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# Branching of hemicelluloses through an azetidinium salt ring-opening reaction



## Mikaela Börjesson<sup>a</sup>, Gunnar Westman<sup>a,b,\*</sup>

<sup>a</sup> Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Kemivägen 10, SE-41296 Gothenburg, Sweden
<sup>b</sup> Wallenberg Wood Science Center (WWSC), Chalmers University of Technology, Kemivägen 10, SE-41296 Gothenburg, Sweden

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#### ABSTRACT

During the last century there has been a steady increase in the number of publications on practical applications of hemicellulose. Due to the water and moisture sensitivity, poor film-forming ability and lack of thermal processability most of the hemicelluloses need to be chemically modified prior to processing into materials. Within this study we present the results of azetidinium salts as a new functional group for conjugation to polysaccharides. The reactivity of three azetidinium salts on xylan, arabinoxylan and galactoglucomannan was investigated. Carbonyl groups were found to be favorable for the reaction with azetidinium salts and thus the glucuronic acid content in the hemicellulose determines the degree of substitution. TEMPO-oxidation of the hemicelluloses was done which successfully increased the degree of substitution. The highly reactive azetidinium salts are easily synthesized from secondary amines and epichlorohydrin and can be used as a new tool toward functionalization of hemicelluloses into the after sought properties.

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#### 1. Introduction

Today the society has become increasingly aware of the limited resource of fossil-fuels and the importance of developing materials from renewable resources with a low environmental impact. The demand for replacing non-renewable products with bio-based products requires knowledge and tools on how to utilize the resources in more efficient ways. Key factors for a shift to hemicellulosebased materials are availability in large quantities at a reasonable price and that the material manufactured from hemicellulose has similar or better properties than the fossil fuel-based material that it will replace. A big difference between fossil fuel-based polymers and biopolymers is the higher content of oxygen in biobased materials. A consequence of the higher oxygen content is that the material has lower heat value, 13.6 MJ/kg; on the other hand it has the advantage that the reactive oxygen containing groups such as carboxylic acids and alcohol functions can be functionalized to tune the properties of the material.<sup>1</sup> During the 21st century there has been a huge increase in the number of publications on practical applications of hemicelluloses where the main applications have been toward hydrophobized materials such as gels, films and reinforcement in composites.<sup>2,3</sup> The overall properties of the

E-mail address: westman@chalmers.se (G. Westman).

hemicelluloses depend on their carbohydrate composition, the degree of polymerization (DP), its branching pattern as well as the chemical structure of added substituents.

Hemicelluloses represent approx. 20-30% of the biomass in nature and are in general shorter than cellulose polymers. The main hemicelluloses found in hardwood are xylans with a DP of 100-400. Xylans consist of a backbone of D-xylopyranose units and are mostly branched with L-arabinofuranose and glucuronic acids and the degree of branching depends on plant species and cell type. The main hemicelluloses found in softwoods are glucomannans, which consist of D-glucopyranose and D-mannopyranose units that are often branched with D-galactopyranose units. The glucomannans are partly acetylated and is shorter than xylans with a DP of ca 100-200. The hemicelluloses found in annual plants such as crops are generally more structurally diverse and complex than the hemicelluloses found in wood. The hemicelluloses in annual plants often consist of mixtures of many different sugar units and can be branched with for example D-xylopyranose, L-arabinofuranose, D-galactopyranose or uronic acid side chains.4,5

Hemicellulose can be utilized from sources like low-value wastewater streams in kraft pulping processes and agricultural crop residues. As said, the type and composition of hemicelluloses are highly dependent on the source, but also the isolation process and chemical reactions during the process can give rise to deacetylation, debranching and/or degradation. As a consequence the hemicelluloses structure depends on its process and fractionation history. Today, isolation of hemicelluloses such as hardwood xylan from the black liquor in kraft pulping processes and galactoglucomannans





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<sup>\*</sup> Corresponding author. Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Kemivägen 10, SE-41296 Gothenburg, Sweden. Tel: +46 (0) 31 772 3072.

