has much higher DS than that of the WAX (0.45 compared to 0.13). This is in agreement with a work of Schwikal *et al.* [116] when they synthesized cationic xylan from birch wood using EPTAC. According to their work, the DS of the products was controlled by adjusting the molar ratio of the cationization reagent to the anhydroxylose units. Their results also showed that the DS of cationic xylans increased significantly with this ratio. This is apparent because the higher amount of the chemicals leads to more side chains being attached onto the xylan backbone.

When comparing the effectiveness of the two reagents, it is hard to consider which one is better because they show different influence to different raw materials. For OSX, for instance, CHPTAC seems to be the better one since it results in the product with higher DS (0.45 compared to 0.26), while with WAX, EPTAC gives better result with DS 0.27 compared to DS 0.13. In addition, when using EPTAC, the two cationic xylans have nearly the same DS, while CHPTAC shows a great difference in DS with 0.45 for OSX compared to DS 0.13 for WAX. However, the unstable property of EPTAC as mentioned earlier is a drawback of this compound, which might limit its application in this field of study.

The cationic xylans were then added into paper with a dosage of 2% based on the dry mass of the pulp. Figure 3.12 demonstrates the tensile and tear index of the papers

with the modified xylans addition.

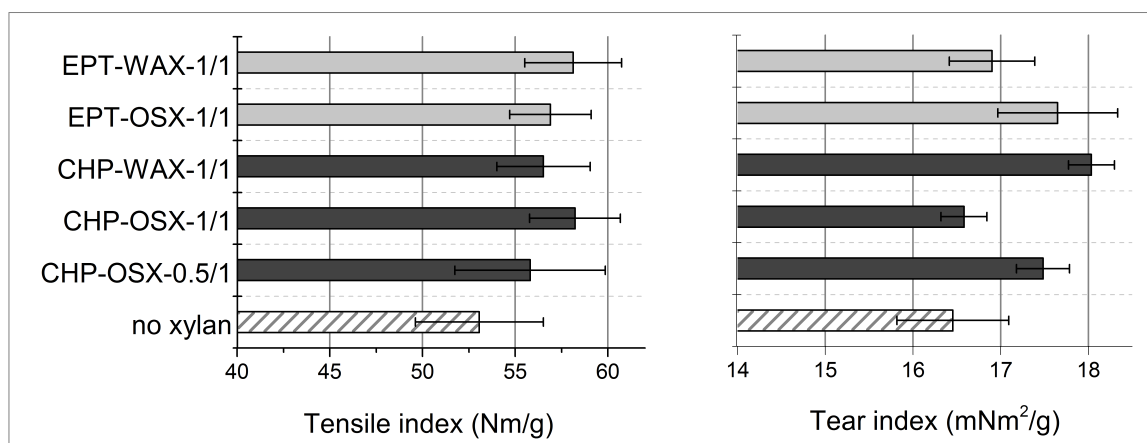


Figure 3.12: *Cationic xylan derivatives and their influence on tensile and tear index of paper sheets; EPT(CHP)-OSX(WAX)-1/1 (0.5/1) stands for the modified oat spelt xylan (wheat arabinoxylan) using EPTAC (CHPTAC) with reagent-to-xylan ratio of 1 : 1 (or 0.5 : 1); milled pulp 19.5 °SR; xylan dosage 2%*

As can be seen from figure 3.12, the tensile strength of the papers with the cationic xylans addition is significantly improved compared to the paper without xylan addition. It is well-known that cellulose fibers carry slightly negative charges on their surfaces. When cationic xylans are added into the pulp, there are electrostatic interactions between these two oppositely charged objects. This interaction contributes significantly to the strength of the fiber network, thus improves the mechanical properties of the final paper, especially tensile and burst index [117, 124]. As discussed earlier in section 3.1.3, the tearing strength of the paper is decreased with increasing cationic xylan content. However, it can be seen that the tear index in this experiment is also improved when compared to the pure paper. The trend is especially true when using the cationic xylans with high DS. These results suggest that the higher cationic materials can improve both tensile and tear index due to stronger electrostatic interactions.

When using CHPTAC to modify the OSX the higher reagent-to-xylan ratio results in the higher tensile index. For instance, when using the ratio of 0.5 : 1, the tensile strength is about 5.2% enhanced, while at the ratio of 1 : 1, this parameter is 9.7% increased. This is in correlation with the DS of the corresponding products as seen earlier in table 3.2 and suggests that higher DS of the cationic xylan might result in higher tensile strength of the finished paper. Whereas, the tear index often has an opposite trend compared to the tensile index since this parameter depends on the strength of individual fibers as mentioned previously in section 1.4.1. However, the role of DS of the cationic xylan in the mechanical properties of the paper in this experiment is not absolutely clear. It can be seen that the tensile index of the paper with EPT-WAX-1/1 (DS 0.27) and that of the paper with CHP-OSX-1/1 (DS 0.45)

addition have nearly the same values although their DSs are much different. The reason behind this could be the difference in structure between the two xylans. On the one hand, the high content of 4-*O*-methyl glucuronic acid of OSX compared to that of WAX [24, 91, 113] might cause internal stability and better dispersion of the xylan on the cellulose fibers [21]. On the other hand, WAX is often contaminated with arabinogalactan-protein as reported by some authors [27, 37]. The WAX, therefore, might not interact effectively with the fibers during the sheet formation.

To sum up, the cationizing reagents (EPTAC and CHPTAC) effectively modify xylans resulting in highly cationic charged derivatives, which are reflected by their DS. However, the reagents seem not to be appropriate for every xylan cationization, i.e., CHPTAC is better used for oat spelt xylan, while EPTAC is more suitable for wheat arabinoxylan modification. The addition of the cationized xylans, especially the derivatives with high DS, significantly improves the tensile and tearing strength of the paper.

3.2.2 Oxidation

The OSX was oxidized with sodium *m*-periodate, hydrogen peroxide, and ozone one in succession. This process is expected to form dialdehyde xylan derivatives as shown in figure 1.13, section 1.2.4. Effectiveness of the oxidation was evaluated through intrinsic viscosity and aldehyde content of the oxidized products. Molecular weight and molecular weight distribution of some samples were also tested.

Xylan oxidation with sodium *m*-periodate

Among the three oxidants, sodium *m*-periodate shows the clearest trends regarding aldehyde content and intrinsic viscosity. When increasing oxidation time, the aldehyde content of the oxidized product is obviously increased, while the viscosity is decreased (figure 3.13). This suggests that the reagent has successfully attacked and cleaved the xylopyranose rings of the xylan chain producing more aldehyde groups with the increment of the reaction time. During the reaction, the polysaccharide is also broken into shorter chains leading to the viscosity reduction of the product.

It is indicated in a work of Tatarkina and co-workers [133] that the xylans (araboglucuronoxylan – so-called arabinoglucuronoxylan – from wheat straw and 4-*O*-methylglucuronoxylan from Platanus wood) were dissolved gradually during the periodate oxidation. According to their results, the oxidation reached the highest level of around 32% of aldehyde content after 96 h of treatment. This result is far higher than the result in this study. On the one hand, the concentration of the periodate solution was 3 times higher (0.3 M compared to 0.1 M in this experiment). On the other hand, in their work, the aldehyde content was determined directly by the oxime method

compared to elemental analysis via oxime reaction in this work. The direct oxime method seemed not to be reliable since it was difficult to record color change when the aldehyde content was too low as observed in some trial experiments. Moreover, as specified by Aspinall *et al.* [6], each xylose unit of xylan consumed 1 mole of sodium *m*-periodate. In this work, although the ratio between the sodium *m*-periodate and the xylose residue was only 1 : 3, the reagent still shows its effectiveness on the xylan oxidation.

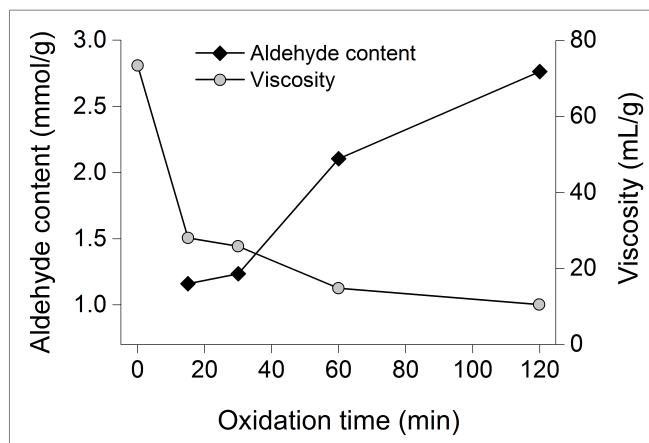


Figure 3.13: *Aldehyde content and intrinsic viscosity of oat spelt xylan after oxidation with sodium m-periodate in correlation with reaction time*

Changes in the structure of the xylan after oxidation are also reflected in its molecular weight as shown in table 3.3. Dramatically decreasing trends observed in both molecular weight and viscosity express a strong relationship between the two parameters. Molecular weight distribution of the OSX after 15 and 120 min oxidation is depicted in figure 3.14.

Table 3.3: *Molecular weight of oat spelt xylan and its derivatives after modification with sodium m-periodate (sp) for 15 and 120 min; data obtained by SEC with RI detector using pullulan standards and glucose for calibration*

<i>Sample</i>	<i>Code</i>	<i>Oxidation time, min</i>	<i>M_w, g/mol</i>
Oat spelt xylan	OSX	-	22000
Periodated oat spelt xylan	OSX_sp_15m	15	5000
Periodated oat spelt xylan	OSX_sp_2h	120	1500

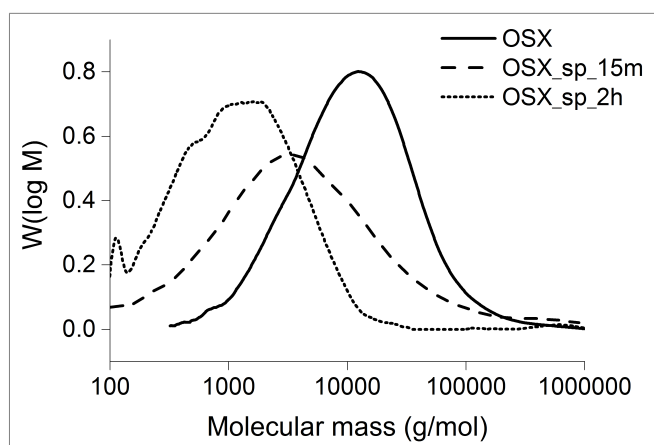


Figure 3.14: *Molecular weight distribution of oat spelt xylan and its derivatives after modification with sodium m-periodate (sp) for 15 and 120 min; data obtained by SEC with RI detector using pullulan standards and glucose for calibration*

Xylan oxidation with hydrogen peroxide

The oxidation of the oat spelt xylan was carried out following the procedure described in section 2.8.1. The aldehyde content and intrinsic viscosity of the oxidized xylan in correlation with the reaction time is illustrated in figure 3.15.

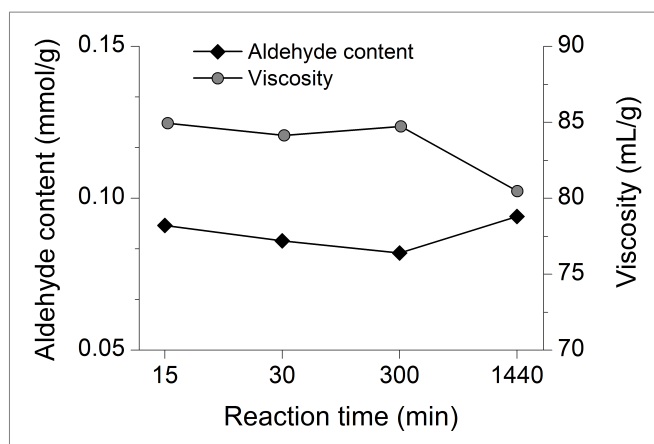


Figure 3.15: *Aldehyde content and intrinsic viscosity of oat spelt xylan after oxidation with hydrogen peroxide in correlation with reaction time*

A slightly decreasing trend in the intrinsic viscosity with increasing reaction time is seen in the above figure. This slight change is also observed in the molecular weight of the oxidized products as shown in table 3.4. The molecular weight distributions of these derivatives are also available in figure 3.16.

Table 3.4: *Molecular weight of oat spelt xylan and its derivatives after modification with hydrogen peroxide (hp) for 15 and 480 min; data obtained by SEC with RI detector using pullulan standards and glucose for calibration*

<i>Sample</i>	<i>Code</i>	<i>Oxidation time, min</i>	<i>M_w, g/mol</i>
Oat spelt xylan	OSX	-	25000
Peroxidated oat spelt xylan	OSX_hp_15m	15	16000
Peroxidated oat spelt xylan	OSX_hp_8h	480	17000

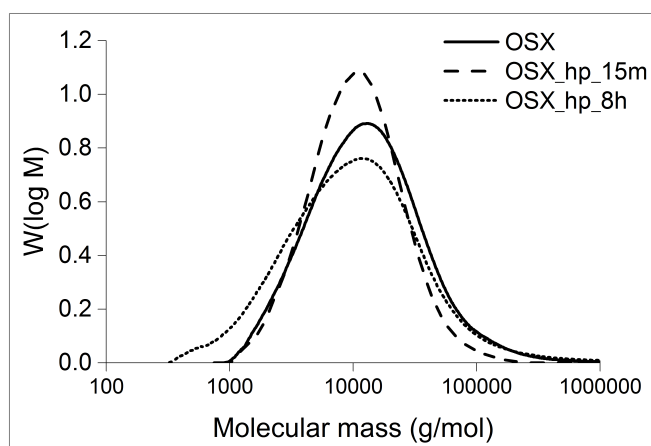


Figure 3.16: *Molecular weight distribution of oat spelt xylan and its derivatives after oxidation with hydrogen peroxide (hp) for 15 and 480 min; data obtained by SEC with RI detector using pullulan standards and glucose for calibration*

According to the data shown in table 3.4, the molecular weight decreases significantly after 15 min of reaction. The parameter stays almost the same when the reaction is prolonged for 8 h. This result suggests that under the used conditions, hydrogen peroxide does not have a strong influence upon the molecular structure of the xylan as sodium *m*-periodate does.

The aldehyde content insignificantly reduces for the first 5 h of reaction but increases slightly at longer period. This can be explained by the role of hydrogen peroxide as a bleaching agent at the very first period of the reaction. This is possible because the OSX in this study still contains a small amount of lignin, which is discussed later in section 3.2.6. After a certain time of oxidation, the formation of aldehyde occurs causing the increase of the aldehyde content of the product.

Xylan oxidation with ozone

When using ozone for xylan oxidation following the procedure described in section 2.8, the same trend in the intrinsic viscosity is observed as in figure 3.17. However, the aldehyde content only starts decreasing after 5 h reaction like in case of using hydrogen peroxide.

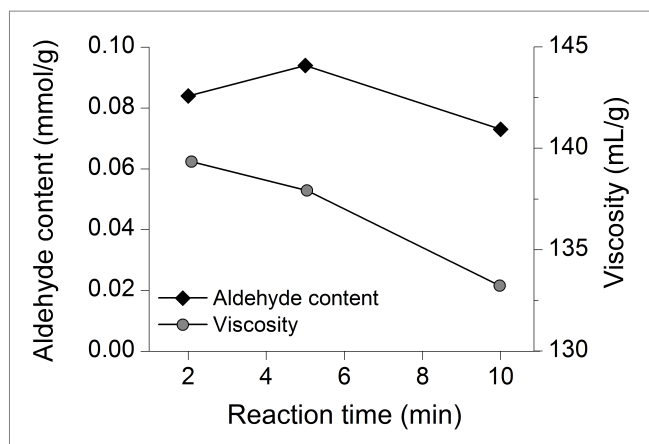


Figure 3.17: Aldehyde content and intrinsic viscosity of oat spelt xylan after oxidation with ozone in correlation with reaction time

The consumption of ozone along with the reaction time shown in figure 3.18 indicates that ozone actually reacts with the polysaccharide. The difference between input and access amount of ozone increases gradually with increasing reaction time, while the aldehyde content is rather low (the highest value is 0.06 mmol/g compared to approx. 3 mmol/g of the periodated xylan). However, the intrinsic viscosity are generally much higher than when using other oxidants.

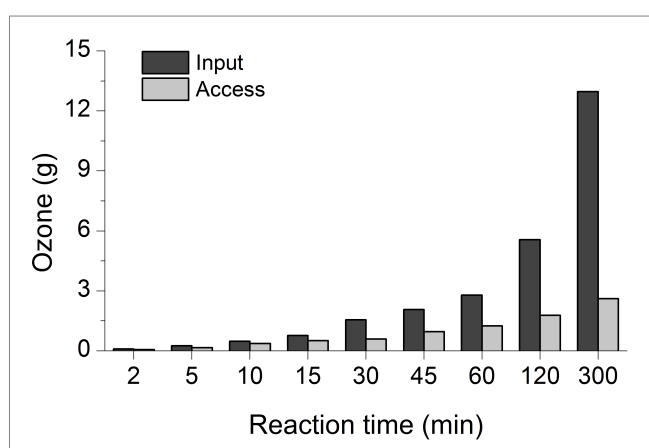


Figure 3.18: Ozone consumption along with reaction time

The molecular weight and molecular weight distribution of the OSX and its modified derivatives after 2 and 45 min reaction with ozone is presented in table 3.5 and figure 3.19, respectively.

