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Characterization and molecular weight distribution of carbohydrates isolated from the autohydrolysis extract of mixed southern hardwoods

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ABSTRACT

In order to utilize hemicelluloses from biomass as a feedstock for production of higher value added products such as biofuels and bio-based chemicals, it is essential to assess the chemical composition of the liquid phase and molecular weight distribution of isolated carbohydrates. The association of hemicelluloses with lignin also plays an important role for utilization of isolated hemicelluloses. In the present study carbohydrates were first extracted from a mixture of southern hardwoods by autohydrolysis at different temperatures for 100 min, and then precipitated after mixing with four volumes of ethanol. Depending on the temperature, all (100% at 130 °C) or a small part (5% at 170 °C) of the oligomeric hemicelluloses present in the extract precipitated together with some lignin and oligo-glucose (glucans). The classical iodine test confirmed that most of the glucans are actually starch. Oligo-glucose is the major component in the precipitate from extracts produced at 150 °C and below, while xylan is the main component at 160 °C. Almost all lignin in the precipitate is in the form of lignin–carbohydrate complexes. The average M_w of the carbohydrates isolated from the extract decreases with increasing autohydrolysis temperature. The average DP of hemicelluloses isolated from the extract decreases from about 71 to 36 over the temperature range from 130 to 170 °C during 100 min autohydrolysis.

1. Introduction

There is a strong interest in large-scale use of biomass as a feedstock for biofuels and biomaterials due to environmental and economical concerns such as the increasing concentration of greenhouse gases in the atmosphere and the high cost of oil respectively. Woody biomass, the feedstock for the forest products industry, holds great potential for production of biofuels and renewable chemicals in a so-called Integrated Forest Products Biorefinery (IFBR) (van Heiningen, 2006). Although, the pulp and paper industry is well placed for utilization of cellulose from wood, hemicelluloses, the most abundant polysaccharide in wood after cellulose (Sjöström, 1993) is presently under utilized since its value is mostly the low heating value obtained by combustion in the Kraft recovery process. Therefore, fractionation of wood into its main components (cellulose, hemicelluloses and lignin) following the biorefinery concept (Myerly, Nicholson, Katzen, & Taylor, 1981) is crucial to obtain the full benefit from the chemical value of hemicelluloses. For example, xylose based oligosaccharides have potential use in pharmaceutical, food, agriculture, and papermaking industries (Campbell, Fahey, & Wolf, 1997; Dohnalek, Ostrom, & Hilty, 1998; Ebringerova & Heinze, 2000; Jaskari et al., 1998; Kayserilioglu, Bakir, Yilmaz, & Akkas, 2003; Okazaki, Fujikawa, & Matsumoto, 1990; Okazaki, Koda, Izumi, Fujikawa, & Matsumoto, 1991; Stone, Melton, & Lewis, 1998).

Several processes have been suggested for removal of hemicelluloses from biomass such as steam explosion, organic solvents, alkali, dilute acid or enzyme treatment and autohydrolysis. Autohydrolysis is of interest because water is the only reagent making it an environmentally friendly, inexpensive process compared to dilute mineral acid prehydrolysis (Conner & Lorenz, 1986). Significant amounts of hemicelluloses (40–80% on hemicellulose basis, depending on the wood type and extraction conditions) can be extracted with hot water. During the autohydrolysis process at elevated temperatures, acetic acid is released by hydrolysis of the acetyl groups in hemicelluloses. This lowers the pH of the wood extract to a range of 3–4 (Brasch & Free, 1965), enhancing hydrolysis and dissolution of the hemicelluloses in wood. Hemicellulose extracts (liquor) after autohydrolysis of hardwoods contain mainly xylo-oligosaccharides, glucans, lignin, acetic acid, uronic acid and dehydration/decomposition products such as furfural, HMF, lactic acid and formic acid (Casebier, Hamilton, & Hergert, 1969, 1973; Garrote, Dominguez, & Parajo, 1999, Lora and Wayman (1978); Tunc & van Heiningen, 2008a, 2008b, 2009). In a recent study (Chen, Lawoko, & van Heiningen, 2010) it was proposed that lignin free xylan and glucan oligomers are the major components dissolved

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Table 1
The chemical composition of original extractives-free SHM (% on wood).

