Comparative Study on the Yield and Characterization of Hemicelluloses Isolated with Hydrothermal Extract and Catalyzed by Acetic Acid from Acacia Wood

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To utilize hemicelluloses from biomass as a feedstock to produce various value-added products, the soluble hemicelluloses must be isolated from the liquid phase with a high yield and purity. In this study, acacia wood was extracted by hydrothermal treatment, catalyzed by acetic acid at 170 °C for different lengths of time, and then precipitated after concentration and mixing with ethanol. Acetic acid led to faster hydrolysis of hemicelluloses, a process that was confirmed by a larger amount of total saccharides than the controlled results. A yield of more than 90% oligosaccharides was achieved in the hydrolysate with 1% (w/w) acetic acid. The maximum precipitate yield obtained was reduced, but a faster increase was observed in the first 30 min at 170 °C depending on the utilization of acetic acid. Analysis of ^{13}C nuclear magnetic resonance (^{13}C NMR) confirmed that the side chains, such as arabinose linked on the xylan chain, were severely broken down, and more dissolved hemicelluloses bonded with lignin (LCC) were present in the precipitates with 1% (w/w) acetic acid. Based on gel permeation chromatography (GPC), a molecular weight of not less than 1900 is suggested when ethanol is used to precipitate the oligosaccharides from hydrolysate.

Keywords: Hydrothermal; Pre-extraction; Hemicelluloses isolation; Acacia; Acetic acid

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INTRODUCTION

The chemical pulping industry is one of the largest manufacturers of lignocellulosic material, from which high strength and whiteness fibers are produced to make various grades of end-use papers. However, due to the increased capacity of the pulp and paper industry, the profitability of this industry is decreasing. Biorefining has been proposed to reshape the pulping industry by utilizing biomass resources more effectively and producing a variety of products (Vila *et al.* 2011; Liu *et al*. 2015). In kraft pulping, about 20% of biomass, mainly hemicelluloses, are dissolved in the black liquor along with lignin and then burned in the recovery cycle to produce energy for the process. However, the heating value of hemicelluloses is half that of lignin (13.6 MJ/kg) (Tunc and van Heiningen 2008). Therefore, instead of burning the hemicelluloses with black liquor, hemicellulose extraction prior to chemical pulping has been widely discussed as a practical approach in the implementation of a pulp mill-based biorefinery. There is great potential for the conversion of hemicelluloses derivatives into high-value added products such as fuels, films, health products, and polymers (Vila *et al.* 2011).

Hydrothermal pretreatment (hot water treatment or autohydrolysis) is widely used to extract hemicelluloses from biomass for producing fermentable sugars and

oligosaccharides (Ertas *et al.* 2014; Batalha *et al.* 2015; Cuevas *et al.* 2015). This method is a simple, low-cost, and attractive technology for extracting lignocelluloses in the kraft pulping (Deb *et al.* 2016), soda, soda-anthraquinone, and soda ethylenediamine processes for producing dissolving pulp (Jahan 2009; Borrega *et al.* 2013).

In implementing pulp mill-based biorefinery processing, the yield of hemicelluloses extracted from hydrothermal pretreatment of biomass greatly determines the efficiency of the process. Furthermore, the additional expenses for the separation of by-products must be justified by the amount and purity of the products recovered from resulting liquor. Early studies showed that raising the intensity of treatment, determined by time and temperature, increased the amount of hemicellulose removed from the lignocellulosic materials. However, the amount of detectable oligosaccharides and monosaccharides in liquor was not increased with the higher intensity of treatment due to further degradation of furfural, as well as other unknown degradation products under intense treatment (Shi *et al.* 2015). High temperatures and short reactions are beneficial for obtaining a high concentration of dissolved hemicelluloses. Hydrothermal treatment also allows hemicellulose to dissolve as oligosaccharides rather than monosaccharides. Yields of more than 90% oligomeric sugars can be obtained from hardwoods and woody biomass using the hydrothermal pretreatment (Leschinsky *et al.* 2009; Sukhbaatar *et al.* 2014). However, in addition to dissolved hemicelluloses, the resulting liquor contains other substances, such as phenolic compounds, furfural, and acetic acid. Additional processing is required before the hydrothermal pretreatment liquor can be utilized to produce various value-added products (Shuai *et al.* 2010; Ximenes *et al.* 2011). The recovery of the dissolved hemicelluloses with a simple and economic method would promote the implementation of this pulp mill-based biorefinery.