Table 3.5: *Molecular weight of oat spelt xylan and its derivatives after oxidation with ozone (oz) for 2 and 45 min; data obtained by SEC with RI detector using pullulan standards and glucose for calibration*

<i>Sample</i>	<i>Code</i>	<i>Oxidation time, min</i>	<i>M_w, g/mol</i>
Oat spelt xylan	OSX	-	25000
Ozonated oat spelt xylan	OSX_oz_2m	2	21000
Ozonated oat spelt xylan	OSX_oz_45m	45	21000

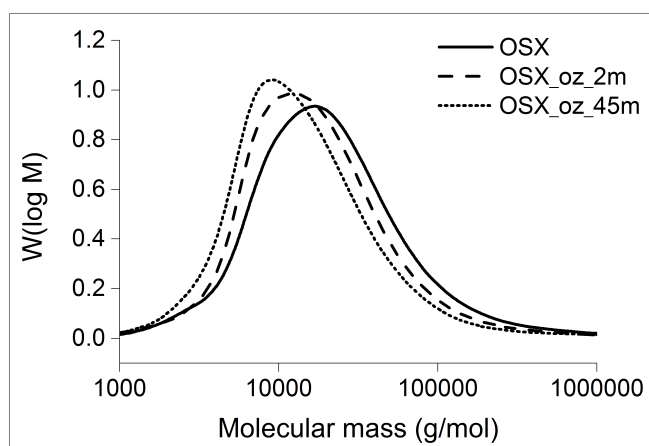


Figure 3.19: *Molecular weight distribution of oat spelt xylan and its derivatives after oxidation with ozone (oz) for 2 and 45 min; data obtained by SEC with RI detector using pullulan standards and glucose for calibration*

Compared with the two other chemicals, ozone provides the least influence in the xylan structure under the used conditions. According to table 3.5, the molecular weight of the xylan reduces slightly after 2 min of reaction and seems to stay the same until 45 min. However, this is partly not in correlation with the dramatically decreasing trend of the viscosity as shown in fig 3.17. Unlike sodium *m*-periodate, hydrogen peroxide and ozone are well-known as bleaching agents in paper technology. Hydrogen peroxide is also used in xylan isolation. The role of these agents in this study is not much clear since their influence is not obvious. In order to use them for xylan oxidation, the used conditions might not be adequate to provoke a big change in the structure of the xylan. More tests will be needed to verify whether these two chemicals are suitable for xylan oxidation or not.

To sum up, among the three used oxidants and under certain conditions, sodium *m*-periodate is the most effective and appropriate reagent for xylan oxidation. The aldehyde content of the oxidized product increases significantly with increasing reaction

time, while the intrinsic viscosity is reduced. Change in structure of the xylan derivatives is also reflected in their molecular weight. Optimal conditions for the periodation should be of interest but beyond the scope of this study.

3.2.3 Amidation

Pure oat spelt xylan was amidated following the procedure described in section 2.8.2. Two concentrations of ammonia solution in isopropanol, i.e., 4 M and 6 M, were used. Corresponding amidated products of the OSX were coded as Amo4M and Amo6M, respectively. The amidation process was assessed through nitrogen content of the final products as illustrated in figure 3.20.

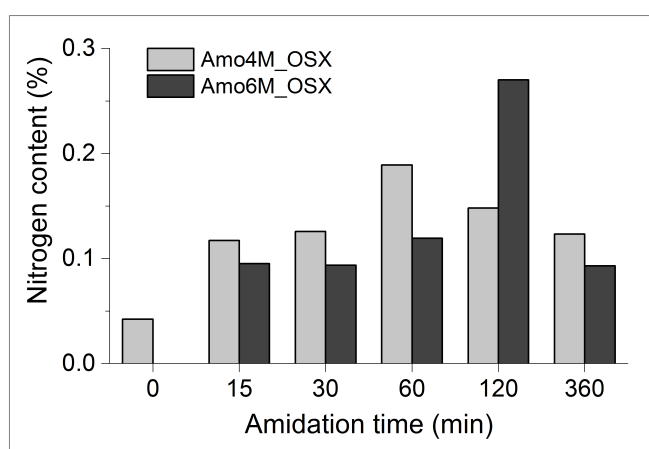


Figure 3.20: *Nitrogen content of amidated oat spelt xylans in correlation with reaction time; Amo4M_OSX and Amo6M_OSX: oat spelt xylans amidated with 4 M and 6 M ammonia solution, respectively*

In general, the nitrogen content is low. This might be the case, because the OSX contains a low amount of carbonyl and/ or carboxylic groups, which are necessary for the amidation. The dependence of the nitrogen content on ammonia concentration is shown apparently in figure 3.20. It can be seen that the nitrogen content of the product reaches the highest value after 60 min when using 4 M ammonia solution, and after 120 min when using 6 M solution. The lower concentration (4 M) shows its advantages over the 6 M solution for the first 60 min of reaction. The trend stays the same for the whole range of the amidation except for the period of 120 min of reaction using 6 M ammonia.

To conclude, for amidation of the oat spelt xylan 4 M ammonia solution is better than 6 M solution. The amidation results in low content of nitrogen in general due to the low content of carbonyl groups in the OSX. The results indicate that the OSX can be amidated best with 4 M of the solution for 60 min.

3.2.4 Two-step modification

Oat spelt xylan (13-Xyl-03) was modified following the two-step procedure illustrated in figure 2.5, section 2.8.3. The OSX was first oxidized with sodium *m*-periodate at room temperature for 15, 30, 60, and 120 min, resulting in oat spelt dialdehyde xylans (OS-DAXs) as intermediate products. In the next step, these OS-DAXs were amidated with 4 M ammonia solution in isopropanol for 60 min, which formed the final products, oat spelt cationic xylans (OS-CatXs). Aldehyde content of the intermediate products and nitrogen content of the final products were determined to evaluate the modification.

Figure 3.21 shows the aldehyde content of the OS-DAXs and the nitrogen content of the OS-CatXs in correlation with the oxidation time.

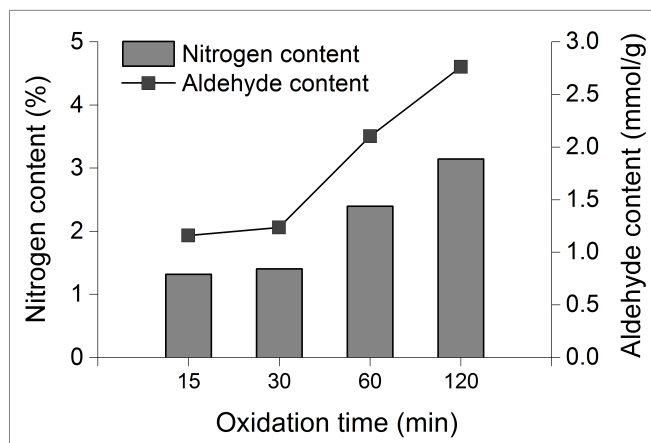


Figure 3.21: *Aldehyde content of oat spelt xylan after the oxidation step in correlation with nitrogen content of the same xylan after the amidation step*

As expected, there is a correlation between these two parameters, which both increase significantly with prolonged oxidation time. This indicates that the longer reaction time, the more cleavages occur and more aldehyde groups are formed. As discussed earlier, the oxidation aimed to activate the xylan molecules by producing aldehyde groups, which are necessary for the amidation reactions to be occurred. As can be seen from the graph, the values of the aldehyde content are quite low, with the highest value of approx. 2.7 mmol/g after 2 h of oxidation. This leads to a correspondingly low content of nitrogen in the final product. As already discussed earlier, the effectiveness of this modification procedure can be increased by changing the oxidation conditions. Amendment of concentration of sodium *m*-periodate or temperature should be of interest for further investigation.

In conclusion, the effectiveness of the two-step modification depends on the oxidation since this step activates the xylan structure to enable it for the amidation procedure

in the next step. The results show a correlation between the nitrogen content of the amidated xylan and the aldehyde content of the intermediate product.

3.2.5 Paper application of xylans and their derivatives

Oat spelt xylan and wheat arabinoxylan were modified using the two-step modification method as described previously. The process results in the intermediate products (DAXs) and the final products (CatXs). All of these xylans were added into the paper one in succession with a dosage of 2% based on the dry mass of the pulp. Short names for these derivatives of the xylans are given in table 3.6. The used milled pulp had the refining degree of 19.5 °SR.

Table 3.6: *Xylan derivatives formed during the two-step modification of oat spelt xylan and wheat arabinoxylan*

<i>Code</i>	<i>Product name</i>	<i>Note</i>
OS-DAX	oat spelt dialdehyde xylan	intermediate product
W-DAX	wheat dialdehyde xylan	
OS-CatX	oat spelt cationic xylan	final product
W-CatX	wheat cationic xylan	

Tensile and tear index of the finished papers with and without oat spelt xylan and its derivatives addition are depicted in figure 3.22.

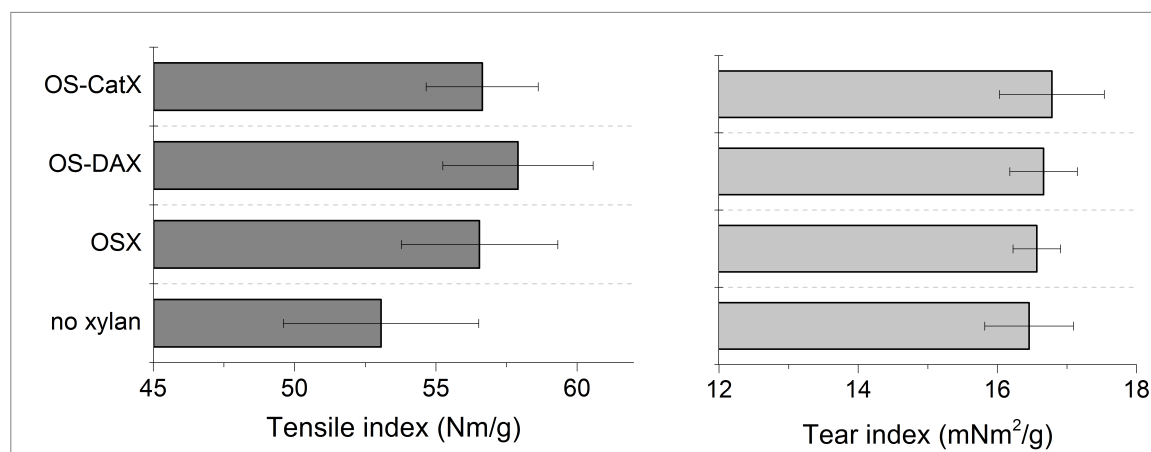


Figure 3.22: *Mechanical properties of papers with oat spelt xylan (OSX) and its derivatives addition; oat spelt dialdehyde xylan (OS-DAX) and oat spelt cationic xylan (OS-CatX) are the intermediate product and final product of the two-step modification, respectively; milled pulp 19.5 °SR; xylan dosage 2%*

The data show a significant improvement in the tensile index and only a small en-

hancement in the tear index. The addition of the OS-DAX provides the paper with the highest tensile strength, which is about 9% improved in comparison with the pure paper. When using the OS-CatX and the unmodified OSX, these enhancements are almost similar with 6.6% and 6.7%, respectively.

A similar trend in the tensile index is observed when using the WAX and its derivatives in paper as illustrated in figure 3.23. The influence of these xylan derivatives on the tensile strength is not much different to the OSX as described earlier. Surprisingly, the intermediate product, wheat dialdehyde xylan (W-DAX) also results in the highest tensile index of the paper. It also gives the highest value in the tear index.

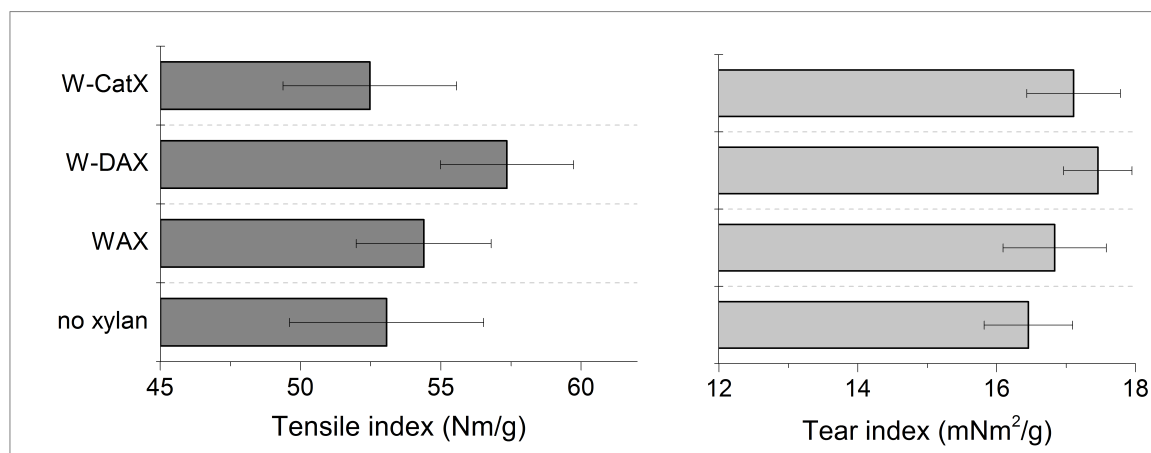


Figure 3.23: *Mechanical properties of papers with wheat arabinoxylan (WAX) and its derivatives addition; wheat dialdehyde xylan (W-DAX) and wheat cationic xylan (wheat cationic xylan (W-CatX)) are the intermediate product and final product of the two-step modification, respectively; milled pulp 19.5 °SR; xylan dosage 2%*

The addition of xylan and cationic derivatives to paper is proved to improve the paper mechanical properties due to two factors. On the one hand, the interaction between the xylan and cellulose fibers is supported by more hydrogen bonds between the functional groups on the surface of these two polysaccharides created. On the other hand, the electrostatic interaction between two oppositely charged objects are believed to play an important role in strengthening the fibers network [21,117]. However, this rule is only confirmed when using xylans modified with some powerful cationization reagents like EPTAC, CHPTAC, and other related compounds. In this experiment, the OSX and WAX were oxidized with sodium *m*-periodate and followed by the amidation. The alkaline environment during the amidation might cause the degradation of the xylan chains leading to the product with low molecular weight. Shortened chains of xylan derivatives are believed to result in the lower tensile index of the paper as seen in this section. The phenomenon observed in the tensile index has a good correlation with the retention capacity of the OSX and its derivatives, which is discussed later in section 3.4.5.

To conclude, the two-step modification of xylan including an oxidation and an amidation step subsequently produces two products, dialdehyde xylan and cationic xylan. The results show that the dialdehyde xylan, which is obtained from the intermediate process can improve the tensile strength of the paper much more effectively than the final cationic product. This finding is quite unexpected and suggests that the amidation step in the two-step method might not be necessary for the modification of OSX and WAX and can be eliminated.

3.2.6 Structural characterization of xylan

Molecular weight and molecular weight distribution

The molecular mass distribution of the OSX obtained by SEC with an RI detector is illustrated in figure 3.24. The data reveal an average molecular weight (M_w) of 22000 g/mol for the OSX.

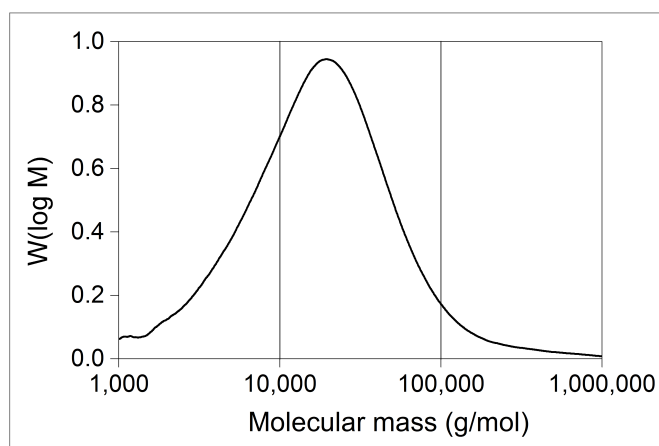


Figure 3.24: *Molecular mass distribution of oat spelt xylan; signal obtained by SEC with RI detector using pullulan standards and glucose for calibration; xylan concentration 5.62 g/L*

This molecular mass of the OSX is in the range of typical xylyans from annual plants, e.g., 28000 – 29000 g/mol for barley hemicellulose [101] and 25400 g/mol for DMSO extracted oat spelt xylan [111]. Whereas, xylyans from hardwood normally have higher molecular weight with around 30000 – 36000 g/mol [29, 66, 96]. However, the molecular weight of oat spelt xylan in general can be different depending on the raw material as well as the extraction method and conditions. For instance, an OSX sample with M_w of 79200 g/mol was isolated by Hettrich *et al.* [43].

Molecular structure of oat spelt xylan

The molecular structure of the isolated oat spelt xylan was confirmed by FT-Raman spectroscopy. Figure 3.25 depicts the Raman spectra of the OSX before and after an

extra-bleaching step.

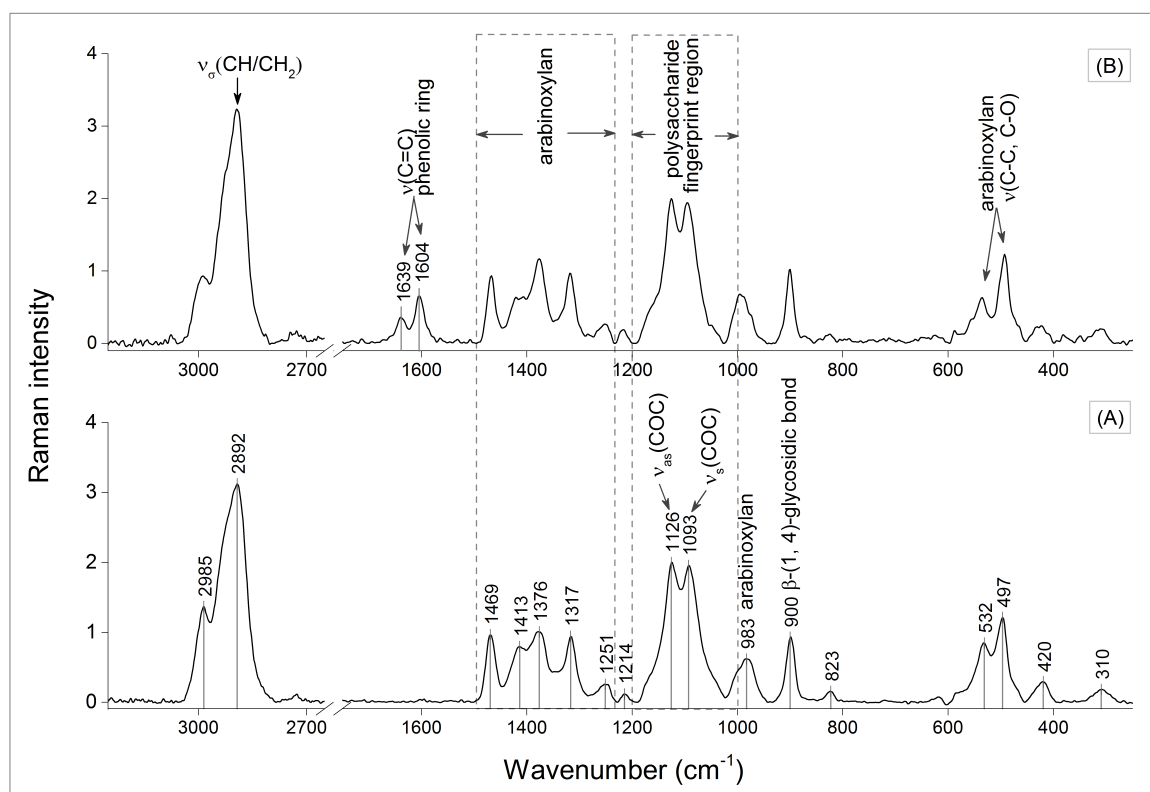


Figure 3.25: *FT-Raman spectra of oat spelt xylan before (B) and after (A) an extra-bleaching step*

Major peaks at 497, 532, 900, 983, 1093 – 1126, and 1251 – 1469 cm^{-1} are observed. These bands are typical in arabinoxylan [7,97,98]. The bands at 497 and 532 cm^{-1} are assigned to C-C and C-O stretching vibrations. The presence of the β -(1, 4)-glycosidic bonds of the xylan backbone is identified by the peak at around 900 cm^{-1} .