Chemical component	Amount (% by wt.)
Arabinose	0.52 ± 0.01
Galactose	1.00 ± 0.01
Glucose	43.66 ± 0.61
Xylose	15.48 ± 0.03
Mannose	2.18 ± 0.05
Lignin	28.61 ± 0.19
Ash	0.38 ± 0.08
AcG	3.30 ± 0.05
UAG	4.47 ± 0.10

AcG: acetyl groups. UAG; uronic acid groups.

in the initial stage of hardwood autohydrolysis, while xylan covalently bound to lignin (i.e. a so-called lignin–carbohydrate complex or LCC) is the major hemicellulose component dissolved in the second stage. The molecular weight of the dissolved components was found to decreases with time in the second stage.

Hemicelluloses are linked to cellulose and lignin via hydrogen and covalent bonds, respectively (Sun, Sun, & Tomkinson, 2004). Chemical bonds between lignin and hemicellulose components can be ester, ether or glycosidic type linkages (Sjöström, 1993). Ether type linkages between lignin and carbohydrates are more common and stable while the ester linkages are easily cleaved by alkali (Sjöström, 1993). These LCCs may explain the recalcitrance of hemicelluloses to completely dissolve during autohydrolysis of wood and lignocellulosic biomass (Tunc, Lawoko, & van Heiningen, 2010; Yang & Wyman, 2008). In order to further process the dissolved hemicelluloses for production of biofuels and new biomaterials, it is important to know the chemical composition and polymeric nature of the dissolved carbohydrates, as well as their association with lignin. Therefore, in the present study hemicelluloses were extracted from a mixture of southern hardwoods with hot water at different temperatures (130–170 °C) for 100 min in a modified Dionex ASE100 extractor. The dissolved carbohydrates were then precipitated by addition of four volumes ethanol to the autohydrolysis extract. The composition and molecular weight distribution of the isolated carbohydrates as well as their association with lignin are discussed.

2. Materials and methods

2.1. Materials

The southern hardwoods mixture (SHM) chips used for the autohydrolysis experiments consists of sweet and black gum (35%), oak (35%), maple (15%), poplar and sycamore (12%) and southern magnolia (3%). The chemical composition of extractives-free SHM is summarized in Table 1. The amounts of cellulose, hemicelluloses, and lignin of the extractives-free SHM are 42.30 ± 0.64, 28.72 ± 0.64 and 28.61 0.19%, respectively.

2.2. Hydrothermal treatment of wood (autohydrolysis)

Autohydrolysis of extractives-free SHM chips was performed at different temperatures from 130 to 170 °C for 100 min in a modified Dionex ASE-100 extractor. The time at temperature is corrected for heat-up time using the Dionex program of the modified ASE-100. The liquor to wood ratio (L/W) was approximately 3.7:1. More details about autohydrolysis of SHM are given in Tunc and van Heiningen (2008a, 2008b).