(GGMs) released and accumulated in process waters in the production of thermo-mechanical pulp is often done by membrane filtration.<sup>6,7</sup> There is also a number of methods available to obtain hemicellulose from different plant sources like extraction with alkali,<sup>8</sup> dimethyl sulfoxide,<sup>9</sup> hot-water treatment,<sup>10</sup> microwave treatment<sup>11</sup> or extrusion.<sup>12</sup>

Although, being such a close relative to cellulose much less development in chemical modification of hemicellulose has been done compared with cellulose. Traditional modification processes are etherification, esterification, graft polymerization and oxidation which are quite often used as an intermediate for further functionalization. These processes are usually used with the aim to increase the hydrophobicity of the material. Successful acylation of hemicelluloses to increase the hydrophobicity has been reported by several groups.<sup>13–19</sup> There are also reports on fluorination of hemicelluloses, however thus far; it seems that the water sensitivity of the trifluoroacetyl group limits the use of these derivatives.<sup>20,21</sup>

Hemicelluloses can find use within the packaging industry,<sup>22,23</sup> food industry,<sup>24</sup> biomedical uses<sup>25</sup> and for chemical production.<sup>26,27</sup> Cross-linked hemicelluloses have also been reported to form hydrogels and are evaluated for drug delivery systems applications.<sup>28,29</sup>

We have looked into azetidinium salts as new coupling reagents that can be used for functionalization of hemicelluloses. Azetidines are found in a diverse range of natural products and serve as a valuable building block for other structural classes. The four member azetidine-ring can be guarternized to an azetidinium ion and in combination with the ring strain, it is a suitable reagent due to its high reactivity.<sup>30</sup> Azetidinium ions can easily be synthesized from secondary amines and epichlorohydrin.<sup>31,32</sup> Because of the many choices of carbon chains or other functional groups that could be attached to the azetidine-nitrogen, there is a big variety of modifications that can be done. Akzo Nobel Chemicals recently filed a patent for production of azetidinium compounds with carbon chains between 1 and 30 carbon atoms.<sup>33</sup> A difference compared to traditional hydrophobization agents from using azetidiniums is that two substituents in a Y-shaped branching will be formed when the azetidinium ring is opened and covalently linked to the target. Hydrophobization and properties tuning in this way will be a complement to the ones formed by ether and ester conjugation to the polysaccharide. Functionalization with Y-shaped chlorohydrins has recently been investigated showing that longer alkyl chains attached to CNC increase hydrophobicity and enhance the dispersion in low-density polyethylene matrices.<sup>34</sup>

There are only a few reports on azetidinium ions in the field of polysaccharides. Most of them are found in polyamide amine epichlorohydrin (PAE), which is a water-soluble resins used as wet strength additive for preparing wet strength papers. In a review article by Espy<sup>35</sup> potential cross-linking reactions of azetidinium groups are discussed as a possible wet strength agent. In the review it is stated that alcohol groups may play a part in cross-linking reactions as well as carboxylate end groups of cellulosic polymers. Carboxymetylated cellulose (CMC) or other carboxyl-bearing dry strength polymers showed an increased effectiveness in reaction with PAE.<sup>36</sup> Also Obokata and Isogai<sup>37</sup> found in their study on PAE that TEMPO-oxidized cellulose under alkaline conditions showed an increased reactivity toward PAE resulting in increased wet strength in the cellulose sheets.

Branching of hemicelluloses through azetidinium salts opens up the possibility to attach different functional groups or length of carbon chains and by that gives new properties to the material depending on how the branching is designed. In this study, three different and relatively small azetidinium salts with a 3-hydroxyazetidin-1-ium chloride structure have been synthesized and used in reaction with the three different hemicelluloses—xylan, arabinoxylan (AX) and galactoglucomannan (GGM)—to evaluate the branching effect on the different hemicellulose structures. AX and GGM were also TEMPO-

#### Table 1

Relative carbohydrate compositions (Rel %) in the different hemicellulose samples assuming the samples only consist of detected sugar units. The glucuronic acid (GlcA) content was determined by NMR analysis