A pair of bands observed at 1093/1126 cm^{-1} is assigned to glycosidic linkages, involving C-O stretching vibration. These are typical peaks assigned to asymmetric and symmetric stretching vibrations of COC and belong to the polysaccharide fingerprint region, which is usually seen in the range of 1000 – 1200 cm^{-1} [7, 109, 122]. These peaks usually appear in xylan samples, including wheat arabinoxylan as well as some hard wood xylans [7,62,122]. When using hulls or husks from annual plants as starting materials for xylan isolation, the products may contain proteins, phenols, and other soluble polysaccharides. As an illustration, a double peak observed at 1604/1639 cm^{-1} is resulted from phenolic ring. According to Kacuraková *et al.* [62], ferulic acid has characteristic adsorption bands at 1517, 1600, 1620, and 1690 cm^{-1} . Furthermore, Himmelsbach and Atkin indicated that one of the typical peaks of ferulic acid appeared at 1602 cm^{-1} , which was almost the same position as lignin at 1601 cm^{-1} [44]. As mentioned before, hemicelluloses from annual plants and cereals usually contain

ferulic acid groups, which are covalently linked to the arabinose residues via ester linkages [57, 129]. However, these ester bonds are easily broken during the xylan isolation using sodium hydroxide and hydrogen peroxide especially when using oat spelt as a raw material [129, 141]. Therefore, this doublet could be identified as the lignin residue. To confirm this hypothesis, an extra-bleaching process was carried out for the xylan using chlorine dioxide as described in section 2.6. As expected, these peaks are absent in the spectrum of the bleached xylan as seen from figure 3.25 (A). Hydrogen peroxide in alkaline environment does not only remove lignin but can also dissolve and hydrolyze hemicelluloses resulting in lower molecular weight of the isolated product. As a result, the bleaching process should only reach a level where the extraction yield is high and the product has an acceptable brightness.

In addition, a very strong, intense band observed at 2892 cm^{-1} is assigned to CH/CH_2 asymmetric stretching vibration, which is also typical for carbohydrates.

Molecular structure of modified oat spelt xylan

The structure of OSX and its derivatives was characterized by FT-Raman spectroscopy. Important peaks and their assignments are listed in table 3.7.

Figure 3.26 depicts Raman spectra of the pure OSX, its derivative OS-CatX modified with the two-step modification method, and the cationic oat spelt xylan modified with CHPTAC (quab-to-xylan ration = 1 : 1, DS 0,45), CHP-OSX-1/1. In both cases, changes observed in the range of $2894/2937\text{ cm}^{-1}$ are characterized as symmetric and asymmetric stretching vibrations of CH/CH_2 . A sharp peak at 3031 cm^{-1} is assigned to the symmetric stretching vibration of $(\text{CH}_3)_3\text{N}^+$ due to the structure of CHPTAC. There is a small amount of lignin, which is shown as a double peak at $1639/1604\text{ cm}^{-1}$ due to the lignin residue after the isolation process. Changes at 1471 cm^{-1} refer to symmetric deformation vibration of CH/CH_2 . While, the peak at 1372 cm^{-1} is assigned to a symmetric deformation vibration of CH/CH_2

Specific diffusion bands are observed at $1122/1093\text{ cm}^{-1}$. These are typical peaks assigned to asymmetric and symmetric stretching vibration of COC. They also belong to the polysaccharide fingerprint area, which is usually seen in the range of $1200 - 1000\text{ cm}^{-1}$.

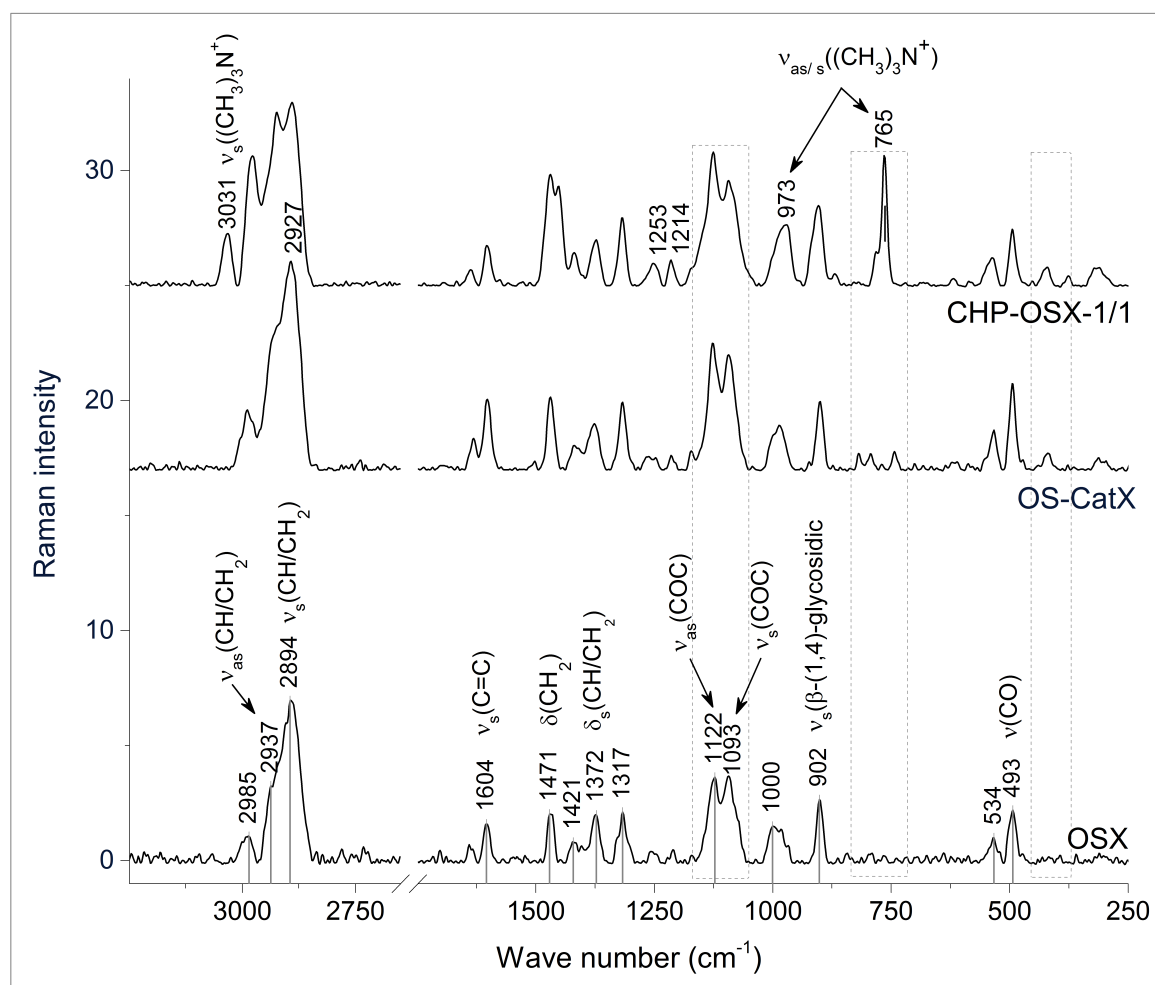


Figure 3.26: Raman spectra of oat spelt xylan and its cationic derivatives obtained by the two-step modification (OS-CatX) and by using CHPTAC (CHP-OSX-1/1); ν , δ respectively stand for stretching and in-plane bending (deformation) vibrations; s = symmetric, as = asymmetric

Another typical band for xylan as well as cellulose is the peak at 902 cm^{-1} . This peak is related to symmetric stretching vibration of β -(1, 4) glycosidic linkage of the xylan backbone. In case of CHP-OSX-1/1, a sharp peak at 765 cm^{-1} is identified as symmetric stretching vibration of $(\text{CH}_3)_3\text{N}^+$ groups from the structure of CHPTAC. These peaks are not observed in the OS-CatX because the OS-CatX in this experiment was modified with sodium *m*-periodate and ammonia. Unlike the Raman spectrum of CHP-OSX-1/1, there is almost no change in the spectrum of the OS-CatX in comparison to the starting material OSX. This is also reflected in the fact that this xylan does not improve the mechanical properties of the paper as discussed previously in section 3.2.5. While, the xylan modified with CHPTAC, CHP-OSX-1/1, can significantly strengthen the tensile index as mentioned earlier (section 3.2.1).

To sum up, the structure of the OSX has been changed after the modification especially when using the cationizing reagent CHPTAC. When using the two-step modification

method developed in this work, the oat spelt cationic xylan shows less changes in functional group than the CHPTAC-cationized OSX. This is in agreement with the mechanical properties of the paper as discussed earlier.

Table 3.7: *Band positions and assignments for Raman spectra of oat spelt xylan and its cationic derivatives obtained by the two-step modification (OS-CatX) and by using CHPTAC (CHP-OSX-1/1) [7, 95, 130]*

Wavelength (cm ⁻¹)			Band assignment
OSX	OS-CatX	CHP-OSX-1/1	
		3031	(CH ₃) ₃ N ⁺ symmetric stretching
2927 (sh)	2927 (sh)	2927 (sh)	CH/CH ₂ asymmetric stretching
2894	2894	2894	CH/CH ₂ symmetric stretching
1064	1064	1064	C=C stretching
1417	1417	1417 (s)	CH ₂ deformation
1372	1372	1372	CH/CH ₂ symmetric deformation
1122	1122	1122	COC asymmetric stretching
1093	1093	1093	COC symmetric stretching
		973	(CH ₃) ₃ N ⁺ asymmetric stretching
902	902	902	β -(1, 4) glycosidic linkage symmetric stretching
		765	(CH ₃) ₃ N ⁺ symmetric stretching
493	493 (s)	493	CO stretching

Note: OSX – oat spelt xylan; OS-CatX – oat spelt cationic xylan modified with the two-step method; CHP-OSX-1/1 – oat spelt cationic xylan modified with CHPTAC using reagent-to-xylan ratio of 1 : 1, DS 0.45; s – strong; sh – shoulder

3.3 Chemical modification of pectin

Due to the glucuronic-acid-rich structure of pectin, only amidation was needed for the modification of this polysaccharide. Three apple pectins, namely AP1, AP2, and AP3 (the information about these pectins is described previously in section 2.1.1) were amidated following the method described in section 2.8.2. The amidation was conducted alternatively for 0.5, 1, 2, and 5 h. The reaction resulted in corresponding amidated products which are coded as APx(0.5), APx(1), APx(2), APx(5), respectively

($x = 1, 2, 3$ in proportion to the AP1, AP2, and AP3). The amidated pectins were analytically characterized through the degree of esterification, acetyl group content, ferulic acid content, and nitrogen content. Structure of the pectins and their modified derivatives were studied by FT-Raman and FT-IR spectroscopy.

3.3.1 Analytical characterization of amidated pectins

Degree of esterification (DE)

As mentioned earlier, the DE is referred to the percentage of methyl carboxylated groups at the C6 of the pectin [120]. This parameter was calculated based on the ferulic group content in the pectin sample, which is determined according to the standard curve of ferulic acid as shown in appendix B. Figure 3.27 plots the DE of the amidated pectins as a function of reaction time.

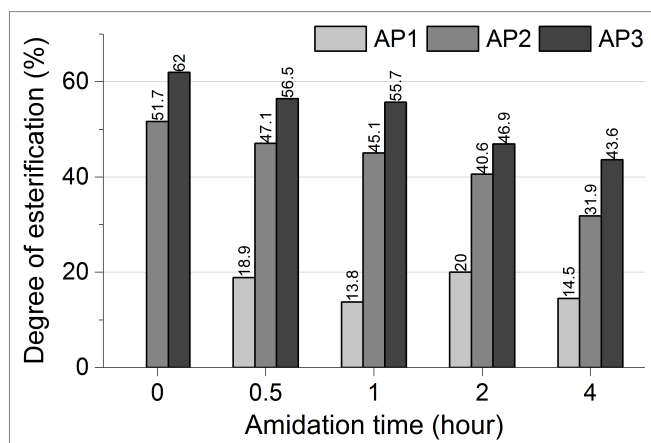


Figure 3.27: Changes in degree of esterification of apple pectins after amidation

According to the data and the terminology mentioned in section 1.3.1, AP2 and AP3 are classified as high-methyl-ester (so-called high-methoxyl) pectins and AP1 is classified as low-methyl-ester (so-called low-methoxyl) pectin. As can be seen from figure 3.27, the DEs of the amidated AP2 and AP3 are significantly decreased with increasing amidation time. During the amidation, the methyl ester groups react with ammonia resulting in amide compounds, which causes the reduction in this parameter. The same trends are observed when high-methoxyl pectins were amidated for different periods of time at 5 °C by Reitsma *et al.* [104]. They indicated that the saponification of the methyl ester groups was very limited in extent and confirmed that the amidation concerned essentially the ester groups. Furthermore, amidation is also known as a deesterification method for producing low-methyl-ester pectins [102].

The DE of the pure AP1 was not measurable. This suggests that the method might not

be suitable for the pectins with too low DE. Moreover, this could also be the reason for a looking of trend in the DE of the AP1 after amidation as it is not obvious.

Acetyl group content

Figure 3.28 shows changes in the acetyl group content of AP2 and AP3 after amidation. Data for AP1 are not available since this analytical method does not seem to work with this pectin and its amidated derivatives. Perhaps, AP1 contains a low amount of acetyl groups that is difficult to determine with the used method.

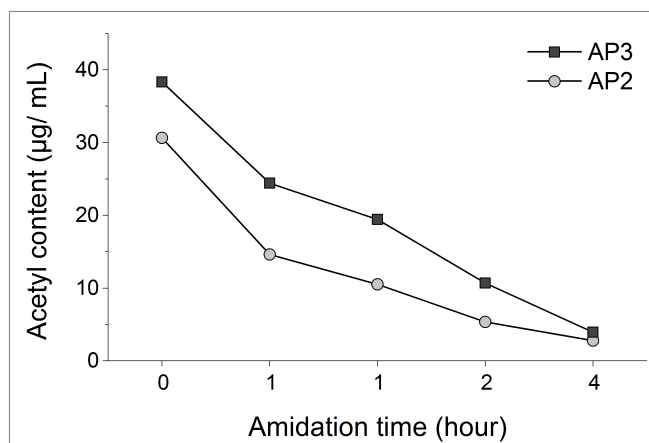


Figure 3.28: *Changes in acetyl group content of apple pectins after amidation*

From the above figure it can be seen that the amount of acetyl groups reduces remarkably with increasing amidation time. This functional group is almost removed after four hours of reaction. Consider the structure of acetyl groups in pectin (as shown previously in figure 1.16), during the reaction with ammonia, the ester bonds are broken. According to the proposed mechanism illustrated in figure 1.19, this reaction produces acetamide and leaves a hydroxyl group on the main chain of the pectin as shown in figure 3.29. During the washing step, acetamide might be washed out. As a result, the content of acetyl groups of the pectin is reduced with increasing amidation time.

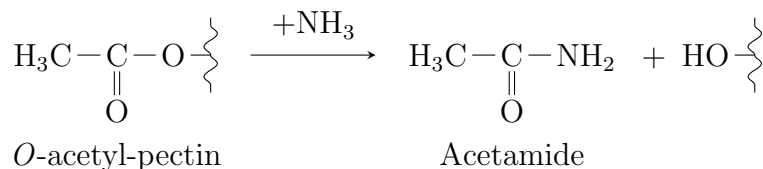


Figure 3.29: *Possible reaction between acetyl group in pectin with ammonia*

Ferulic acid content

Ferulic acid is present in pectin as a side chain that is linked via ester bonds. The content of ferulic acid of the amidated pectins as a function of amidation time is

presented in figure 3.30.

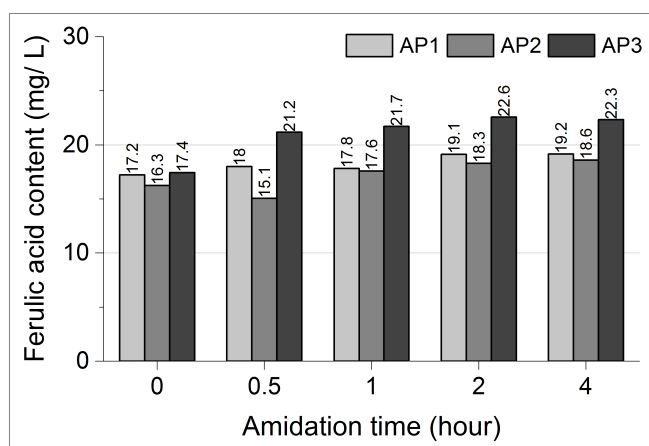


Figure 3.30: *Changes in ferulic acid content of apple pectins after amidation*

At first glance, the three pure AP1, AP1, and AP3 have nearly the same content of ferulic acid with approx. 16 – 17 mg/L. The content of this group does not change much in AP1 and AP2, whereas a slightly increasing trend is observed in case of AP3. In fact, ferulic acid is not influenced by the ammonia solution, however, during the amidation, the content of the acetyl and methyl groups reduces significantly. This apparently leads to the reduction of the molecular weight of the polysaccharide. Hence, the ferulic acid content tends to increase slightly with increasing amidation time.

Nitrogen content

Nitrogen content is one of the important parameters used to evaluate the effectiveness of the amidation process. Figure 3.31 depicts the nitrogen content of the three apple pectins as a function of amidation time.