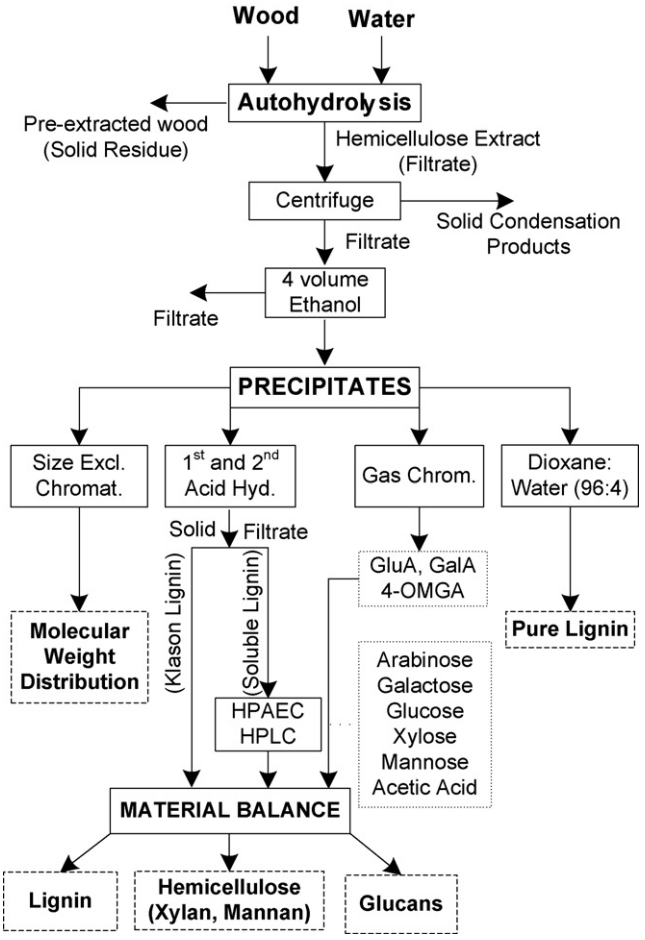

Fig. 1. Experimental design for the isolation and analysis of the isolated carbohydrates from the hemicelluloses extract.

2.3. Experimental procedures

The experimental analysis scheme applied to the precipitates isolated from the hemicellulose extract of the SHM is summarized in Fig. 1. The details about the procedures used to analyze the hemicelluloses extract are reported elsewhere (Tunc & van Heiningen, 2008a). Precipitates containing mostly carbohydrates were obtained by addition of four parts of ethanol to the autohydrolysis extract. Then the precipitates were analyzed for the presence of hemicelluloses, glucans and lignin. The molecular weight distribution of the precipitates redissolved in 0.5 M NaOH was determined. In addition, the amount of so-called "pure-lignin" was determined by extracting the precipitates with 96/4 dioxane/water. Details are given in the sections below.

2.4. Analysis of carbohydrates precipitated from hemicellulose extract

Carbohydrates were isolated from the extract by mixing one volume of extract with four volumes of ethanol and allowing to stand overnight. The precipitates were separated by centrifugation, washed with ethanol and then freeze dried. The freeze dried precipitates were analyzed for carbohydrates, lignin, acetyl groups, uronic acid content and presence of starch.

The monosugar content of hydrolysate produced by the two-step hydrolysis of precipitates with 72 and 4% sulfuric acid (Davis, 1998) was determined by High Performance Anion Exchange Chromatography with Pulse Amperometric Detection (HPAEC–PAD).

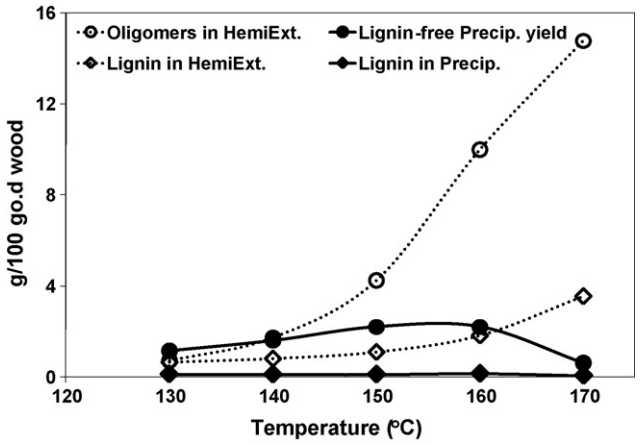

Fig. 2. The lignin-freee yield of EtOH precipitates, the yield of oligomers in the extract and lignin amount in the precipitates and extract vs. temperature.

Acetic acid in the hydrolysate was determined by HPLC using a refractive index detector and BIO-RAD Aminex HPX-87H column. The mobile phase used was 5 mM H_2SO_4 with a flow rate of 0.6 ml/min and the oven temperature was 60 °C.