Sample	Ara <sup>a</sup>	Gal <sup>a</sup>	Glc <sup>a</sup>	Xyl <sup>a</sup>	Man <sup>a</sup>	GlcA <sup>b</sup>
Xylan	0.7	0.3	3.1	82.5	0.0	13.4
NaOH-xyl	1.0	1.4	3.3	81.0	0.1	13.2
AX	20.9	1.1	7.5	70.5	0.0	-
NaOH-AX	19.2	1.7	10.2	68.7	0.1	-
TEMPO-AX	9.1	1.4	5.8	83.5	0.2	-
GGM	2.1	14.3	16.9	0.1	66.6	-
NaOH-GGM	2.1	10.9	16.4	0.5	70.0	-
TEMPO-GGM	2.0	16.3	16.9	0.7	64.2	-

<sup>a</sup> Content detected by ICS.

<sup>b</sup> Content detected by NMR.

oxidized to investigate the reactivity of the azetidinium salts. TEMPOoxidations are known to oxidize primary alcohol functionalities of several polysaccharides. Recently, it has been reported that TEMPOmediated oxidation is an efficient method for the oxidation of GGM.<sup>38,39</sup>

#### 2. Experimental procedure

## 2.1. Materials

Three hemicelluloses were used in the study; a commercial hardwood-xylan (beech wood, Sigma-Aldrich), arabinoxylan (AX) extracted from barley husk (Lyckeby Culinar, Sweden) following the procedure described by Höije et al.<sup>40</sup> and galactoglucomannan (GGM) from spruce kindly received as a gift from Stora Enso (Sweden). The hemicelluloses have been analyzed with carbohydrate analysis and NMR to determine the sugar content composition in the samples (Table 1). All chemicals used for the synthesis of azetidinium salts (diethylamine, dihexylamine, morpholine and epichlorohydrin) or treatments of the hemicelluloses with NaOH-activation or TEMPO-oxidation (2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and bis(acetoxy)iodobenzene (BAIB) as co-oxidant) were purchased from Sigma-Aldrich and used as received.

## 2.2. Preparation of N,N-dialkylazetidinium salts

Preparation of the different azetidinium (Az) salts following previously known synthesis.<sup>32,41–44</sup> A general reaction scheme is shown in Fig. 1.

The general procedure for preparation of azetidinium salts was dropwise addition of 0.1 mol epichlorohydrin to a solution of 0.1 mol secondary amine and solvent. The temperature was kept below 5 °C during the addition of epichlorohydrin due to an exothermic reaction. The reaction mixture was stirred in an ice bath for 1 h and additional 47 h at room temperature. The solution was purified with diethyl ether and water and solvent was removed in vacuum. Fig. 2 shows the molecular structure of the different azetidinium salts used in this study. For more details see supporting information.

#### 2.3. Pre-treatment of hemicellulose

A pre-treatment of the hemicelluloses was performed before reaction with the azetidinium salts, where a 17.5 wt% NaOH-solution



Fig. 1. A secondary amine is converted to azetidinium chloride through reaction with epichlorohydrin.



Fig. 2. The molecular structure of the azetidinium salts used in the study. I: 1,1-diethyl-3-hydroxyazetidin-1-ium chloride prepared from diethylamine, II: 1,1-dihexyl-3-hydroxyazetidin-1-ium chloride prepared from dihexylamine and, III: N-morpholino-3-hydroxyazetidin-1-ium chloride prepared from morpholine.

was used to activate the hydroxyl groups in order to increase the reactivity of the polysaccharides.<sup>45–47</sup> Ten grams of hemicellulose was suspended in 35 mL deionized water and heated under reflux for 15 min before cooled down to room temperature and 10 mL 17.5 wt% NaOH-solution was added to the mixture. After 60 min dimethyl ether was added to obtain a heterogeneous mixture and the NaOH-hemicellulose was filtrated and washed with ethanol.

A TEMPO-oxidation was performed on AX and GGM to introduce carbonyl groups in the polymers following a procedure described by Nylander.<sup>48</sup> Dried hemicellulose (1 g) was dissolved in 6.45 mL distilled water and 19.35 mL acetonitrile was added giving a two-phase solution. The solution was cooled on ice and 3.48 g BAIB (10.8 mmol) and 0.12 g (0.768 mmol) TEMPO was added and the solution was magnetically stirred for 2 h at 0 °C and additional 4 h at room temperature. The mixture was thereafter precipitated in cold ethanol followed by washing and filtration with ethanol.