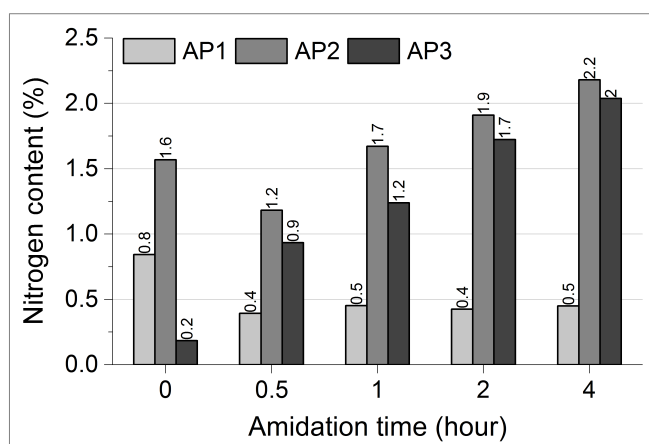


Figure 3.31: *Changes in nitrogen content of apple pectins after amidation*

The nitrogen content of AP3 increases remarkably with increasing amidation time. The reason is straightforward and in correlation with the decrease of the DE as discussed

previously. First of all, the pectin is classified previously as high-methyl-ester pectin. During the amidation, the more methyl ester groups are deesterified, the more nitrogen can be induced resulting in the formation of more amide groups. In case of AP2, also a high-methyl-ester pectin, the nitrogen content decreases after 30 min of reaction and then increases gradually for the rest of the reaction time. For AP1, the higher nitrogen content in the starting material might be from measurement error since it is just about 0.8% compared to 0.4 – 0.5% of the amidated one. This is understandable because this pectin contains very low content of acetyl and methyl ester groups.

To sum up, the amidation has influenced the functional groups of the three apple pectins. The degree of esterification and the content of acetyl group decrease with increasing reaction time, which result in the increase of the nitrogen content after the amidation. The method for determination of DE and acetyl groups content are not suitable for low-methyl-ester pectins like AP1.

3.3.2 Paper application of pectins and their derivatives

Pure apple pectins with different DEs (AP1, AP2, AP3) and their modified derivatives were added to the paper during the paper making process. Different dosages of pectin, 2, 5, and 10%, based on the dry mass of the pulp were used. However, the application of these pectins in paper only worked with 2 and 5%. At higher content (i.e., 10%), the pulp suspension became so thick that water could not be drained normally to form a uniform sheet. Therefore, the results are shown only for 2 and 5% dosages of the pectins. In each case, 4 – 5 replicates resulting in the corresponding number of sheets were produced.

The tensile and tear properties of the final papers with AP1, AP2, and AP3 addition versus amidation time are depicted in figures 3.32, 3.33, and 3.34, respectively. These graphs show the influence of the amidated pectin derivatives on the paper strength.

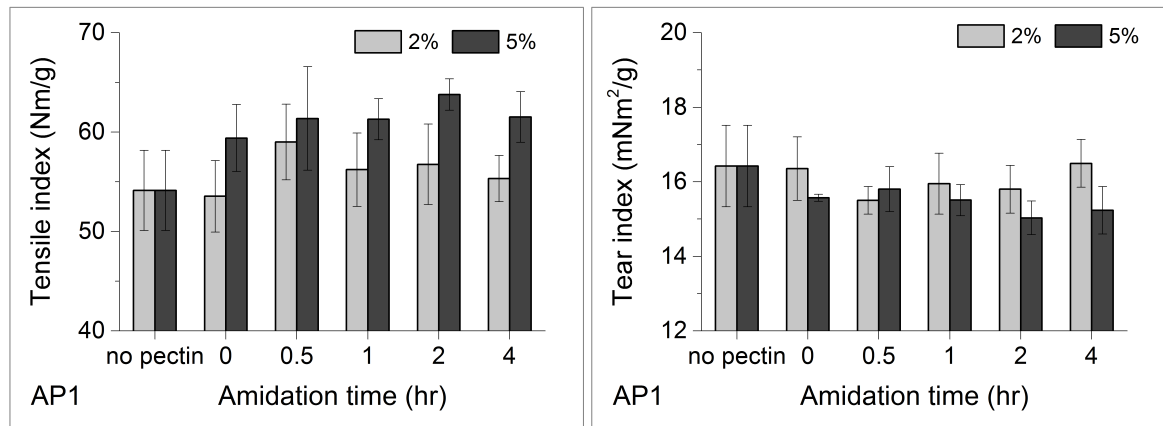


Figure 3.32: Influence of amidation time of the apple pectin AP1 on paper mechanical properties

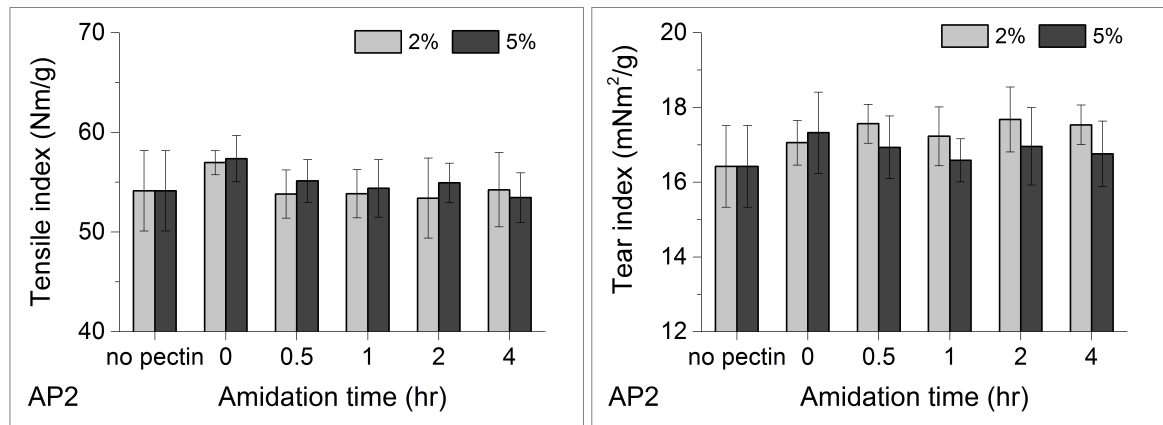


Figure 3.33: Influence of amidation time of the apple pectin AP2 on paper mechanical properties

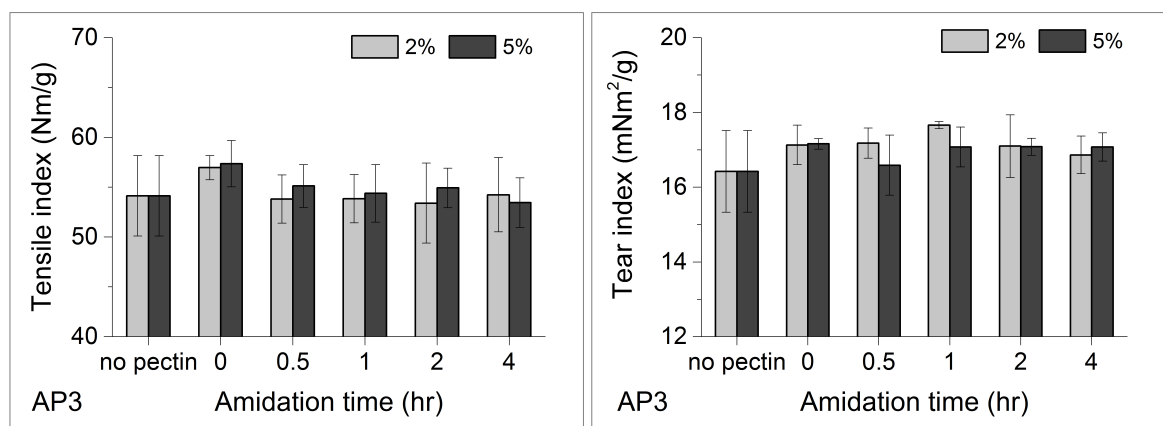


Figure 3.34: Influence of amidation time of the apple pectin AP3 on paper mechanical properties

Among the three varieties of apple pectins, only AP1 improves the tensile strength. This enhancement is more apparent when 5% of the pectin was used as seen in figure 3.32. However, these results are not in correlation with the nitrogen content as expected since the AP1 has the lowest nitrogen content compared to the other two pectins. In contrast, AP2, AP3, and their amidated derivatives do not bring any enhancement to the paper strength although their DEs are rather high. The different in structure of the three apple pectin, i.e., acetyl and methyl groups content, are believed to cause this phenomenon. The low DE of AP1 might lead to the fact that the pectin has more OH groups for the formation of hydrogen bondings with the cellulose. These results are similar to the case of OS-CatX and W-CatX, which were modified with the two-step modification. As discussed previously in section 3.2.5, the cationic xylans modified with the same amidation process do not enhance the paper strength. This finding suggests that the amidation process conducted in this work might not be suitable for the modification of the xylans and high-methyl-ester pectins. The low-methyl-ester pectin (e.g., AP1 and its amidated derivatives) can be used in paper with the dosage of 5%.

To sum up, among the used pectins, only the pectin with low DE (AP1 and its amidated derivatives) can enhance the paper mechanical properties. The results also suggest that the amidation might not be suitable for the pectins with higher DE (e.g., AP2, AP3, and their amidated derivatives) since only the original pectins slightly improve the paper strength. In addition, when using apple pectin in paper, the dosage should not be higher than 5% since the pectin solution becomes very viscous at high concentrations. However, although this finding could be scientifically interesting, the application of apple pectin in paper appears to be less promising and should, therefore, needs to be carefully considered.

3.3.3 Structural characterization of pectin

A combination of FT-IR and Raman spectroscopy is the preferred method for the complete analysis of carbohydrates structure instead of using each method separately [132]. FT-Raman and FT-IR spectroscopy are evident to compensate for each other. FT-Raman spectroscopy is sensitive to homo-nuclear molecular bonds like C–C, C=C, or C≡C since it depends on polarizability of a molecule. The method is, therefore, more suitable for complex skeletal structure of polysaccharides, e.g., glycosidic bonds and pyranoid rings. In contrast, FT-IR spectroscopy is sensitive to hetero-nuclear functional group vibrations and polar bonds since it depends on changes in the dipole moment. Hence, the FT-IR spectroscopy is an excellent application to detect functional groups and side chains, e.g., hydroxyl groups, carboxyls, esters, and amides.

Most important peaks and band assignments are listed in table 3.8.

Table 3.8: *Band positions and assignments for FT-IR and FT-Raman spectra of apple pectin [28, 119, 130, 132]*

Frequency (cm ⁻¹)	Assignment	IR	Raman
~ 3500	$\nu(\text{O-H}), \nu(\text{N-H})$	3524-3447	
2900 \pm 200	$\nu(\text{C-H})$ aliphatic	2941-22	2956-44
1745 \pm 10	$\nu(\text{C=O})$ ester	1755-51	1749-47
1670 \pm 20	amide I, $\nu(\text{C=O})$		1680
~ 1600	$\nu(\text{C=C})$ skeleton		1612-1592
	$\nu_{as}(\text{COO}^-)$	1626-12	
~ 1355	$\delta(\text{CH}_3)$ acetate		1336-30
	$\delta(\text{C-O-H})/ \delta(\text{CH}_2)$ twist		1324
~ 1230	amide III/ $\nu(\text{C-O})$ acetate	1239-33	1231
1100 \pm 50	$\nu(\text{C-O-C})$ glycosidic linkage		1151-47
1160-970	Nonlocalized, highly coupled modes of polysaccharide backbones; fingerprint region of polysaccharides	1148 1101 1016	1106 1079 1048 981-79
~ 950	$\delta(\text{CH}_2)/ \delta(\text{CCH})/ \delta(\text{COH})$		925
~ 850	$\nu_{as}(\text{C-O-C})$ glycosidic linkage		858-52
~ 800	$\delta(\text{NH}_2)$ wag	833-29	836-32
	$\delta(\text{NH}_2)$ wag		813-07
700 \pm 50	$\delta(\text{R-O-H})$		717-15
684	$\nu_s(\text{C-O-C})$ glycosidic linkage		686-84
441	$\delta(\text{C-O-C})$ glycosidic linkage		445-41
336	$\delta(\text{C-O-C})$ glycosidic linkage		339-33

Note: ν , δ are respectively designated stretching and in-plane bending vibrations; s and as stand for symmetric and asymmetric

Figure 3.35 illustrates FT-Raman spectra of the apple pectin AP1 and its derivatives.

The derivatives were obtained by amidation for 0.5, 1, 2, and 4 h resulting in four products, which are coded as AP1(0.5), AP1(1), AP1(2), and AP1(4), respectively. The FT-Raman spectra of the AP2, AP3, and their derivatives are presented in appendix C, D.

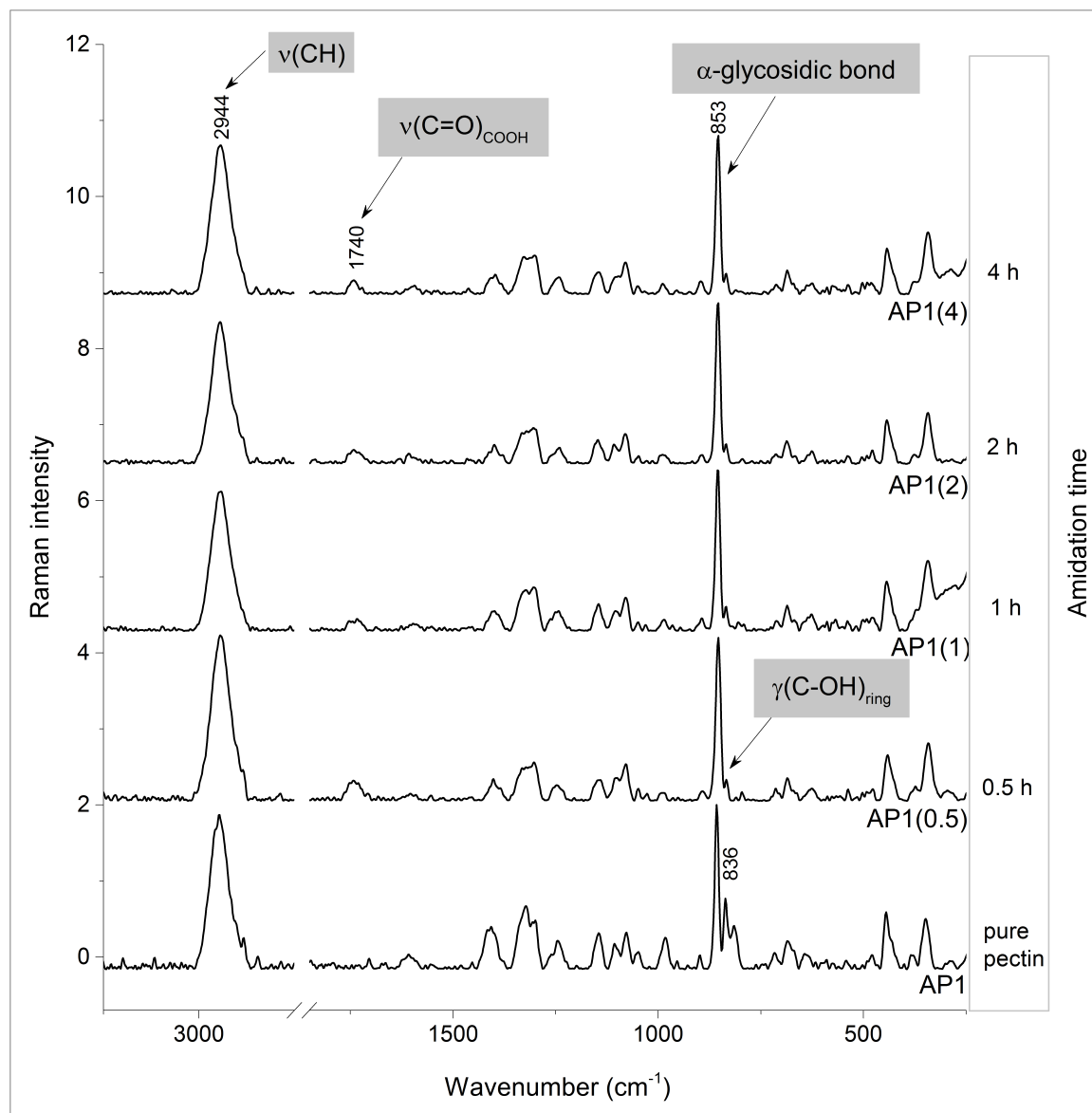


Figure 3.35: *FT-Raman spectra of apple pectin AP1 and its derivatives after 0.5, 1, 2, 4 h of amidation; ν , δ , γ are designated stretching, in-plane bending, and out-of-plane bending vibrations, respectively*

Some changes in the backbone structure of the pectin and its amidated derivatives are seen from the figure. Two highlight and intensive peaks at 2944 and 853 cm^{-1} are assigned to stretching vibration of CH bonds of pyranoid ring carbons and stretching vibration of COC of the glycosidic linkage of the backbone. These two peaks do hardly change since the reaction seems to happen only at carbonyl and/ or carboxyl groups. The peak at 853 cm^{-1} is also characterized as a typical band of pectin and can be used

for normalization in the Raman spectra [45]. When comparing the four derivatives with the starting material (AP1), the most obvious changes can be observed at 1740 and 836 cm^{-1} . The former is assigned to stretching vibration of C=O of carboxylic acid. However, this peak is not only present in the spectra of the derivatives of AP2 and AP3 but also in those of these original pectins (appendix J – N). In this case, it can not be concluded that the amidation causes this phenomenon. Two bands at 836 and 813 cm^{-1} of the AP1, which are assigned to out-of-plane vibration of hydroxyls C–OH are less intensive when compared to the derivatives. This might be due to the appearance of NH_2 groups after the amidation since deformation vibration of this group is also observed at this position.

Figure 3.36 visualizes the FT-IR spectra of AP1 and its two derivatives, which were obtained after 0.5 and 4 h of amidation. For other pectins and their modified products, the spectra are available in appendix I and J. As shown in figure 3.36, a broad and intense band appearing in the range of 3600 – 3200 cm^{-1} , which is not seen in Raman spectra is assigned to stretching vibrations of OH groups of hydroxyls or bound water. A peak at 2941 cm^{-1} is dominated by stretching vibrations of CH of esters and acids. Particularly, this peak is assigned to OCH_3 from methyl esters of galacturonic acid [72]. A big change observed at around 1736/ 1741 cm^{-1} is assigned to asymmetric stretching vibration of COO^- of ester. This peak becomes more intensive in the derivatives and can be resulted from the hydrolysis reaction during the amidation as described earlier in section 1.3.3, figure 1.19. A strong band at 1612 cm^{-1} appearing in the IR but very weak in the Raman spectrum of the AP1 is assigned to asymmetric stretching vibration of COO^- [132]. A noticeable change observed at 1250 cm^{-1} is assigned to stretching vibration of COC of acetyl group [132].