The uronic acid (GalA, GluA and 4-OMGA) content of the freeze dried precipitates was analyzed by gas chromatography after acid methanolysis and silylation (Sundberg, Sundberg, Lillandt, & Holmbom, 1996). So-called pure (free) lignin in the precipitates was determined by extraction with 96% (v/v) dioxane (Björkman, 1957) followed by UV–vis analysis at 280 nm. The acid insoluble lignin content in the precipitate, or Klason lignin, was measured according to the method by Effland (1977), while the acid soluble lignin content was determined by Tappi method 250.

The classical iodine test was performed in order to verify the presence of any starch in the precipitates.

2.5. Molecular size distribution

Size exclusion chromatography (SEC) was utilized to measure the molecular size distribution of the precipitates redissolved in 0.5 M NaOH. The analysis was performed by Lenzing AG, Austria. The details of the SEC system are as follows: The stationary phases are 1 PSS MCX 1000 Å column (10 μ, 8 mm × 50 mm), 2 PSS MCX 1000 Å columns (10 μ, 8mm × 300 mm) and 1 PSS MCX 100,000 Å column (10 μ, 8mm × 300 mm). The analysis was performed at room temperature. The mobile phase was 0.5 M NaOH at a flow rate of 1 ml/min. Another pump was used to feed 0.1 ml/min of 0.5 M NaOH for continuous purging of the reference cell of the RI-detector. The detector system consists of a UV-detector (Kontron 430) at 280 nm, a RI-detector (Shodex RI-101) and a Viscosity-Detector (WGE Dr. Bures ETA-2010). The other devices in the SEC are a degasser (ERC-3415a), two pumps (Kontron 4200, two pulse dampers (Shodex DP-1) and an autosampler (Kontron 465). The software utilized are Dionex Chromeleon Version 6.80 and PSS WinGPC Unity. The delay time between the UV- and RI-Detector is 0.30 min. The mobile phase flow is split after the UV-Detector; one half goes to the RI and the other half to the Viscosity-Detector.

3. Results and discussion

3.1. Isolation of carbohydrates from the extract

The lignin-free yield of freeze dried precipitates obtained from the hemicelluloses extract after addition of EtOH was plotted against autohydrolysis temperature in Fig. 2. The oligomeric yield of carbohydrates in the hemicelluloses extract generated during the hydrothermal processing (autohydrolysis) of wood was also shown in Fig. 2. It should be noted that the bound acetyl and uronic acid groups are included in the oligomer yield.

Fig. 2 shows that although the extraction yield of oligomeric carbohydrates increases sharply and continuously with increasing temperature, the lignin-free yield of the precipitate reaches a maximum at around 160 °C. It is also clear from Fig. 2 that the ethanol precipitate represents only a small fraction of the oligomeric carbohydrates isolated from the extract obtained at 160 and 170 °C. However, at 130 and 140 °C the precipitate yield is similar to that of the oligomeric carbohydrates in the hemicellulose extract. The low recovery yield of carbohydrates from the liquid phase by ethanol addition was observed earlier for autohydrolysates (Casebier et al., 1973) and for kraft black liquor (Simonson, 1971). This suggests that further acid hydrolysis of the extracted carbohydrates is significant at 160 and 170 °C, resulting in relatively low degree of polymerization (DP) oligomers which remain soluble upon ethanol addition. It is also clear from Fig. 2 that lignin content of the extract increases with increasing temperature while the amount of lignin precipitated with addition of EtOH is almost constant and represents only a small fraction of lignin present in the extract.

The content of oligo-glucose (glucans) and hemicelluloses (xylan and mannan) of the precipitates and the extract calculated according to the equations below and plotted as a function of temperature in Fig. 3.