## 2.4. Modification of hemicellulose with azetidinium salts

Since the azetidinium salts as well as the hemicelluloses used in the study have slightly different solubility a general method that works well for all hemicelluloses and azetidinium salts used in this study was developed. It was found that a general process as follows gave the best yields. Five grams of hemicellulose, 2–3 mol eq. azetidinium salt depending on hemicellulose (1 mol equiv./–OH functionality) and a 50 g solution of DMSO/toluene (90:10) were added to a round bottom flask. A condenser was attached to the flask and the solution was stirred and kept at 90 °C for 22 h. The mixture was allowed to cool to room temperature before the modified hemicellulose dropwise was precipitated in cold ethanol during stirring. The modified hemicellulose was filtrated, washed with ethanol, redissolved in deionized water and precipitated again in ethanol followed by filtration and washing several times with ethanol before drying at room temperature.

Fig. 3 shows suggested reaction schemes for the three hemicelluloses where the hemicelluloses, AX and GGM have been TEMPOoxidized before reaction with azetidinium salts.

## 2.5. Hydrolysis of hemicellulose

The modified hemicelluloses were hydrolyzed to its monosaccharides by a sulfuric acid hydrolysis.<sup>49</sup> Three milliliters of 72%  $H_2SO_4$ were added to 200 mg dried sample and put under vacuum for 15 min before placed in a water bath at 30 °C for 1 h. Eighty-four grams of distilled water was added and the samples were put in an autoclave for 1 h at 125 °C and 20 bar. To analyze the carbohydrate content the hydrolyzed samples were filtered and diluted with distilled water to a concentration of 200 mg/L.

## 2.6. Characterization

<sup>1</sup>H NMR and <sup>13</sup>C NMR were recorded on a Varian MR-400 (400 MHz) spectrometer. DMSO- $d_6$  was used as solvent for the azetidinium salts and the hemicellulose samples were dissolved in

 $D_2O$  . The glucuronic acid content in hemicellulose was analyzed with NMR according to Westbye et al.  $^{50}$ 

Fourier-Transform Infrared (FTIR) spectroscopy was recorded on a PerkinElmer Spectrum One instrument using the potassium bromide (KBr) pellet technique with 10 wt% sample. FTIR was analyzed between 4000–400 cm<sup>-1</sup> and 32 scans collected.

Neutral carbohydrate composition was analyzed on acid hydrolyzed hemicellulose samples with a high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), using an ion chromatography system ICS 3000 (Dionex) equipped with a gradient pump, isocratic post column pump, column oven and autosampler. The different sugars detected with the available system were L-arabinose (Ara), D-galactose (Gal), D-glucose (Glc), D-xylose (Xyl) and D-mannose (Man).

The degrees of substitution (DS) were determined from the amount of nitrogen in the samples measured by elemental analysis at Mikrolab Kolbe in Germany. The DS according to the number of nitrogen  $(DS_N)$  were calculated using Eq. (1)

$$DS_{N} = (M_{(Average hemicellulose unit)} \times N) / (1401 - M_{Az-reagent} \times N)$$
(1)

where  $M_{(Average hemicellulose unit)}$  and  $M_{Az-reagent}$  are the molecular weights of the average anhydrous sugar units in the hemicellulose polymer and the azetidinium reagents respectively, and %N is the amount of nitrogen in percent measured by elemental analysis. The maximum DS for the different hemicelluloses are dependent on the available reaction sites per sugar unit which were estimated from the carbohydrate analysis presented in Table 1.

Thermogravimetric analysis (TGA) was used to detect thermal changes in the hemicellulose samples. A TGA/DSC 3+ Star System (Mettler Toledo) was used where 10 mg sample was heated from 25 °C to 500 °C under N<sub>2</sub> atmosphere with a heating rate of 5 °C/ min. The extrapolated onset temperature and the inflection point calculated from the 1st derivate were measured using the STARe Excellence Software.