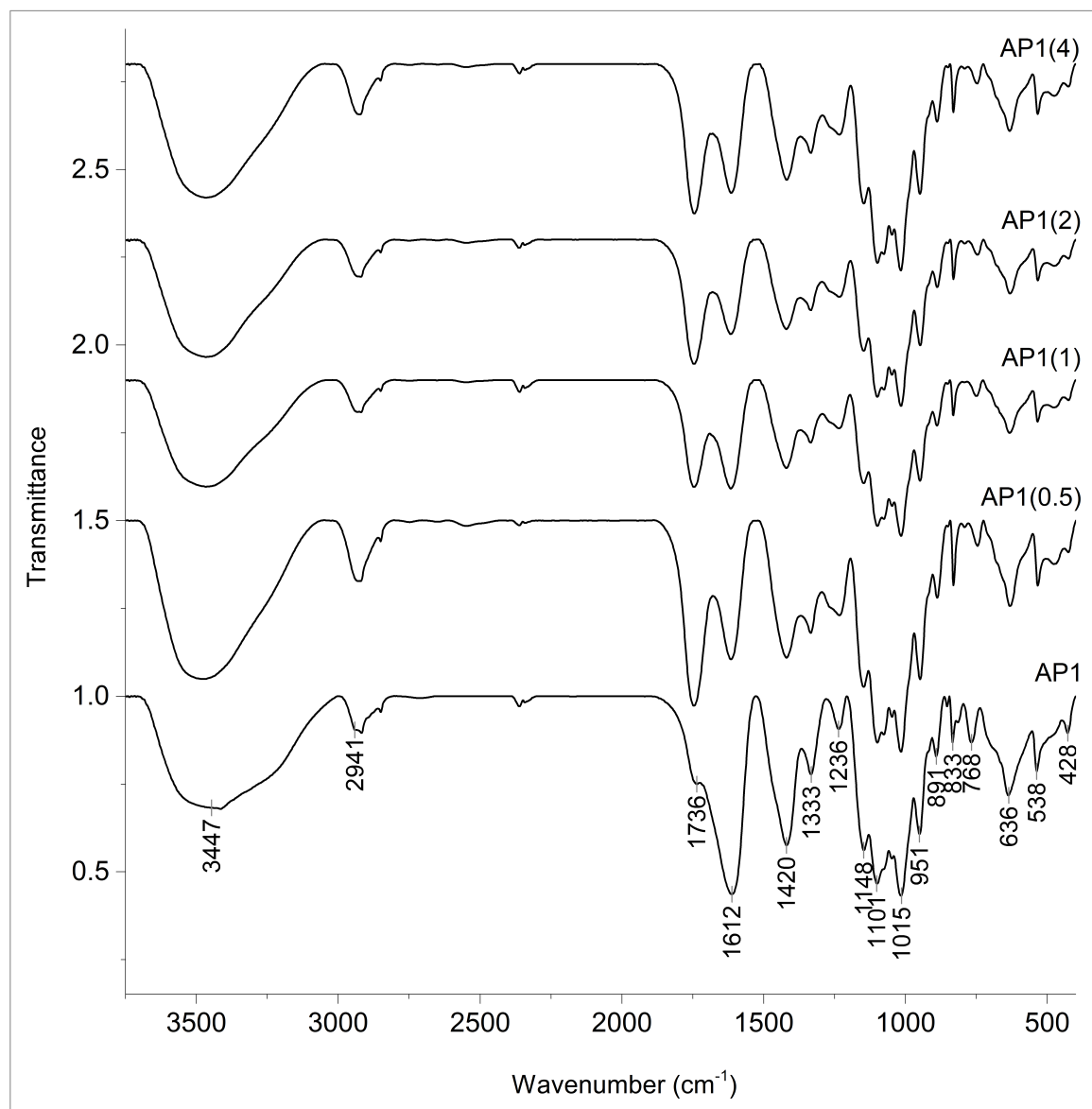


Figure 3.36: *FT-IR spectra of apple pectin AP1 and its derivatives after 0.5, 1, 2, 4 h of amidation*

3.4 Relative retention capacity of xylan on paper

In this work, a quantitative method to determine the adsorbed amount of xylan and its retention on paper after was developed. There are many reasons that lead to the fact that not all the xylan added into pulp slurry can be remained on the finished paper. This method aims to quantify how much xylan can be retained on the final paper sheets. In the end, it is allowed to evaluate the role of the adsorbed xylan in mechanical properties of the paper.

In this experiment, milled pulp with the refining degree of 18 °SR was used as cellulose substrate. Specific amounts of pure OSX (13-Xyl-01) ranging from 0 – 10% based on

the dry weight of the pulp were dispersed in warm water. The xylan suspensions were added into the pulp slurry during the paper making process following the procedure described in section 2.2.

3.4.1 Sugar composition of oat spelt xylan

The pure OSX was hydrolyzed with TFA under mild conditions following the procedure described in section 2.12.3. Major sugars, including xylose, arabinose, mannose, and galactose were analyzed with HPLC according to the method described in section 2.12.4. The xylose and arabinose are the most concerned components because they contribute the major part of xylan. Other sugars like glucose cannot be determined precisely because the mild hydrolysis method cannot degrade glucose completely. To analyze glucose, total hydrolysis should be used. However, the total hydrolysis is too strong for xylose that it converts xylose into furfural, which is not the purpose of this study. The sugar composition of the OSX is presented in table 3.9.

Table 3.9: *Major sugar composition of oat spelt xylan*

Single sugar [%]	Xylose	Arabinose	Galactose	Mannose
Oat spelt xylan	73.4 ± 3.5	6.6 ± 0.7	0.4 ± 0.0	-

As seen from the table, xylose constitutes a major part of the neutral sugars in the OSX with approx. 73%. This value is in the range of typical xylose content of OSX as indicated by other authors, for instance, Saake *et al.* [111] (76%), Sarbu *et al.* [112] (81%), and Kabel *et al.* [61] (77%). A small amount of arabinose (6.6%) indicates the presence of arabinose side chains attached to the backbone of this xylan. The xylose-to-arabinose ratio implies that one arabinose side chain substitutes every eleventh xylose unit of the xylan backbone. A ratio of 10 was also detected by Deutschle *et al.* [21] when they characterized an OSX sample. Galactose contributes only an insignificant amount, while mannose is not detected. The low content of galactose is assumed from arabinogalactan, which could be covalently linked to protein present in the xylan [21, 32]. The rest of sugar composition could be glucose, which cannot be detected correctly with the used method, and other sugars.

3.4.2 Sugar composition of papers with xylan addition

The paper sheets with and without xylan added were also hydrolyzed with TFA and their sugar composition was analyzed by HPLC. Figure 3.37 depicts the sugar contents of the papers with 0 – 10% of xylan addition.

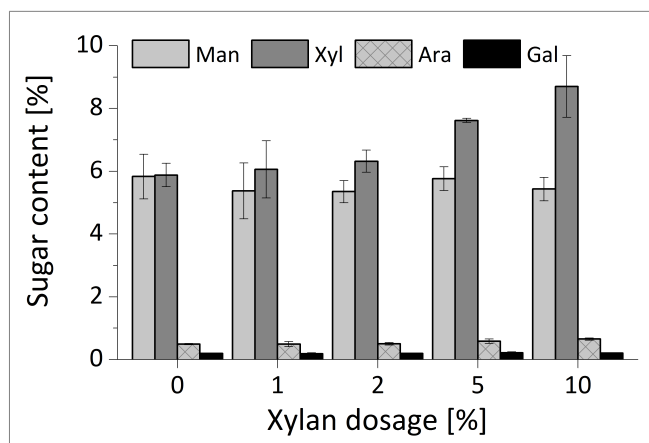


Figure 3.37: *Sugar composition of papers with and without oat spelt xylan addition*

As shown in the figure, xylose and mannose are the two major sugars in the paper samples. The xylose content increases significantly with increasing xylan dosages. On the one hand, this trend in xylose content is more obvious than the other sugars because xylose constitutes the major part of the xylan with 73% as presented in table 3.9. On the other hand, the addition of xylan into paper is believed to create more hydrogen bonds with cellulose fibers resulting in increased amount of xylose content in the finished paper sheet. There is almost no change in mannose since this sugar is not present in the xylan used on its own (data shown in table 3.9). Thus, the values only represent the preexisting mannose present in the paper itself. Galactose does not seem to be affected by xylan content since this sugar constitutes only a small part of the xylan as well as the paper sheets.

3.4.3 Relative retention capacity of xylan on paper

As mentioned earlier, the relative retention capacity of the OSX on paper is defined as the relation between the adsorbed amount and actual amount of xylose present in the xylan added to the paper. The determination of this parameter is based on xylose as this sugar makes up the major part of the xylan. The relationship between the amount of xylan retained on the paper and the xylan dosages is illustrated in figure 3.38.

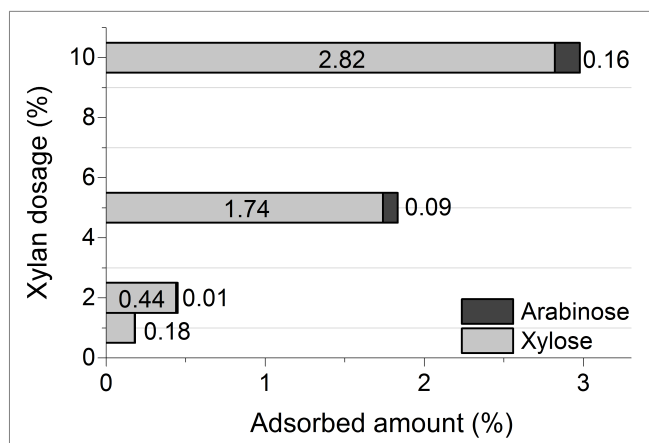


Figure 3.38: Adsorbed amount of xylose and arabinose in correlation with xylan dosage added into paper

It can be obviously seen that the adsorbed amount of xylan increases with increasing xylan addition. It reaches the highest value of 2.8% when 10% of xylan was added. This is in agreement with the xylose content of the papers with different dosages of xylan addition as shown in figure 3.37. However, it was indicated in a work of Han *et al.* that the amount of adsorbed xylan started to decrease when adding more than 8% of xylan [38]. This slight difference could be explained by properties of the used pulp and xylan as they used hardwood pulp and birch wood xylan compared to softwood pulp and oat spelt xylan in this work. In addition, the molecular weight, degree of substitution, and substitution patterns were assumed to influence the adsorption of xylan onto cellulose fibers [61,64,73,87]. Further investigation of this issue is of interest but beyond the scope of this study.

The relative retention capacity of xylan as a function of the xylan dosage is depicted in figure 3.39. Surprisingly, the highest amount of xylan retained on paper does not result in the highest relative retention capacity of the xylan. Nearly 48% of xylose remained on paper when 5% of xylan added, which is corresponding to 1.7% of the adsorbed amount of xylan. This is also the highest value among the used dosages of xylan in this work. Above this point (i.e., 10% of xylan dosage), the retention capacity starts to decrease. Arabinose, the sugar that appears once at every eleventh xylose unit of the xylan backbone, also shares the same trend with the highest retention capacity of approx. 18% when 5% of xylan was added. This phenomenon can be explained by a hypothesis of adsorption limitation of xylan on cellulose fibers. It is well known that paper is formed by creating a network of fibers through hydrogen bonds, which can be improved by the addition of xylan. This process, however, can have a saturation state. When this limitation is reached, xylan can no longer create linkages with the fibers. These results indicate that higher amounts of xylan addition (i.e., from 10% onwards) result in lower retention capacity of xylan in paper although the tensile strength can

still be improved. This has a special meaning in paper industry since the cost for xylan isolation and its application as a paper additive is still rather high.

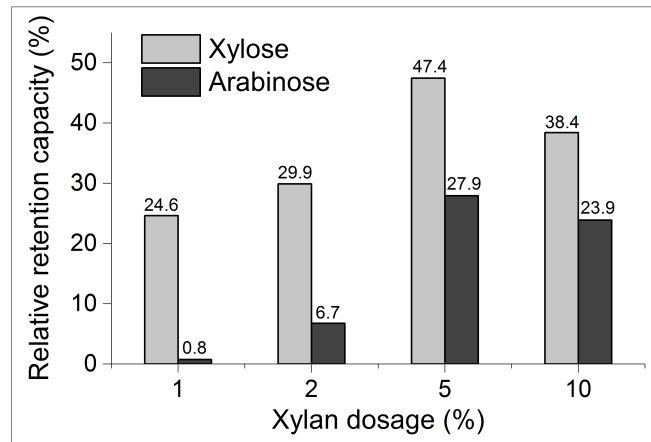


Figure 3.39: *Relative retention capacity of xylose and arabinose in correlation with xylan dosage added into paper*

To sum up, these results indicate that 5% of xylan can be considered as the best dosage for xylan adsorption onto paper. However, further experiments on papers with higher dosages of xylan addition, e.g., 12 – 15%, should be conducted to determine the saturation level of xylan adsorption onto cellulose and prove the proposed hypothesis.

3.4.4 Influence of xylan addition on mechanical properties of paper

The mechanical properties of the papers with xylan addition made in the previous experiment are also tested. The tensile and tear index of these samples are illustrated in figure 3.40.

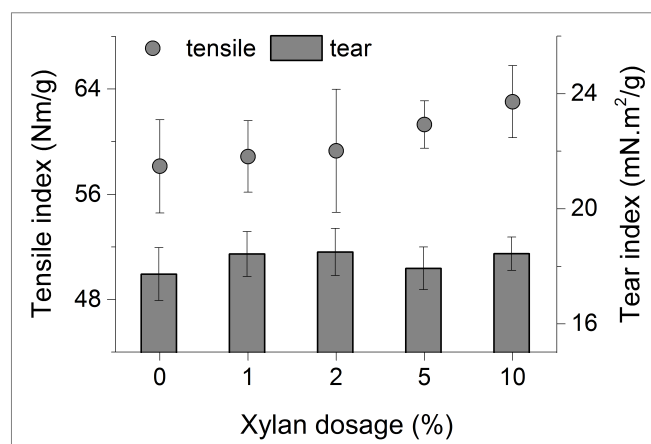


Figure 3.40: *Tensile and tear index of paper using different dosages of xylan*

Xylan dosage has a clear influence on the paper strength as discussed in section 3.1.3.

This feature is once again proved as can be seen from this figure. In accordance with the shown data, the tensile index slightly increases with increasing xylan dosages, while the tearing resistance is insignificantly influenced. This is obvious because tensile strength is dependent on the degree of fibers bonding, which is basically based on the hydrogen linkages. The addition of xylan to the paper is believed to strengthen the fibers network by increasing the number of hydrogen bonds between the polysaccharides [38]. Whereas, tear resistance strongly depends on the strength of individual fibers. The addition of xylan in this case might not bring any improvement to tear index since xylan is a polysaccharide with lower molecular weight in comparison to cellulose. This is also in agreement with the adsorbed amount of the xylan on cellulose as presented in section 3.4.3. These results confirm that higher amounts of xylan adsorbed on paper lead to higher tensile strength of the paper. Similar trends have been published previously by Ramírez *et al.* [103] when they investigated the sorption of corncob and oat spelt arabinoxylan onto softwood kraft pulp, and later by Han *et al.* [38] when birch xylan was used to modify eucalyptus pulp.

Consider figure 3.39 again, when 5% of the xylan was used, 47% of it can stay remained in the paper. The retention capacity is dropped to 38% when 10% of the xylan was added, while the tensile index still keeps increasing. These results suggest that there should be a saturation level for tensile index in correlation with the relative retention capacity of the xylan.

According to the results, it is possible to conclude that the relative retention capacity of the oat spelt xylan on paper depends on xylan dosages under the used particular conditions. The amount of xylan adsorbed on paper increases with increasing xylan dosages used. This is also reflected in the improvement of the tensile index of the papers. However, the retention capacity of the oat spelt xylan does not follow the same trend as the adsorbed amount since it reaches the highest value when only 5% of xylan was added. This finding suggests that very high amounts of xylan addition may not yield any further advantages to the adsorption, perhaps due to the saturation level being reached. In addition, the role of xylan in improvement of paper properties in correlation with remaining percentage of xylan on cellulose has been proved, especially for the tensile strength. The proposed method for determination of xylan adsorption on paper can be readily used with xylans from annual plants, like oat spelt and wheat straw. These findings may provide a practical approach to using oat spelt xylan as an alternative additive in paper technology in consideration of economic issues.

3.4.5 Relative retention capacity of modified xylans on paper

The xylan derivatives obtained from the two-step modification (presented in section 3.2.5) were added into paper. As discussed, the intermediate products (i.e., OS-DAX, W-DAX) provide the papers with better tensile strength than the final product (i.e., OS-CatX, W-CatX). In this section, the relative retention capacity of these xylan derivatives on the papers is also investigated using the developed method. The data are shown in table 3.10.

Table 3.10: *Sugar content of paper with oat spelt xylan and its derivatives addition in correlation with relative retention capacity of xylan on paper*

Samples	Sugar content (% \pm SD)				Retention capacity (%)
	Mannose	Galactose	Xylose	Arabinose	
Paper w/ o xylan	5.46 \pm 0.19	0.12 \pm 0.00	5.39 \pm 0.54	0.48 \pm 0.02	
Paper + OSX	5.51 \pm 0.06	0.15 \pm 0.05	5.45 \pm 0.66	0.51 \pm 0.04	3.28
Paper + OS-DAX	5.51 \pm 0.13	0.15 \pm 0.02	5.65 \pm 0.13	0.48 \pm 0.01	27.20
Paper + OS-CatX	5.47 \pm 0.21	0.15 \pm 0.00	5.49 \pm 0.79	0.45 \pm 0.03	5.79

Note: OS-DAX (oat spelt dialdehyde xylan) and OS-CatX (oat spelt cationic xylan) are the intermediate and the final product, respectively, of the two-step modification of the oat spelt xylan; papers were made from milled pulp with the refining degree of 19.5 °SR; xylan dosage 2%.

As can be seen, the paper with OS-DAX addition has the highest retention capacity of xylan in comparison to the papers with pure OSX and the OS-CatX addition. This is in good agreement with the tensile indices of these papers as presented in section 3.2.5. The finding suggests that the dialdehyde derivatives might have a better ability to interact with the cellulose fibers in comparison to the cationic ones. So far, the mechanism of this interaction is not clear and can be of interest for further investigation.