Glucans(oligo-glucose) content (G);

$$G(\%) = Glu \times \left(\frac{162}{180}\right) - \frac{Man}{b} \times \left(\frac{162}{180}\right) \quad (1)$$

Xylan content (Xn) (for explanation of value of 0.6, see reference Genco, Busayasakul, Medhora, & Robbins, 1990);

$$Xn(\%) = Xyl \times \left(\frac{132}{150}\right) + Ac + UA \times \left[\frac{190}{176} + 0.6 \times \frac{132}{176}\right] \quad (2)$$

Glucomannan (mannan) content (Gm);

$$Gm(\%) = Man \times \left(\frac{162}{180}\right) \times \left[1 + \frac{1}{b}\right] + Gal \times \left(\frac{162}{180}\right) \quad (3)$$

(b = 1.6 for hardwood (Janson, 1974) in Eq. (1) and Eq. (3))Where;

Ac : g acetyl groups per 100 g o.d wood
b : empirical constant (Eqs. (1) and (3))
Gal : g galactose per 100 g o.d wood
Glu : g glucose per 100 g o.d wood
Man : g mannose per 100 g o.d wood
UA : g uronic anhydride per 100 g o.d. wood
Xyl :g xylose per 100 g o.d wood

Fig. 3 shows that with addition of EtOH only a small fraction of oligomeric hemicelluloses (xylan and mannan) available in the extract generated at high temperatures ($T > 150$ °C) can be isolated while the majority of oligo-glucose available in the reaction medium can be recovered. It is interesting to see in Fig. 3a that the amount of precipitated oligo-glucose generated at low temperatures is slightly higher than the oligo-glucose in the extract. The possible explanation is that the amounts of oligo-glucose in the extract generated at low temperature were underestimated due to degradation of glucose during the secondary acid hydrolysis (Tunc & van Heiningen, 2008a).

It is obvious from Fig. 3a that the oligo-glucose yield of precipitates reaches a maximum around 150 °C while the hemicellulose (xylan and mannan) yield reaches a maximum at 160 °C (Fig. 3b and c). The explanation for the occurrence of the maxima are the interaction of two opposing processes with increasing temperature; increasing extraction yield of oligomeric carbohydrates, and the depolymerization of the oligomers to lower DP compounds

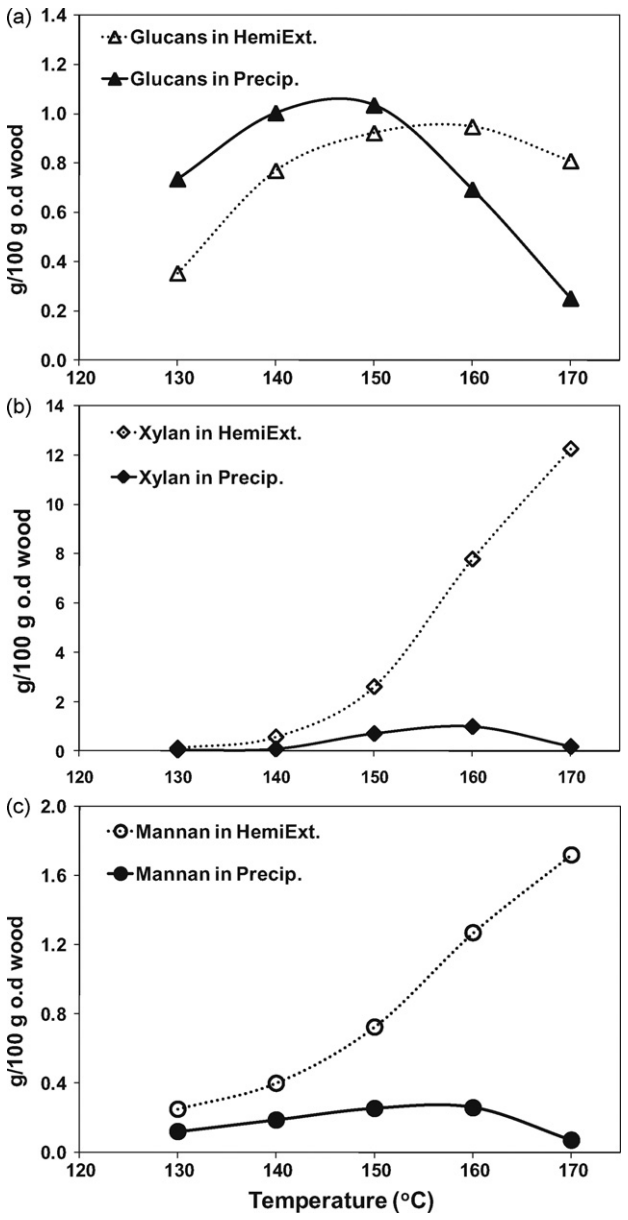

Fig. 3. Composition of precipitates and hemicelluloses extract as a function of temperature after 100 min autohydrolysis, (a) glucans, (b) xylan, and (c) mannan.