## 3. Results and discussion

## 3.1. Azetidinium salts

The azetidinium chlorides prepared from secondary amines and epichlorohydrin were obtained in yields above 70%. The salts were analyzed with <sup>1</sup>H NMR and <sup>13</sup>C NMR in DMSO-d<sub>6</sub>. The synthesis and NMR-analysis of the different azetidinium chlorides are presented in detail in supporting information.

#### 3.2. Carbohydrate content in hemicellulose

The sugar composition in the hydrolyzed hemicellulose samples was determined from a neutral carbohydrate analysis and the relative percent (Rel %) of the sugars was calculated assuming only the detected sugars were present (Table 1).

The glucuronic acid content in beech xylan was measured by <sup>1</sup>H giving approximately 13% glucuronic acids. The glucuronic acid



Fig. 3. Suggested reaction schemes for the reactions between (a) xylan and Az-salt, (b) AX and Az-salt after a TEMPO-oxidation, and (c) GGM and Az-salt after a TEMPO-oxidation.

content in AX and GGM was too low to be detected by NMR and corresponds to literature, AX and GGM should not contain any or just a very small amount of uronic acids.<sup>51,52</sup> Different pre-treatments for hemicelluloses and how it affects the carbohydrate composition are listed in Table 1. The NaOH-activation did not change the carbohydrate composition for xylan and AX significantly but degraded the  $\alpha$ -glycosidic linkage between the galactose side chain and the glucose–mannose backbone in GGM, which is known to be degraded during oxidation or alkali treatments of the polymer.<sup>53</sup>

A TEMPO-oxidation under neutral conditions was performed to introduce carboxylate groups in the AX and GGM polymers without degrading the polymers too much. A traditional TEMPO-oxidation process was also performed following published procedures<sup>38,39</sup> but the oxidation of the hemicelluloses was thwarted by unknown reasons. However, TEMPO-oxidation with BAIB as co-oxidant gave successful oxidations of the hemicellulose materials. Table 2 shows

#### Table 2

The concentrations (mg/L) of the detected sugars in the carbohydrate analysis before and after TEMPO-oxidation of AX and GGM

Sample	Ara	Gal	Glc	Xyl	Man	Total residue (mg/L)
AX	29.4	1.5	10.5	99.1	0.0	140.6
TEMPO-AX	11.0	1.6	7.0	100.4	0.2	120.2
GGM	2.8	18.8	22.2	0.1	87.4	131.3
TEMPO-GGM	2.1	17.8	18.5	0.8	70.1	109.3

the concentrations (mg/L) of the neutral carbohydrate analysis of TEMPO-oxidized AX and GGM. For the AX samples mainly the arabinose groups are changed due to the oxidation of the primary hydroxyl group, present only in the arabinose units. For the GGM samples the largest reduction in detected sugars is seen in the glucose and mannose sugars and only a small change in the detected concentrations of galactose. This means that the oxidation primary occurs in the glucose–mannose backbone of GGM. The arabinose concentration for TEMPO-oxidized AX is reduced by 63% meaning that 63% of the arabinose groups are either oxidized or degraded whereas the number of reduced sugars involved in the TEMPO-oxidation of GGM is 17%.

## 3.3. FTIR analysis of hemicelluloses

FTIR analysis on hardwood xylan (Fig. 4a) shows a broad absorption band at 3600–3100 cm<sup>-1</sup> and at 2925 cm<sup>-1</sup> showing the hydroxyl groups and CH<sub>2</sub> stretching in the xylose ring respectively. A small absorption band at 1711 cm<sup>-1</sup> corresponds to carboxylic groups, which indicates the presence of glucuronic acids in the sample. The absorption band at 1044 cm<sup>-1</sup> is typical ring vibrations and stretching vibrations of C–OH side groups.<sup>54</sup> The band at 1167 cm<sup>-1</sup> represents the glycosidic bonds (C–O–C) and the absorption band at 896 cm<sup>-1</sup> is typical for  $\beta$ -glycosidic linkage. Other absorption bands detected are 1614 (absorbed water) and 984 (C–O) cm<sup>-1.55</sup> FTIR analysis on azetidinium salt branched xylan



Fig. 4. FTIR spectrums of (a) xylan, (b) Az-DEA modified xylan, (c) Az-DHA modified xylan, and (d) Az-morph modified xylan.