3.5 Evaluation of paper making process through standard deviation and values distribution

There are two types of error associated with an experimental result, which are “precision” and “accuracy”. The “precision” error relates to the random error distribution, which is associated with a particular experiment or event with a particular type of experiment. While, the “accuracy” error relates to the existence of systematic errors, such as the differences in laboratories, equipment, measurements, researchers, for instance. The

objective of a good experiment is to minimize these both errors. In fact, for the precision errors, the statistic analysis method can be used to evaluate, while the accuracy one can be minimized by improving the experimental skills of researchers, tight control of the experimental conditions and other objective factors.

As mentioned in section 2.3, all of the experiments related to paper making were carried out in 4 – 5 replicates resulting in the corresponding numbers of paper sheets. The tensile, tear, and burst indices of these paper sheets were then measured. Theoretically, the values between the replicates should be very similar. However, the difference is rather high. In this section, the values distribution among the replicates and their standard deviation (SD) were discussed. In order to evaluate the difference in measured values among replicates, or accuracy error in another word, a test was carried out in which the milled pulp with refining degree of 24 °SR was used. Two batches of pulp were conducted with 10 replicates per batch resulting in total of 20 sheets at the end. Normally, the tensile strength is measured in both machine direction and cross direction. However, with the lab-scale paper making machine, the sheet was formed in a round shape. Thus, the tensile strength was measured with two repetitions instead of MD and CD resulting in 20 values of tensile index per batch. The tearing strength was measured once per sheet, which results in 10 values of tear index per batch. Data distribution as well as mean and SD of the tensile and tear index are displayed in figure 3.41 and figure 3.42, respectively.

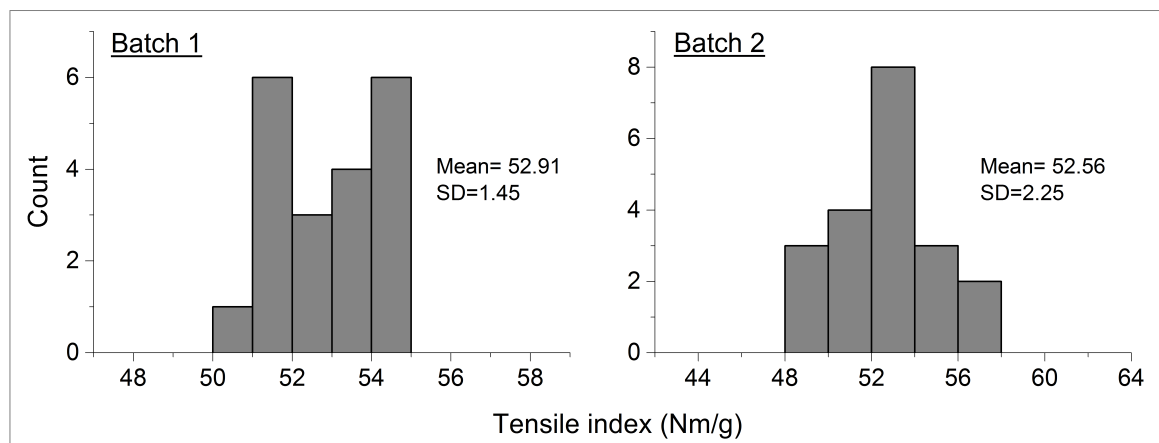


Figure 3.41: Values distribution of tensile index; data obtained from 2 batches with 10 replicates per batch

As can be seen from figure 3.41, in batch 1 the most recorded values are either at 51 – 53 or 54 – 55 Nm/g, while in batch 2 they concentrate at 52 – 53 Nm/g. This makes the variability of batch 2 greater than that of batch 1. The mean values of the two batches are relatively close, while the SD differs from each other. This difference in average tensile index between the two tested batches is believed to result from the

operation and, thus, cannot be eliminated.

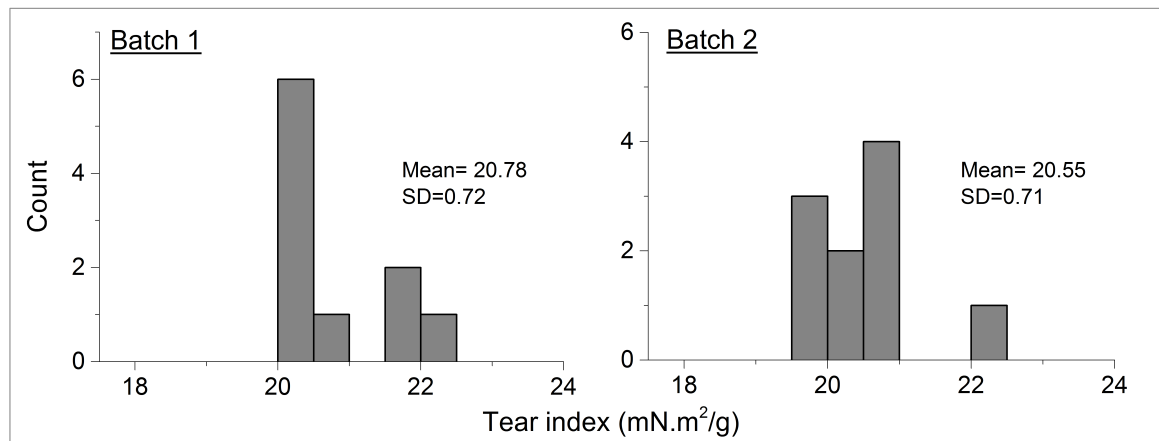


Figure 3.42: Values distribution of tear index; data obtained from 2 batches with 10 replicates per batch

According to figure 3.42, the mean and DS values of the tear index of the two batches are very similar, while their distribution rules are very different. In batch 1, most of the values are at 20 – 20.5 mNm²/g, but higher values also appear at a lower frequency. The most measured values in batch 2 are dispersed widely with many data at 19.5 – 21 mNm²/g. In general, the two batches have almost the same average tear index with 20.8 and 20.6 mNm²/g, respectively.

To conclude, this distribution of tensile and tear index is also encountered in all the experiments afterwards. This could be explained by machinery error from the paper making machine, equipment and other unpredictable factors that can not be avoided. Therefore, the accuracy error in this case is acceptable and the results presented in this work are reasonable.

4

Summary and perspective

Plant-derived polysaccharide is an attractive natural compound that offer a variety of promising potential since it is abundant, sustainable, and renewable. This material has a wide range of applications, and using it as paper additive is one of them. Over many centuries, paper has always played a very important and irreplaceable role in human life. It is an incredible, recyclable, and plant-based product that connects people to many important things in life in many ways.

The application of the plant-based polysaccharides as paper additives is now drawing a great attention. Among many kinds of known natural polysaccharides, xylan and pectin have only been recently studied. However these compounds are becoming more fascinating than ever due to their special structures and many hidden characteristics, which might be discovered by chemical modification for different purposes. Xylan and its cationic derivatives have been found to be able to enhance paper mechanical properties as they strengthen the fibers network through hydrogen bonds and electrostatic interactions. However, most of the previous work used wood xylans and modified them with organic chemicals, for instances, 2,3-epoxypropyltrimethylammonium chloride (EPTAC), (3-chloro-2-hydroxypropyl)trimethylammonium chloride (CHPTAC), 1,2-dimethoxyethane, sodium monochloroacetate. The modification process required a complicated procedure with waste treatment afterwards. This work aimed at investigating and developing a method for xylan and pectin modification, which only used simple and common inorganic chemicals. All reactions were conducted at ambient conditions, i.e., room temperature and neutral environment, which make it easier to upscale for application in the near future.

To fulfill the goals, xylan and pectin originating from agricultural residues, namely oat spelt and apple pomace were used. The reason behind this choice is to utilize waste and turn them into value-added products instead of burning or burying them

as traditionally. Oat spelt, a by-product of oat flake manufacturing, is a potential source for xylan isolation as it contains high amount of hemicellulose and low content of lignin. Apple pomace, a residue from fruit and juice processing, is already known as one of the main sources of pectin production. Prominent objectives and findings of the project are presented in the following paragraphs:

Investigation of the influence of original xylans and built-in cationic xylans on paper properties

For this purpose, xylans from different sources, including beech, birch wood, oat spelt, and wheat straw and their cationic derivatives were added into stock during a paper making process at laboratory scale. The cationic xylans used in this experiment were modified with the well-known procedure using cationizing reagents, including EPTAC and CHPTAC. The xylans were added into the pulp at different dosages ranging from 0 to 10% based on dry weight of the pulp. Tensile, tear, and burst strength of the finished paper were tested for an evaluation. The results show that oat spelt xylan is the best paper additive at any content in comparison to the others since it always gives the highest tensile values.

The tensile index is also dependent on the xylan content used. In case of using pure xylan, the tensile strength reaches its highest value at 2% of added xylan and then reduces significantly. However, a continuously increasing trend with raising xylan content is observed in case of using cationic xylan. The tear index is slightly decreased in both cases. There is almost no change in the tensile and tear index when pure xylan and cationic xylan are added into un-milled pulp.

The degree of substitution of xylan also plays an important role in paper properties. The xylans with higher DS result in higher tensile indices, while the tear index stays almost the same. In addition, homogenization time of xylan in the pulp slurry does not have significant influence on the paper formation.

Modification of oat spelt xylan and application of the cationic modified xylan to paper

It is obvious that cationic xylan prepared using the cationizing reagents (e.g., EPTAC, CHPTAC) can strengthen the paper due to the special structure of these substances. In this work, xylan was modified via oxidation and/ or amidation and a combination of these two processes. The oxidation was conducted with three common oxidizing chemicals, namely sodium *m*-periodate, hydrogen peroxide, and ozone. Aldehyde content and intrinsic viscosity were used to evaluate the oxidation process. Among the

used chemicals, sodium *m*-periodate shows its prominent features with clear trends in both aldehyde content and intrinsic viscosity along with the oxidation time. The aldehyde content increases gradually while the viscosity decreases. The reduction of the viscosity of the oxidized xylans in all cases are also reflected in the decrease of their molecular weight. No clear trend for aldehyde content is observed when using hydrogen peroxide or ozone. Further investigation to find out the optimal conditions of the periodation, e.g., reagent concentration in correlation with reaction time and pH as well as solid-liquid ratio is, therefore, necessary. If using the other two oxidants, catalysts should be considered to stimulate the production of $\text{HOO}\cdot$ / $\text{HO}\cdot$ or $\text{O}\cdot$ radicals for more effective reaction with the xylan.

The pure xylans and also cationic derivatives were amidated using different concentrations of ammonia solution in isopropanol for different periods of time. Nitrogen content of the products was tested for evaluation. The amidation using lower concentration of ammonia solution seems to be more effective and the cationic derivatives tend to react with the agent faster than the unmodified substances.

The two-step modification method is a combination of these two mentioned processes, in which the oxidation was carried out with sodium *m*-periodate and followed by the amidation using ammonia solution in isopropanol. The experiment results in an intermediate product, i.e., dialdehyde xylan, and a final product, i.e., cationic xylan. Aldehyde content and nitrogen content were tested for evaluation. There is an agreement between the aldehyde content of the intermediate product and the nitrogen content of the final product. The higher content of aldehyde groups results in higher content of nitrogen after the amidation.

Xylans modified using the two-step modification method were added into paper to judge the method. The findings show that the papers with the intermediate product – dialdehyde xylan – give higher values in the tensile index than that with the final product – cationic xylan. This suggests that the oxidation step might not be necessary and can be eliminated. The same phenomenon is observed when using wheat arabinoxylan. This is also confirmed through sugar content data obtained by HPLC analysis as it also shows the same trend in xylose content.

Amidation of pectin and paper application

Three apple pectin samples with different degree of esterification, AP1, AP2, and AP3, were used. The amidation process was carried out using ammonia solution in isopropanol for different reaction time. The modification results in reductions of acetyl content, degree of esterification, and an increase in nitrogen content, which are in

an agreement with changes in their structure as shown in FT-IR and FT-Raman spectra. According to the results, only the pectin with low DE (AP1) gives a slight enhancement in tensile index when 5% of this pectin is used. In addition, due to the viscous property, maximum 5% of pectin can be added since at higher doses, the pulp suspension becomes so viscous that water cannot be drained normally during the sheet formation. Further studies on pectins from other sources, such as sugar beet or citrus should be of interest since they are significantly different in chemical and physical properties. However, the study could be scientifically interesting, but the application of pectin in paper appears to be less promising and should, therefore, need to be carefully considered.

Development of a quantitative method for xylan retention determination

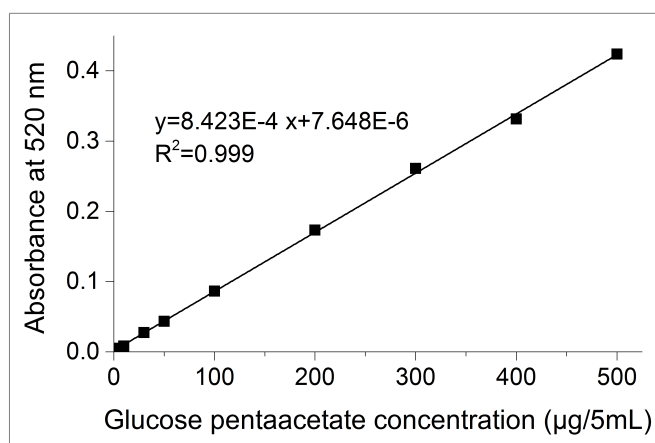
Adsorption and retention of xylan on paper are believed to be the most important reasons for xylan-cellulose interaction. In this work, a new method was developed to quantitatively determine the remained amount of xylan in paper. The principle of this method is mainly based on sugar analysis using high performance liquid chromatography. The original oat spelt xylan was added into paper at dosages of 0 to 10%. The paper sheets were hydrolyzed with tri-fluoroacetic acid under mild conditions in order to produce single sugars, including xylose, arabinose, mannose, and galactose for HPLC analysis. The results shows that the relative retention capacity of the oat spelt xylan on paper depends on xylan dosages under the used conditions. The adsorbed amount of xylan increases with increasing xylan dosages, which results in the improvement of the tensile strength of the paper. However, the tensile strength of the paper has its saturation level in correlation with the relative retention capacity of the xylan. This findings provide a practical approach to the application of xylan as a paper additive in consideration of economic issues.

To conclude, the current study provides a new approach to chemical modification of xylan and pectin for paper application. The advantage of this work is that it only uses simple and common chemicals, which requires no complicated treatment of waste afterwards. Further investigation to find out the optimal conditions for the xylan modification should be taken into account. In addition, deeper studies on changes in structure of xylan during the modification should be of interest. The quantitative method for xylan retention determination should be developed for further application of not only pure xylan but also other materials as well as their ionic derivatives.

Appendix

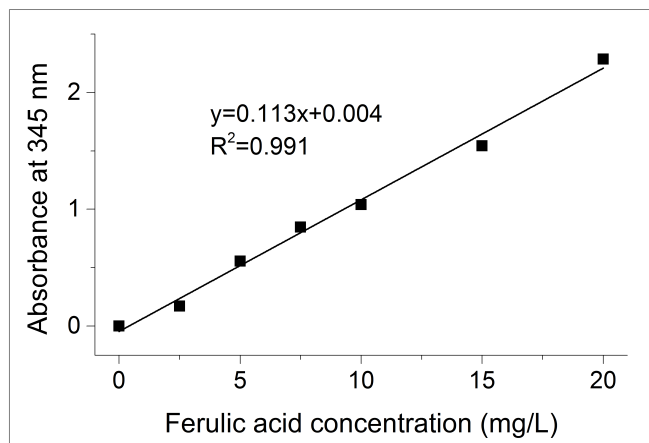
A Standard curve for glucose pentaacetate concentration

This standard curve was built according to the procedure described in section 2.11.1. It was used for determination of acetyl group content in the apple pectins.