which do not precipitate in the ethanol solution. It is further seen in Fig. 3a and b that oligo-glucose is the main component at 150 °C and lower temperatures, while xylan is dominant at 160 °C. Fig. 3c shows that mannan is the minor carbohydrate as to be expected for hardwoods. The amount of xylan and oligo-glucose decreases dramatically at 170 °C most likely due to further hydrolysis to a lower DP of the dissolved oligomers.

3.2. Composition of precipitates

The chemical composition of the precipitates are summarized in Table 2 in form of anhydro-monosugars, uronic acids, acetyl groups and "free" and LCC-lignin. The pure "free" lignin was obtained by 96% dioxane extraction. The LCC-lignin was calculated as the difference between the Klason plus acid soluble lignin and the "free" lignin. It is obvious that the amount of "free" lignin in the precipitate was negligible, i.e. the majority of lignin in the precipitate is LCC-lignin.

It is clear from Table 2 that glucose is the most abundant sugar in the precipitate. Since xylose is the most abundant sugar in the extract at higher temperatures (Tunc & van Heiningen, 2008a), it appears that the oligo-glucose is preferentially precipitated by ethanol dilution, implying that the DP and/or resistance to acid hydrolysis is higher for the extracted oligo-glucose than that of the dissolved xylan. The composition of xylose in the precipitates reaches a maximum around 160 °C. About 10% of the precipitates isolated from the hemicelluloses extract at low temperatures ($T \leq 150$ °C) are in form of pectin because galacturonic acid content of precipitates is approximately 10% up to temperature 150 °C. It is apparent from table that precipitated xylan (hemicelluloses) is completely deacetylated at mild extraction conditions ($T < 150$ °C), but is acetylated at high temperatures ($T > 150$ °C). The precipitates also include minor amounts of arabinose, rhamnose, mannose, glucoronic acid and 4-OMGA. Approximately 6–8% of the precipitates is galactose.

3.3. Classical iodine test

The freeze dried precipitates were dissolved in water and the classical iodine test performed on the precipitate solutions. In all cases the colorless precipitate solutions turned dark blue by addition of a few drops of an iodine solution, indicating the presence of starch in the precipitates. Therefore, it is concluded that at least some of the oligo-glucose in the precipitates are actually starch. Starch was earlier found to be present in beech sapwood and the heartwood (Dietrichs, 1964). The solubility of starch in water is high, while that of cellulose oligomers with a DP higher than eight is very low (Gray, Converse, & Wyman, 2003). Since the oligo-glucan has a DP of about 500 (see below) this suggests that most of the glucan is starch.

3.4. Molecular weight distribution in hemicellulose extract

The RI- and UV-detector responses of the precipitates obtained using SEC are plotted against time in Fig. 4 to verify the existence of LCCs. Two or three peaks depending on the temperature of autohydrolysis are shown in Fig. 4. Based on the UV and RI signals these three peaks at low molecular weight (~200), medium molecular weight (~10,000) and high molecular weight (~100,000) are assigned to lignin, hemicellulose and oligo-glucose (glucans) respectively. Therefore the high molecular weight oligo-glucose is identified as starch and not cellulose due to the fact that cellulose is

Table 2
The chemical composition of precipitates isolated from the hemicellulose extract.