(Fig. 4b–d) shows a new absorption band at 1745 cm<sup>-1</sup> corresponding to carbonyl groups (C==O) such as saturated esters. Due to the formation of an ester the branching reaction between azetidinium salts and xylan occurs on the carboxylic acid group present in the glucuronic acids. According to literature,<sup>35–37</sup> the azetidiniums containing wet strength resin, PAE, are more prone to react with –COO<sup>-</sup> groups compared with secondary –OH groups, which further support that the reaction takes place on the glucuronic acids in the xylan samples. Changes in FTIR spectra can also be seen as a narrowing in the area between 3600 and 3100 indicating structural changes of the –OH groups. Suggested reaction schemes for the reaction between xylan and azetidinium containing groups are shown in Fig. 3a.

AX and GGM do not have any carbonyl groups available and as a consequence FTIR analysis on modified AX and GGM samples does not show any changes in the carbonyl group absorption band area (1760–1665 cm<sup>-1</sup>). For both AX and GGM, primary alcohols are present on the side groups additional to this GGM also has primary hydroxyl groups in the backbone. The primary –OH groups are selective for a TEMPO-oxidation and introduction of carbonyl groups to AX and GGM can increase the reaction efficiency. Figs. 5 and 6 show the TEMPO-oxidation of AX and GGM respectively and the reaction between hemicellulose and Az-DEA salt.

In Fig. 5 it is clearly seen that the TEMPO-oxidation has introduced carbonyl groups to the polymer due to the new absorption band at 1740 cm<sup>-1</sup>. The azetidinium reaction between TEMPOoxidized AX and Az-DEA salt is shown in Fig. 5d and the absorption band at 1740 cm<sup>-1</sup> is shifted to 1744 cm<sup>-1</sup> indicating structural changes of the carbonyl groups due to formation of an ester bonding. Fig. 5b shows the azetidinium reaction for non-oxidized AX where no carbonyl groups are present. Suggested reaction schemes for the reaction between TEMPO-oxidized AX and azetidinium containing groups are shown in Fig. 3b.

The native GGM is partly acetylated which is shown as a distinct peak at 1736 cm<sup>-1</sup> in Fig. 6a and will be present as long as the sample is not treated with alkali such as during NaOH-activation.<sup>5,56,57</sup> The TEMPO-oxidation introduces a broader absorption band area around 1740 cm<sup>-1</sup> due to the presence of both acetylated groups and –COO<sup>-</sup> groups caused by the oxidation. The broadening can also be observed in the Az-DEA modified oxidized sample in Fig. 6d. Suggested reaction schemes for the reaction between TEMPO-oxidized GGM and azetidinium containing groups are shown in Fig. 3c.

#### 3.4. Degree of substitution

The amounts of nitrogen in the samples were measured with elemental analysis and used to calculate the degree of substitutions  $(DS_N)$  using Eq. (1). The results are presented in Table 3 and the carbohydrate composition has been used to calculate the maximum DS. Since carboxylate groups are favorable prior to hydroxyl groups the maximum DS, regarding the number of carboxylic acids available per sugar unit, is determined to 0.13 for xylan. AX and GGM did not have any measurable amounts of carboxylic acid groups present and the reaction efficiency is calculated from the number of primary alcohols available per sugar unit (for un-oxidized samples), giving a maximum DS of 0.3 for AX and 1.0 for GGM.

DS is highest for the xylan samples due to the glucuronic acids present in the polymer chain whereas both AX and GGM samples have too low glucuronic acid content to be detected. Even though GGM has a higher amount of primary –OH groups with one primary –OH group on every sugar unit compared with AX, which only has one primary –OH on the branched arabinose groups, the DS is lowest for the GGM samples. Thus, the primary –OH groups do not seem to have any prominent role in the reaction but as seen in Table 3, some of the –OH groups are substituted in AX and GGM samples. Primary –OH groups are on the other hand favorable in oxidation reactions and the introduction of carbonyl groups could make it possible to control the AX and GGM modifications.