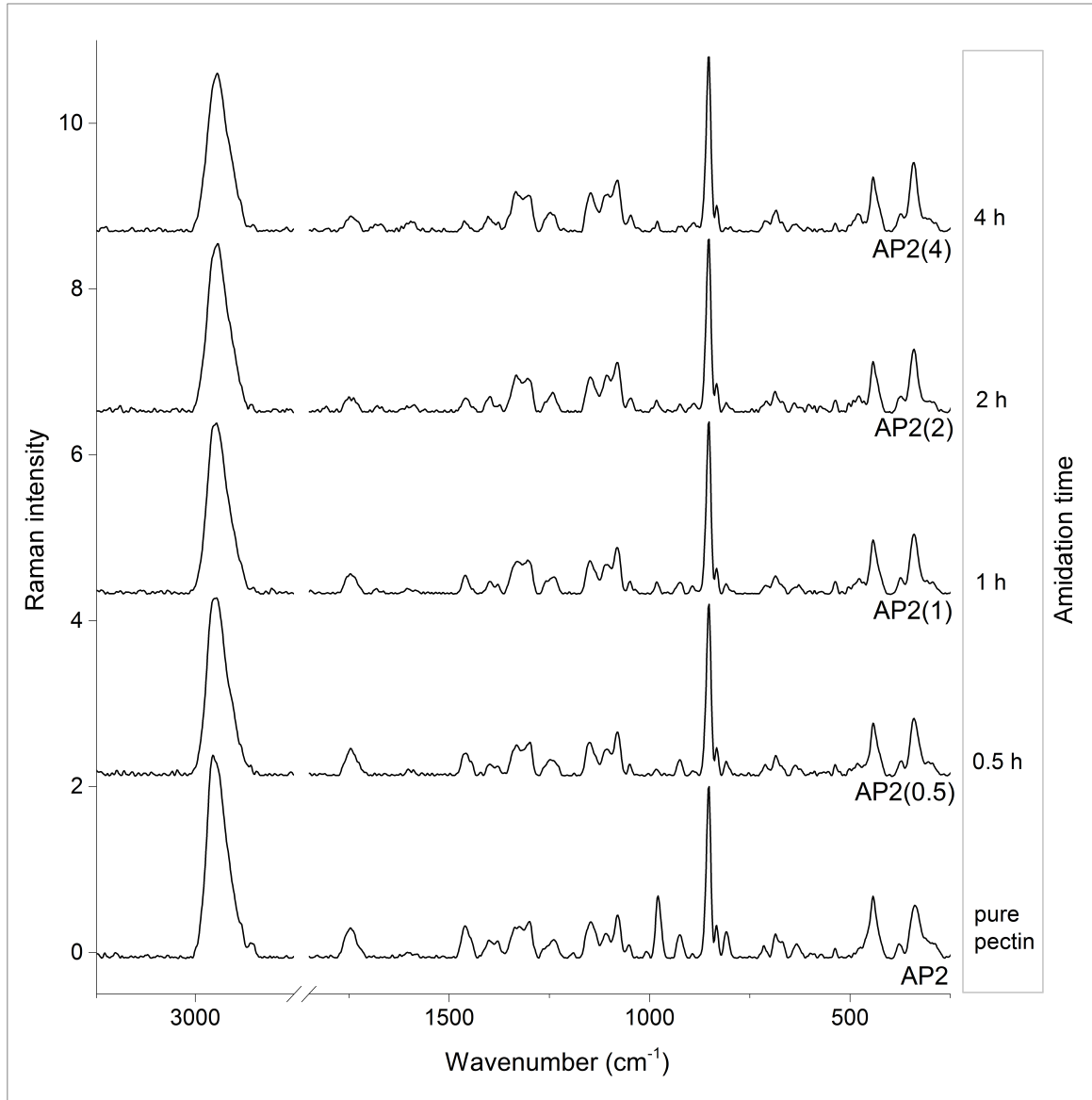


B Standard curve for ferulic acid concentration

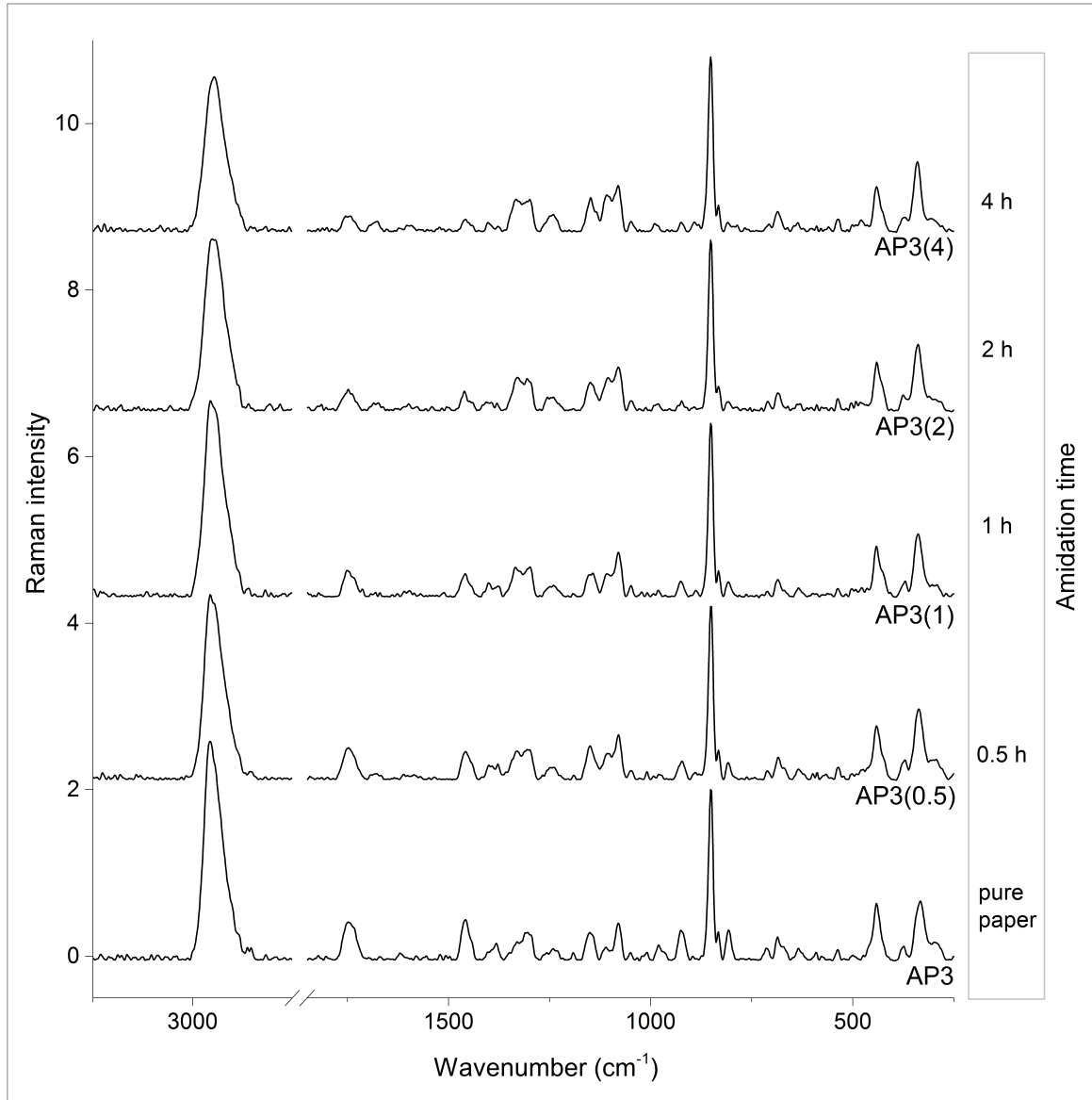
This standard curve was built according to the procedure described in section 2.11.2. It was used for determination of ferulic acid content in the apple pectins.



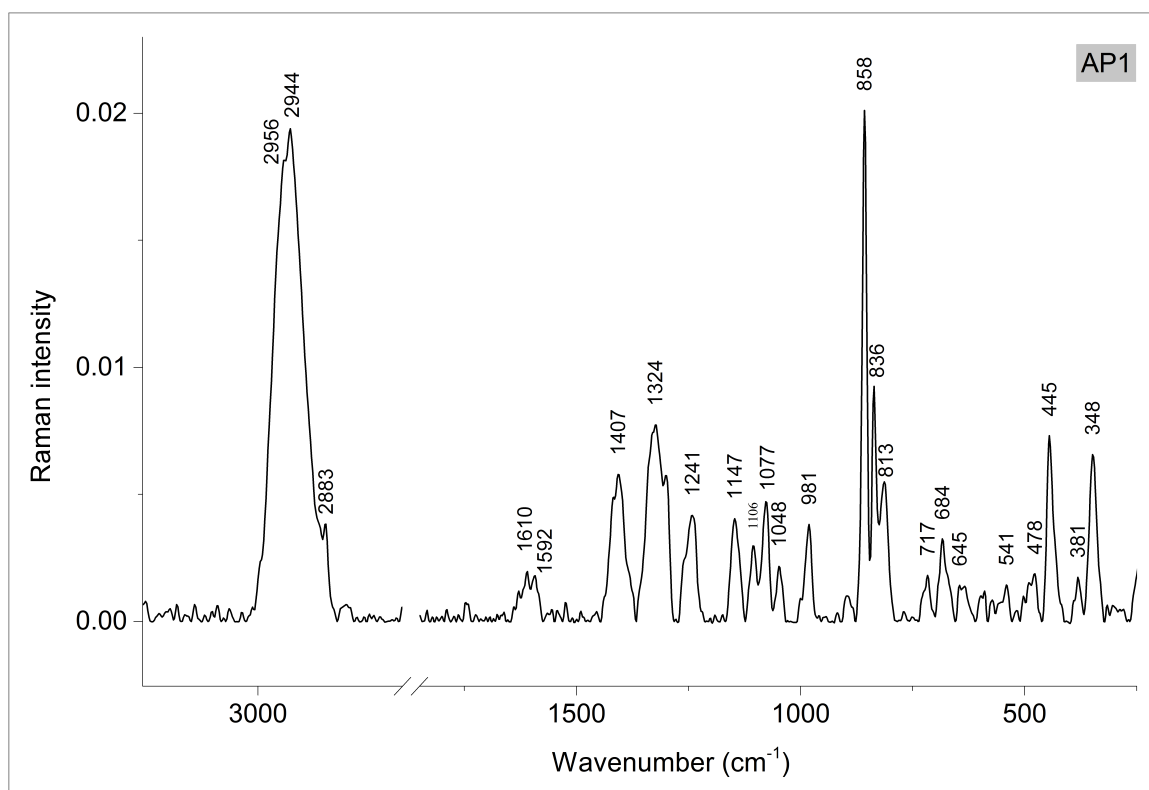
C Raman spectra of the apple pectin AP2 and its amidated derivatives

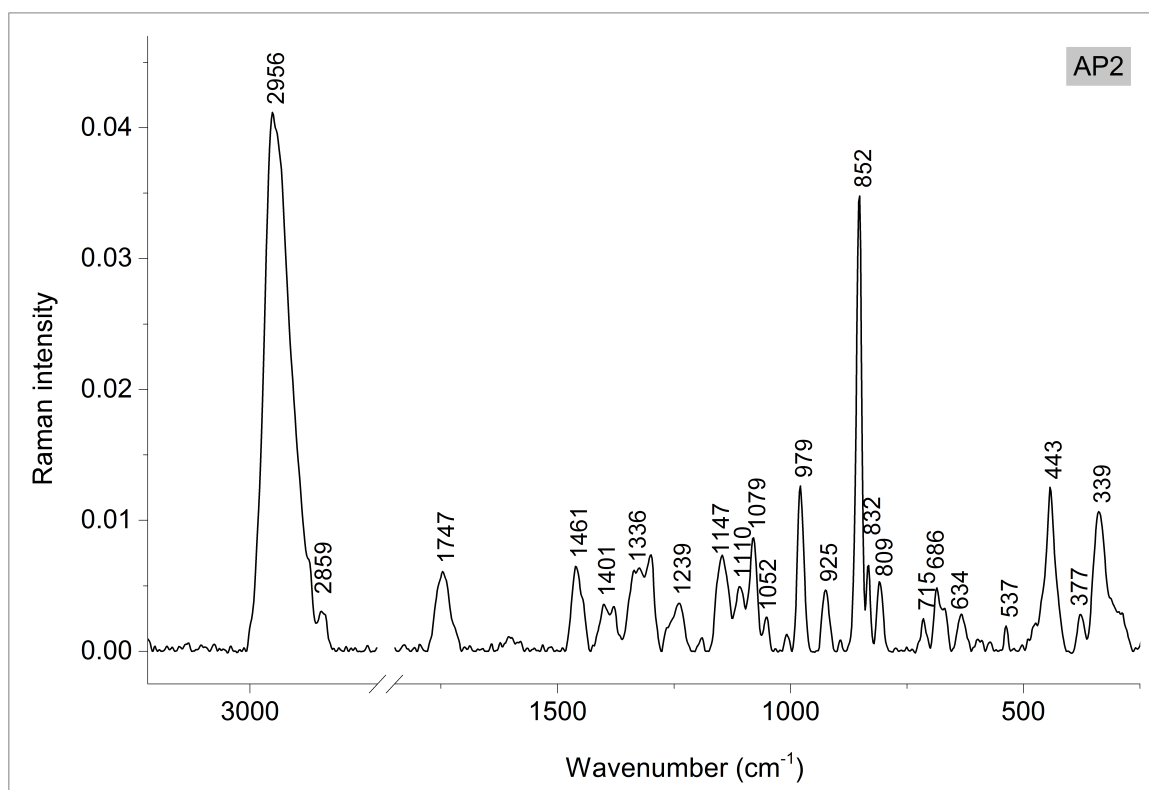


D Raman spectra of the apple pectin AP3 and its amidated derivatives

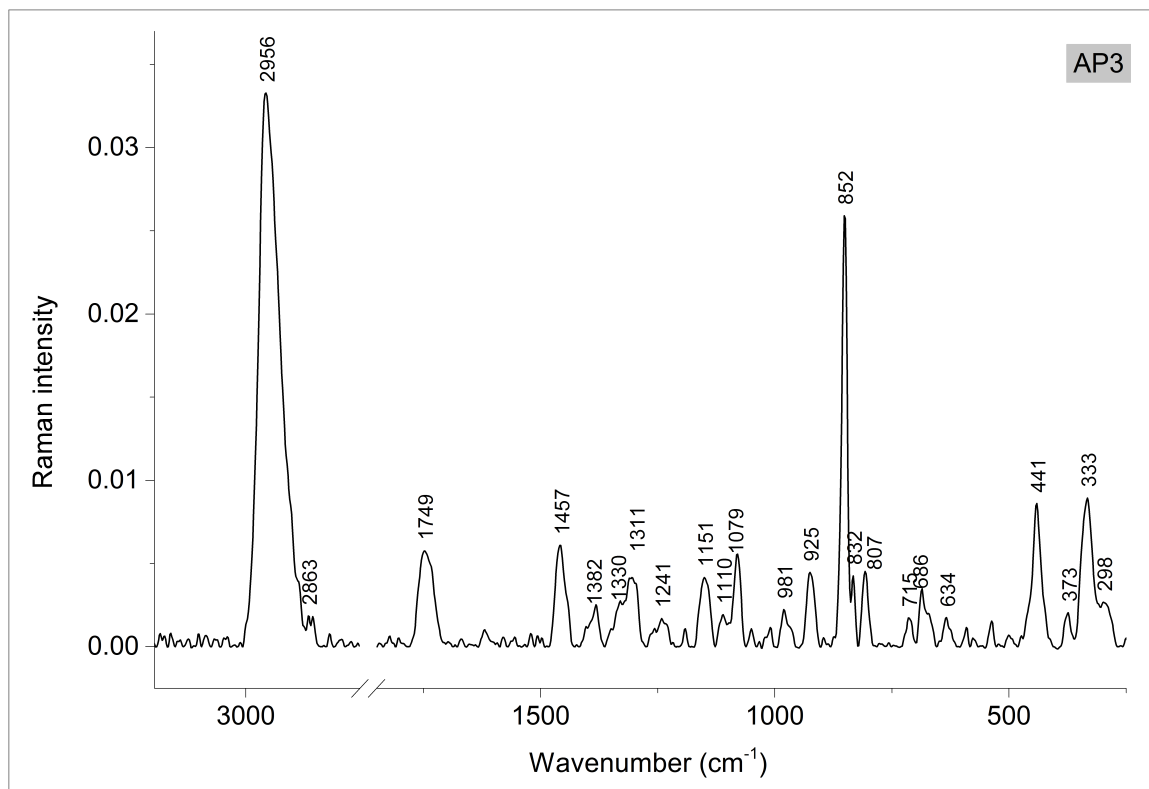


E Raman spectrum of the apple pectin AP1

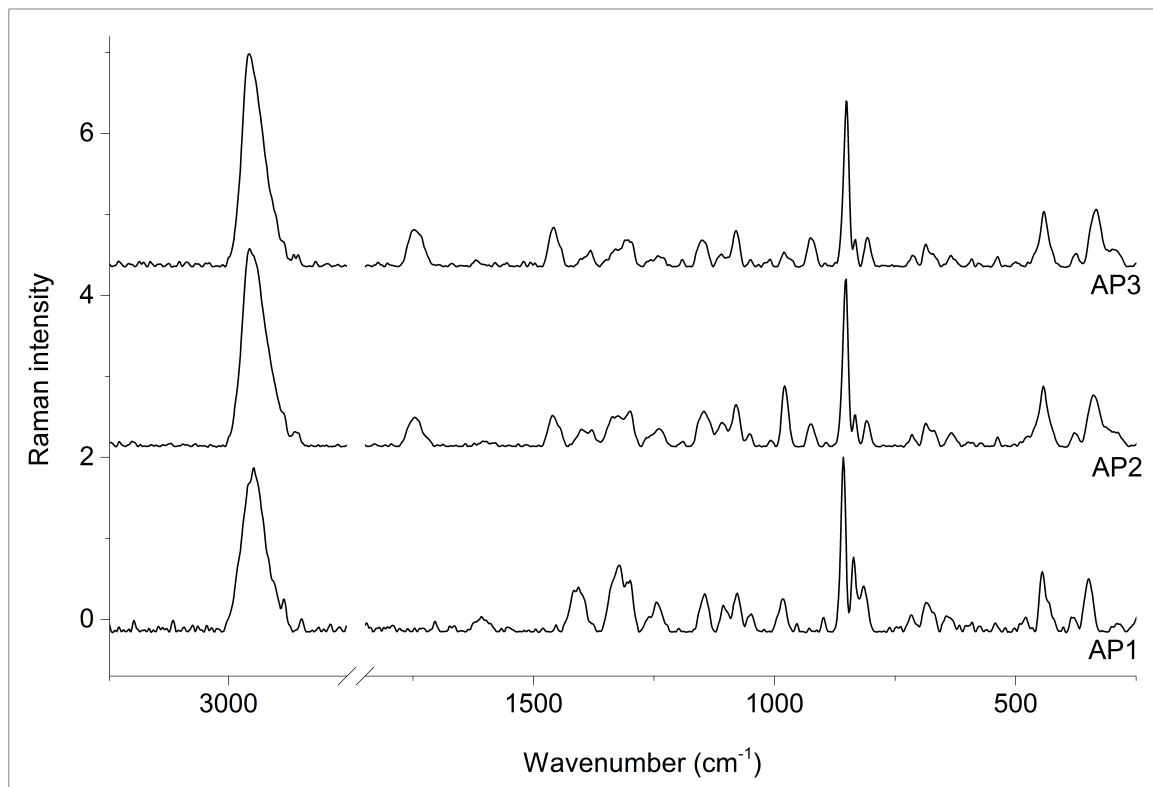


F Raman spectrum of the apple pectin AP2

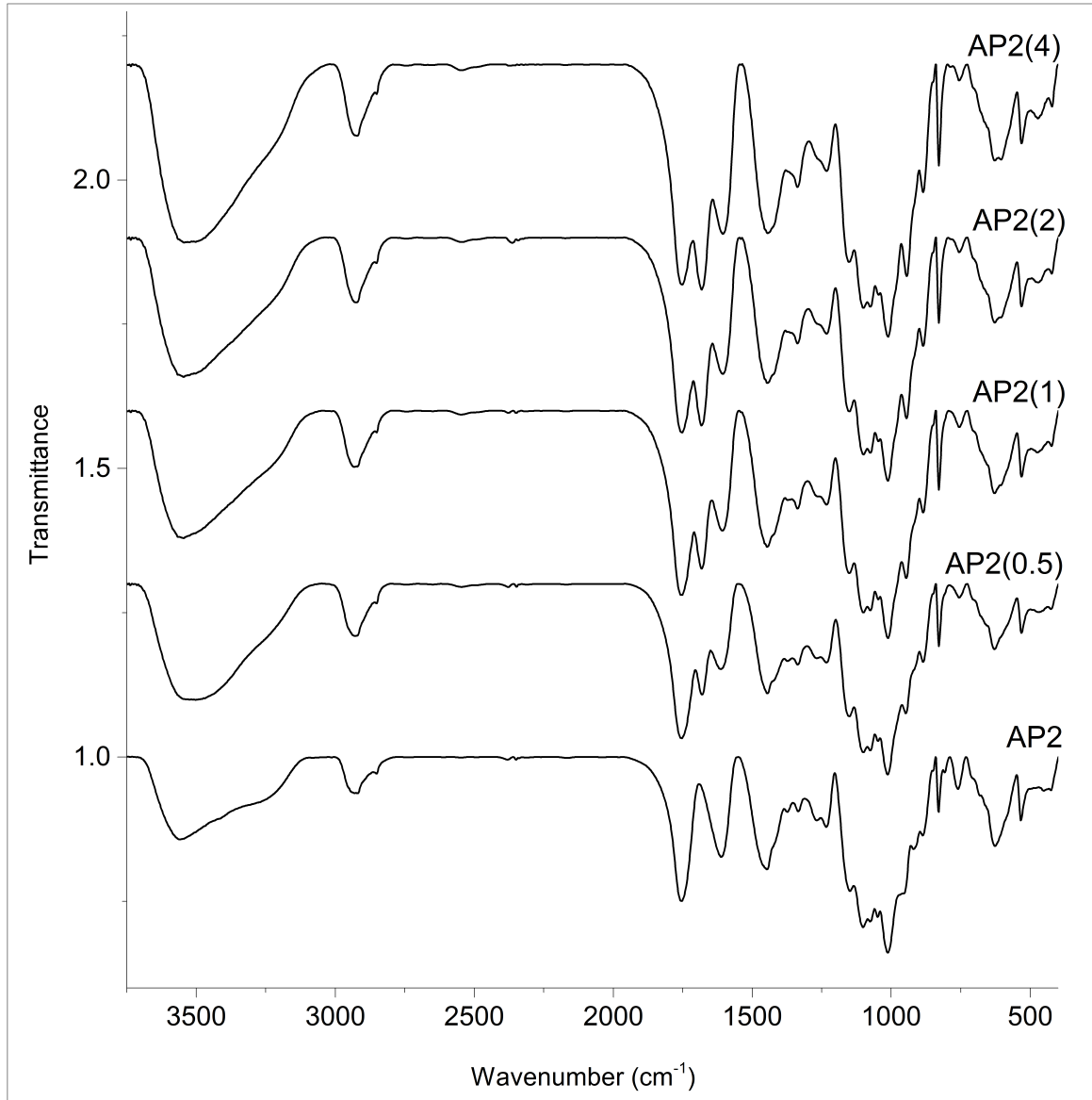
G Raman spectrum of the apple pectin AP3