T (°C)	Anhydro-monosugars (%, based on od EtOH precipitates)											
	Ara	Rha	Gal	Glu	Xyl	Man	Free-lig	LCC-lig	GluA	GalA	4-OMGA	AcG
130	3.0	2.5	7.1	59.1	1.9	1.5	0.2	9.2	1.3	10.9	1.3	0.0
140	1.7	2.0	8.1	60.0	4.1	1.8	0.2	6.3	0.9	10.2	0.7	0.0
150	1.5	2.4	7.6	46.2	24.7	2.1	0.1	4.6	1.4	10.4	3.1	2.6
160	0.7	1.2	6.0	31.4	34.0	3.1	0.4	5.9	0.9	3.9	3.2	4.6
170	0.6	0.8	5.7	39.6	24.2	3.2	0.8	7.7	0.7	3.2	2.0	1.1

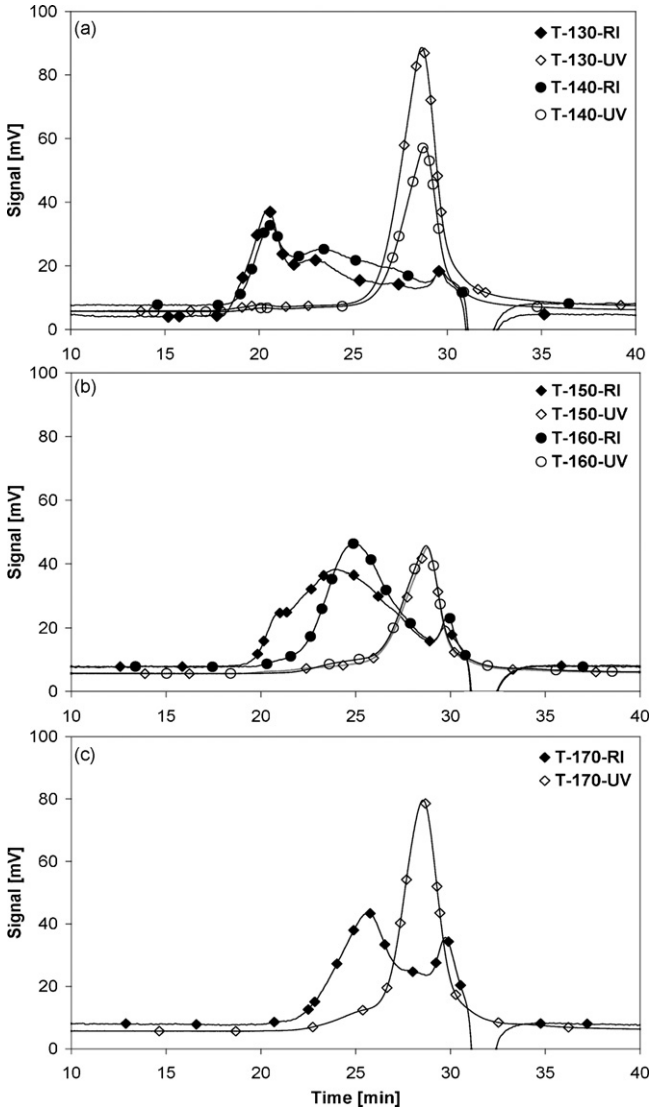

Fig. 4. RI and UV signals of precipitates as a function of time, (a) autohydrolysis temperatures of 130 and 140 °C, (b) autohydrolysis temperatures of 150 and 160 °C, and |(c) autohydrolysis temperature of 170 °C (Symbols are only added for clear identification).

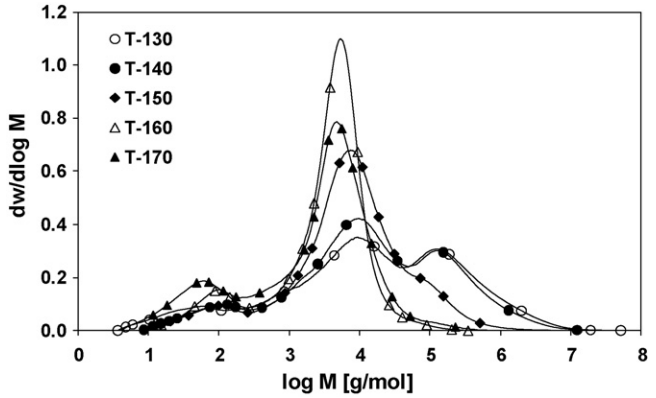

Fig. 5. Molecular weight distribution of precipitates isolated from heimicellulose extract generated at temperatures from 130 to 170 °C (symbols are only added for clear identification).