From Table 3 it is also noted that the smallest azetidinium compound, Az-DEA, gave the highest substitution for all hemicelluloses and the more bulky ones gave lower DS. For reagents with larger functional groups sterical effects could influence the reaction efficiency.



Fig. 5. FTIR spectrums of (a) arabinoxylan (AX), (b) Az-DEA modified AX, (c) TEMPO-oxidized AX and, (d) Az-DEA modified TEMPO-oxidized AX.

#### 3.5. Effects of different pre-treatments of hemicellulose

As stated before, the reactions with azetidinium groups are favorable to carbonyl groups and the introduction of carbonyl groups through TEMPO-oxidation is therefore expected to increase the reaction efficiency, compared with the DS values present in Table 3. Table 4 summarizes the different pre-treatments that were done on the different hemicelluloses which further were reacted with Az-DEA salt for comparison of the reaction efficiency. DS was calculated from the amount of nitrogen found in the elemental analysis using Eq. (1). The TEMPO-oxidation is selective to primary alcohols and Table 4 shows both the reaction efficiency for a fully oxidized polymer and for the actual amount of oxidized groups in the polymers, shown as the numbers in brackets in Table 4.

The 17.5 wt% NaOH-activation increased the reaction efficiency for xylan and AX but did not have an evident effect on GGM, probably due to the alkaline degradation of the galactose units. On the other hand, the introduction of carbonyl groups through TEMPOoxidation had a significant influence on the reaction efficiency for both AX and GGM, which further proves the favorable of carbonyl groups prior hydroxyl groups.

Xylan samples were almost fully substituted while AX and GGM had a much lower substitution degree. Reaction efficiencies in Table 4 are calculated for both fully oxidized samples and the actual amount



Fig. 6. FTIR spectrums of (a) partly acetylated GGM, (b) Az-DEA modified GGM, (c) TEMPO-oxidized GGM, and (d) Az-DEA modified TEMPO-oxidized GGM.

#### Table 3

Degree of substitution (DS) for the different Az-modification reactions of the three hemicelluloses: xylan, AX and GGM. The reaction efficiencies are calculated from the maximum DS of available reaction targets in the hemicelluloses

Sample	Pre-treatment	%N	$DS_N$	Reaction efficiency (%) <sup>a</sup>
Az-DEA-xyl	NaOH-act.	1.10	0.13	96.85
Az-DHA-xyl	NaOH-act.	0.82	0.10	75.01
Az-morph-xyl	NaOH-act.	0.99	0.11	86.85
Az-DEA-AX	NaOH-act.	0.58	0.06	19.47
Az-DHA-AX	NaOH-act.	0.44	0.05	15.07
Az-morph-AX	NaOH-act.	0.36	0.04	11.80
Az-DEA-GGM	NaOH-act.	0.46	0.06	5.66
Az-DHA-GGM	NaOH-act.	0.08	0.01	0.95
Az-morph-GGM	NaOH-act.	0.31	0.04	3.75

<sup>a</sup> The reaction efficiency is dependent on the maximum DS for the hemicellulose that has been determined from the carbohydrate composition to 0.13 for xylan, 0.3 for AX and 1.0 for GGM.

of oxidized groups in the polymers. The oxidation reaction seems to work better on AX than GGM and the oxidation procedure could be more investigated. In both cases the oxidation increased the reaction efficiency. By controlling the number of carbonyl groups it should also be possible to control the number of substituents.

The DS values presented in both Tables 3 and 4 are relatively low compared with other reactions on hemicelluloses, for example the etherification with 2,3-epoxypropyl trimethyl ammonium chloride (EPTMAC) giving a similar ring opening as for azetidinium ions. DS on etherified birch xylan has been reported to vary between 0.17 and 1.64 depending on process parameters<sup>45</sup> and the etherification with EPTMAC on AX from barley husk has reported DS values ranging from 0.03 to 0.2.<sup>58</sup> One significant difference with the etherification with EPTMAC compared with azetidinium ions is that EPTMAC reacts with both primary and secondary hydroxyl groups, giving more available substitution targets.