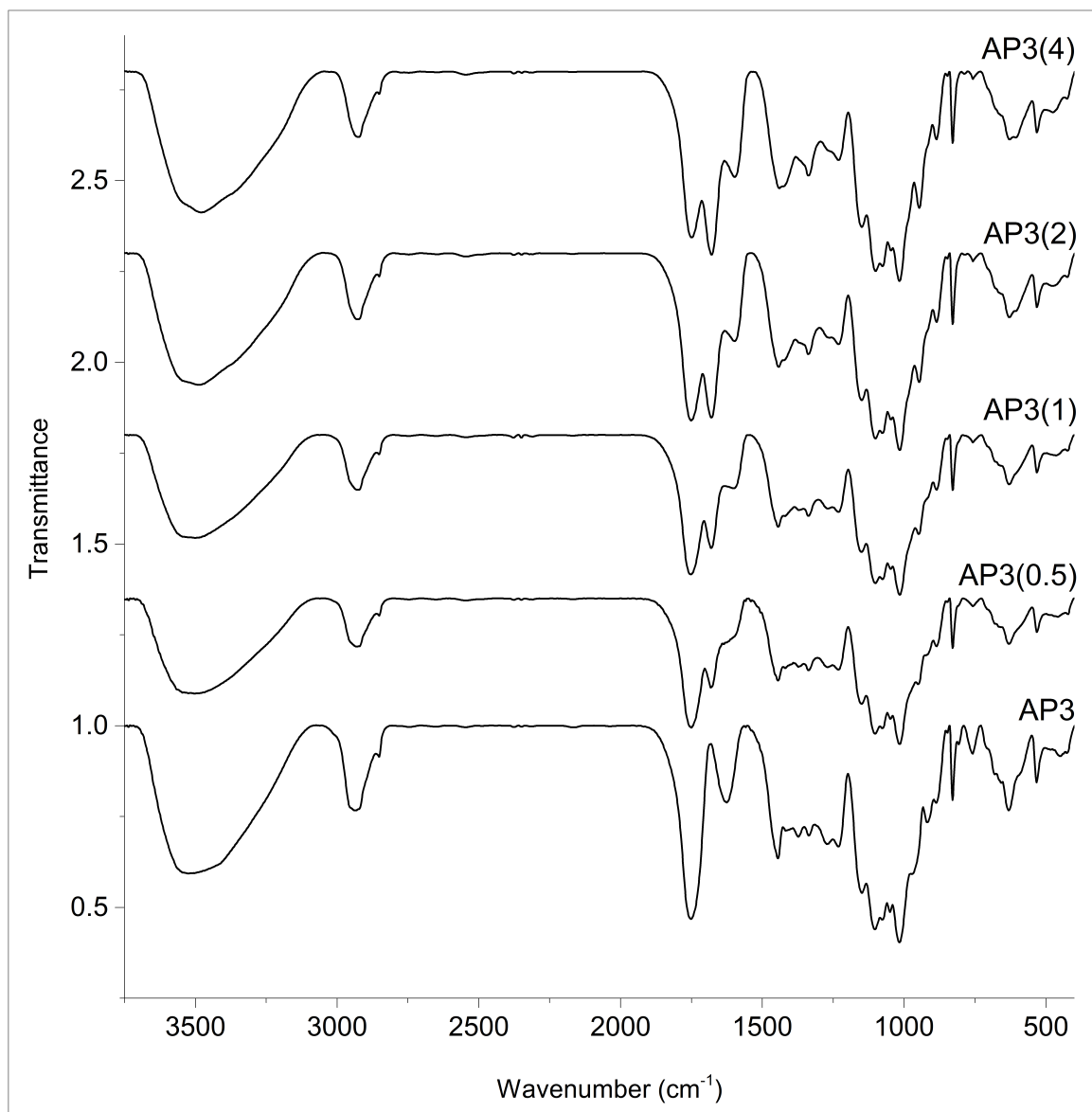
H Raman spectra of the three apple pectins, AP1, AP2, and AP3



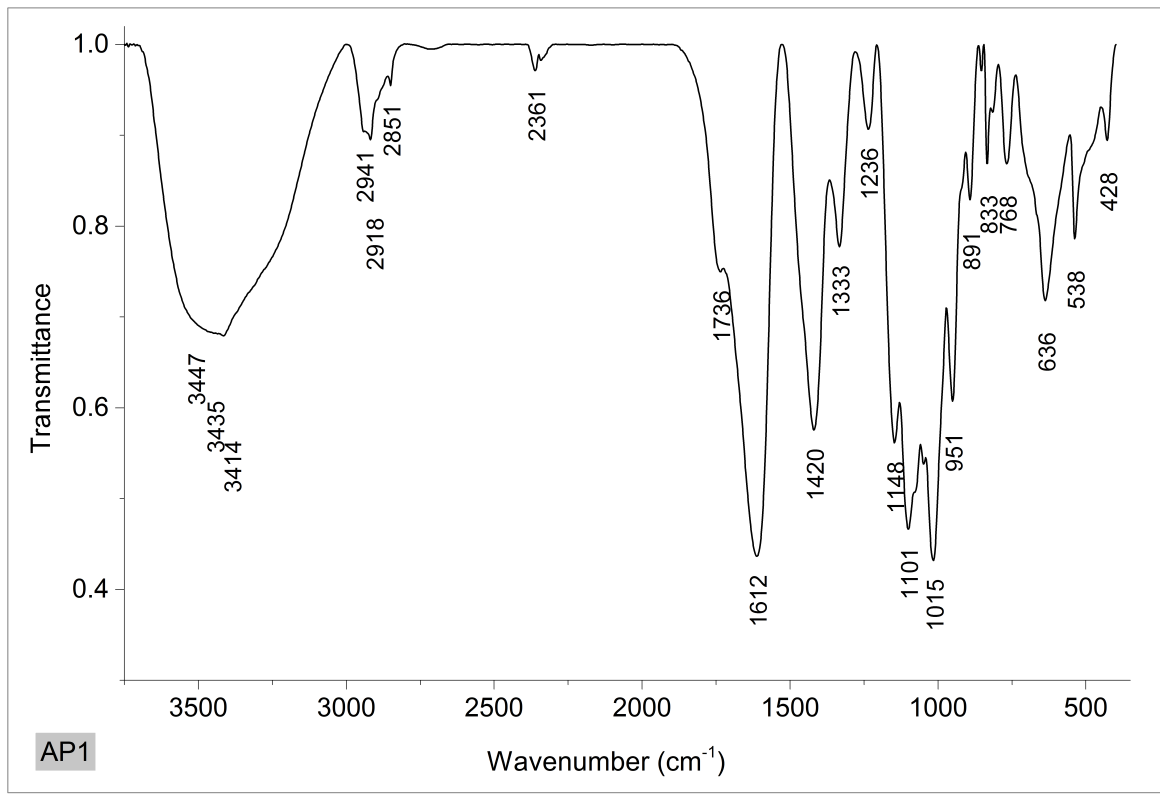
I FT-IR spectra of the apple pectin AP2 and its derivatives



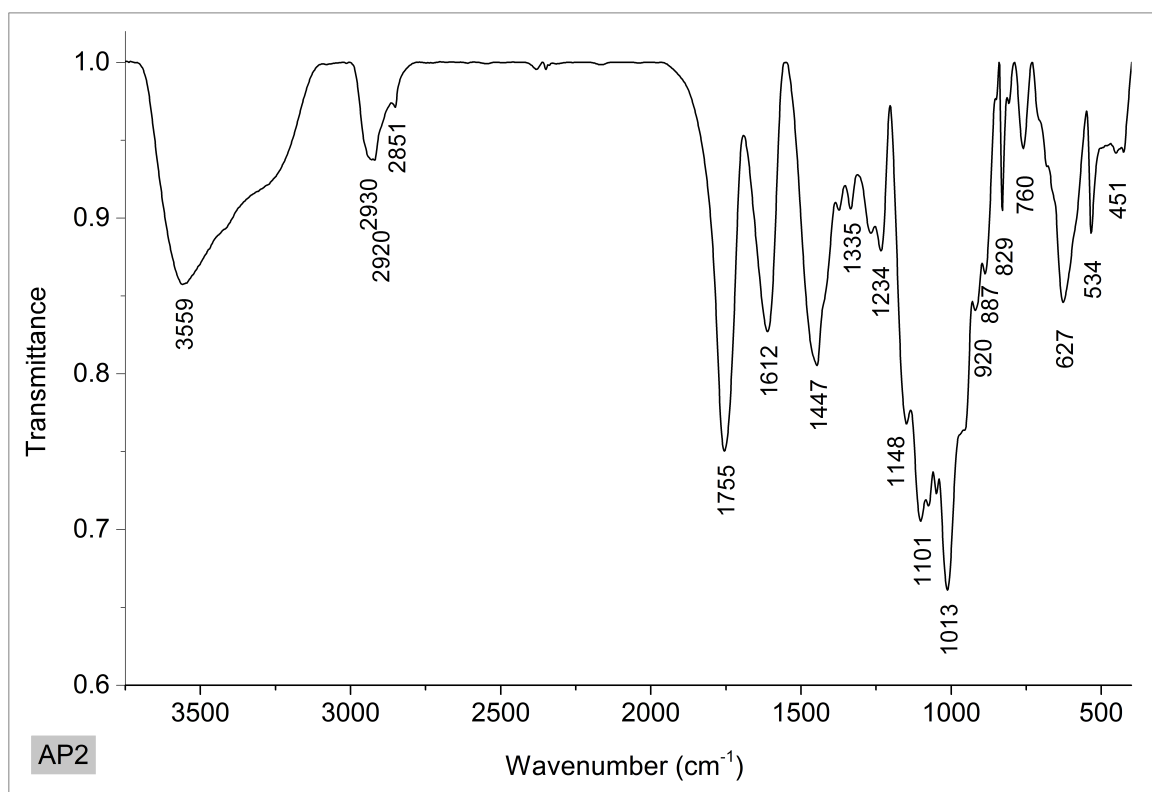
J FT-IR spectra of the apple pectin AP3 and its derivatives



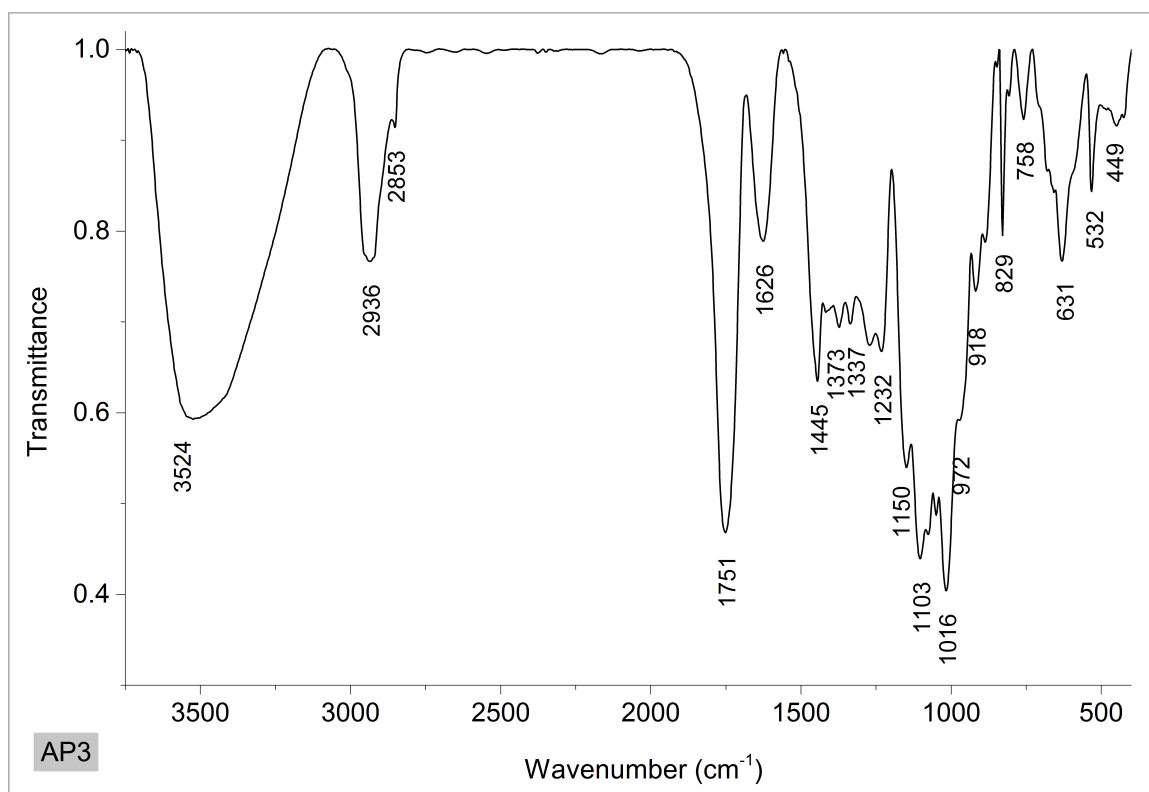
K FT-IR spectrum of the apple pectin AP1



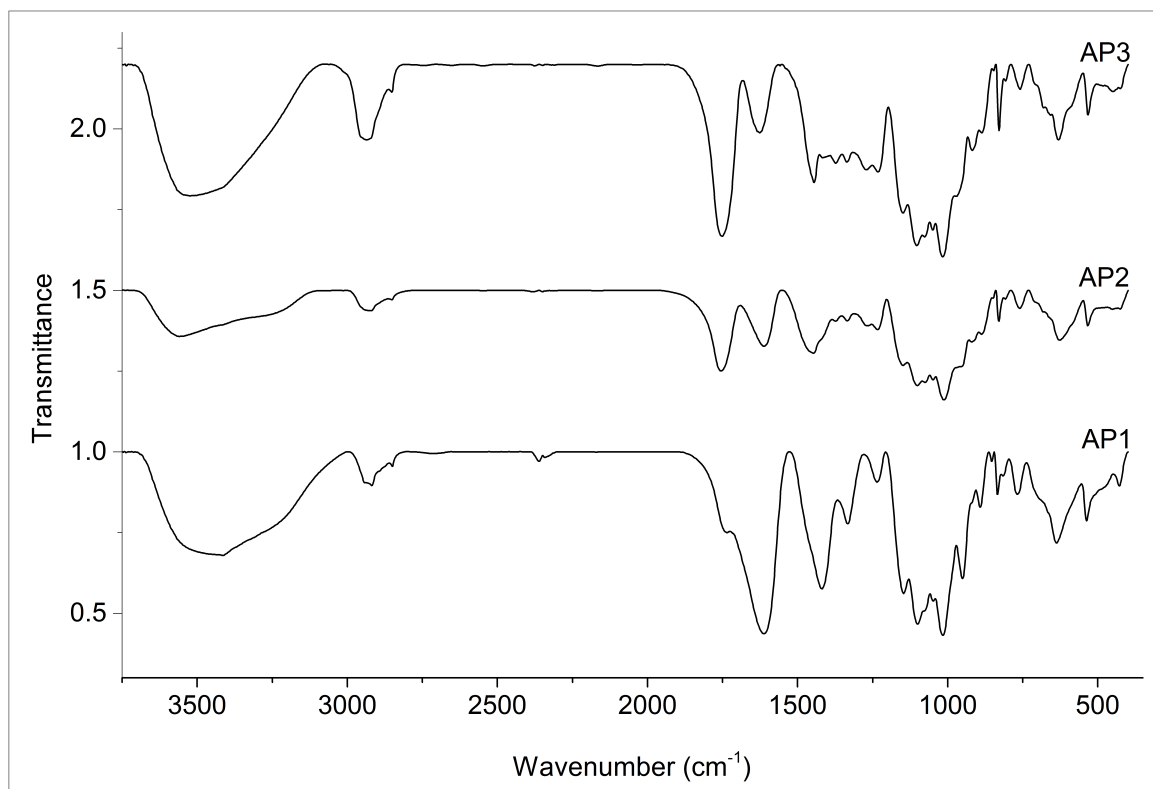
L FT-IR spectrum of the apple pectin AP2



M FT-IR spectrum of the apple pectin AP3



N FT-IR spectra of the three apple pectins, AP1, AP2, and AP3



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