Table 3
The average M_w and DP of precipitates isolated from the hemicellulose extract.

T (°C)	Hemicellulose		Oligo-glucose (Woody starch)	
	M_w (Da)	DP	M_w (Da)	DP
130	9414	71	132,033	815
140	9320	71	125,345	773
150	7424	56	79,979	493
160	5434	41	–	–
170	4761	36	–	–

$DP_{hemicellulose}=M_w/132$; $DP_{woodystarch}=M_w/162$.

only partly water soluble up to DP of 7–8 (Sixta, 2006) while starch is soluble in hot water.

The high M_w component eluting at about 20 min, i.e. starch (oligo-glucose), is the most abundant species in the precipitate at low temperatures (130–140 °C) and is not present at 160 and 170 °C. Since there is no UV response in this area, lignin is not associated with woody starch. From the RI signal in Fig. 4 it can be concluded that hemicellulose elutes between 21 and 29 min, while lignin is separated between 29 and 31 min. The single UV signal peak, indicative of the presence of lignin appears mostly between 26 and 32 min. Therefore, it can be concluded that only the low M_w hemicelluloses are bound to lignin representing the LCCs. However, it is also clear from Fig. 4 that the majority of the carbohydrates represented by the high M_w carbohydrates (starch and hemicelluloses) are not associated with lignin.

The molecular weight distribution of the different precipitates was obtained by SEC and is depicted in Fig. 5. It also shows that the high M_w component peak disappears at 150 °C, and is absent at 160 and 170 °C autohydrolysis. This indicates that oligo-glucose (starch) is degraded to lower M_w components by acid hydrolysis at high temperatures (T > 150 °C).

The average molecular weight of hemicellulose in the precipitates is listed in Table 3. It was reported that the DP of oligomeric carbohydrates isolated from southern pine wood chips after water prehydrolysis was about 23 (Casebier et al., 1969). Table 3 clearly shows that the molecular weight decreases with increasing temperature. Based on this result it may be inferred that the DP of hemicellulose in the precipitates decreases from 71 to 36 after autohydrolysis at 130 and 170 °C respectively.

4. Conclusions

A fraction of the dissolved carbohydrates was precipitated by adding four volumes of ethanol to the hemicellulose extract. The precipitate represents only a small fraction of the oligomeric carbohydrates in the extract obtained at 160 and 170 °C. However, at 130 and 140 °C the precipitate yield is similar to that of the oligomeric carbohydrates in the extract. This suggests that further acid hydrolysis of the extracted carbohydrates is significant at 160 and 170 °C, resulting in relatively low DP oligomers which remain soluble upon ethanol addition. This behavior was confirmed by SEC. The classical iodine test validated the presence of starch in the precipitates. Oligo-glucose is the main component in the ethanol precipitate at 150 °C and lower temperatures, while xylan is dominant at 160 °C. This means that oligo-glucose is preferentially precipitated, consistent with the higher DP and resistance to acid hydrolysis for extracted oligo-glucose than that of dissolved xylan. At 170 °C the amount of xylan and oligo-glucose decreases dramatically due to acid hydrolysis of the dissolved oligomers to low DP. Almost all the lignin in the precipitate is associated with carbohydrates in the form of LCC-lignin, representing 5–8% of the precipitates. This implies that only lignin covalently bound to the oligomers end up in the precipitate. SEC analysis shows that the lignin in the precipitates is associated with low molecular weight hemicelluloses as LCC. However, the majority of the carbohydrates (starch and hemicellulose) in the precipi-

tates are free of lignin. The average M_w of hemicellulose in the precipitates decreases with increasing autohydrolysis temperature. The DP of hemicellulose in the precipitates decreases from approximately 71 to 36 after autohydrolysis at 130 and 170 °C respectively.

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