#### Table 4

Degree of substitution (DS) for different pre-treatments of hemicelluloses; no treatment at all, 17.5 wt% NaOH-activation or TEMPO-oxidation. The reaction efficiencies are calculated from the maximum DS of available reaction targets in the hemicelluloses

Sample	Pre-treatment	%N	$DS_N$	Reaction efficiency (%) <sup>a</sup>
Az-DEA-xyl	None	1.06	0.12	92.62
Az-DEA-xyl	NaOH-act.	1.10	0.13	96.85
Az-DEA-AX	None	0.54	0.06	18.51
Az-DEA-AX	NaOH-act.	0.58	0.06	19.47
Az-DEA-AX	TEMPO-ox.	1.49	0.17	55.97 (92.39) <sup>b</sup>
Az-DEA-GGM	None	0.47	0.06	5.74
Az-DEA-GGM	NaOH-act.	0.46	0.06	5.66
Az-DEA-GGM	TEMPO-ox.	1.00	0.13	13.09 (77.91) <sup>b</sup>

<sup>a</sup> The reaction efficiency is dependent on the maximum DS for the hemicellulose that has been determined to 0.13 for xylan, 0.3 for AX and 1.0 for GGM. <sup>b</sup> The numbers in brackets referring to the reaction efficiency for 63% oxidized primary hydroxyl groups in AX and 17% oxidized primary hydroxyl groups in GGM. The ability to control DS is favorable to understand the reaction and an optimization regarding concentration of azetidinium salts might have increased DS further for all samples, which was the case for the etherification with EPTMAC described above, where the excess of 10 mol equiv. EPTMAC/anhydroxylose unit was necessary for a DS of 1.64. On the other hand, the Y-shaped azetidinium reagent contributes with two alkyl chains per reaction target and a lower DS could have a higher impact on the material properties.

## 3.6. Thermal effects of modified hemicelluloses

The decomposition temperatures for the three hemicelluloses and its modified form were evaluated by TGA, as shown in Fig. 7. All modified samples differ from its native hemicellulose indicating successful reactions. For the modified xylan samples (Fig. 7a) all modified samples showed an increase in onset temperature as well as a more rapid decomposition compared with native xylan. Only the TEMPO-oxidized samples are shown for the AX and GGM samples due to the higher DS for those samples compared with the non-oxidized samples (Fig. 7b and c). The results are varying for the two hemicelluloses but in both cases the onset temperatures are decreased; and for the GGM sample the decomposition rate is slower where most of the material decomposes at a higher temperature compared with native GGM.

Thermal stability is often necessary for heat processing of materials like composite manufacturing and the longer alkyl chains with six carbons show a larger increase in degradation temperature compared with the shorter 2-carbon chains. Even longer alkyl chains could influence the thermal stability further.

#### 4. Conclusions

This study shows that it is possible to attach different azetidinium containing groups on hemicelluloses containing carbonyl groups such as in glucuronic acids or after a TEMPO-oxidation of polymers containing primary alcohols. Carbohydrate analysis of different pretreated hemicelluloses shows that alkali conditions degrade the  $\alpha$ -glycosidic linkage between galactose and the glucose-mannose backbone in GGM and a TEMPO-oxidation with more neutral conditions, such as the TEMPO/BAIB-oxidation was chosen to avoid degradation of the polymers.

The three different azetidinium containing reagents that were synthesized were used in attempt to study the reaction between different hemicelluloses and to understand the chemistry to control the reaction. There is a huge variety of azetidinium containing salts that can be synthesized and used for modification of hemicellulose to increase the value of the so far less explored hemicellulose polymers. By knowing where on the polymer the reactions occur it is easier to control the reaction and by studying different azetidinium groups with different functionalities the properties of



Fig. 7. TGA curves for the three hemicelluloses and its modifications: (a) xylan, (b) arabinoxylan (AX), and (c) galactoglucomannan (GGM).

the modified hemicelluloses can be tailored and for example longer alkyl chains increase hydrophobicity. Based on structure-property relationship hemicellulose derivatives from azetidinium salts have an interesting spectrum in possible applications including barriers or films for packaging solutions or carrier of drugs due to the azetidinium reagents commonly used in drug development. Additionally, the reaction conditions need to be further optimized to reach a more energy efficient process.

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## Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.carres.2016.04.005.

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