The hydrolysis of hemicelluloses has been studied in the past, evaluating the composition of sugar species, the hydrolysis kinetic models and further degradation into non-sugars substances (Garrote *et al.* 1999; Mittal *et al.* 2009; Moniz *et al.* 2013). However, there is little information on the effect of hydrothermal treatment conditions on the efficiency of dissolved hemicellulose recovery. The use of organic acid, such as oxalic acid, as catalysts for the selective depolymerisation of biomass has recently attracted increasing interest (Stein *et al.* 2011). Acetic acid is generally present in the hydrolysate due to the release of acetate group bounded on hemicelluloses during hydrolysis, so it can provide certain options as catalysts for biomass fractionation due to the cleaner concerns because no additional chemical is introduced to the hydrolysate. *Acacia* trees are an important economic forest asset that is widely distributed in Southeast Asia and South China. The area is quite suitable for fast growth of hardwoods due to rainy and warm weather. Together with eucalyptus, the acacia tree has been evaluated as potential biomass for bioethanol production (Ferreira *et al*. 2011; Ko *et al*. 2012). In this study, acacia wood chips were subjected to hydrothermal treatment with and without acetic acid at 170 °C. Ethanol solvent was used to precipitate the dissolved hemicelluloses from the resulting hydrolysate. The major aim was to investigate the effect of pretreatment conditions on the recovery efficiency of dissolved hemicelluloses. The second aim was to compare the influence of acetic acid addition on the controlled hydrothermal treatment by determining monosaccharide and oligosaccharide concentration, as well as the recovered yield with ethanol precipitation from the resulting hydrolysate. Finally, the recovered hemicelluloses from hydrothermal treatment were characterized.

EXPERIMENTAL

Materials

Vietnam acacia wood chips were provided by a kraft pulping mill located in Shandong, China. The wood chips were smashed into powder after a chips sampling preparation according to TAPPI T257-cm85 (1985). Powders between 40 to 60 mesh were collected and stored in a ground-glass stoppered bottle for chemical component analysis. Standard substances for the sugar determination of raw material and hydrolysate, including xylose, arabinose, mannose, glucose, and galactose were purchased from Sigma-Aldrich (Shanghai, China). A glucan molecular weight series (Waters Corporation, Beijing, China) was used to construct a calibration curve. The equipment used included an ion chromatography unit (Dionex-5000, Dionex Corporation, Sunnyvale, USA), NMR instrument (Bruker Advance III 400 MHz, Karlsruhe, Germany), GPC analyser (Waters HPLC Breeze, Milford, MA, USA), and UV spectrophotometer (Varian Cary 300, Palo Alto, USA).

Methods

Hydrothermal treatment

The hydrothermal treatment was conducted in a heated oil bath with 4 stainless steel vessels of 500 mL. The temperature was fixed at 170 °C with a liquor (water or 0.25% to 3.0% (w/w) acetic acid solution)/solid ratio of 4:1 (75 g wood chips). Cooking time was recorded from 0 min when the oil temperature reached at 170 °C. After the set cooking time arrived, the cooking vessel was removed from the heat source and cooled. The hydrolysate was separated with filter paper and stored at 4 °C.

Precipitation of hemicelluloses from hydrolysate

The hydrolysate was concentrated by vacuum evaporation (IKA RV10, Staufen, Germany) at 40 °C under 0.1 MPa. Afterward, the concentrated hydrolysate was mixed with ethanol at an 8:1 ethanol: hydrolysate volumetric ratio to precipitate hemicelluloses. The precipitated hemicelluloses were centrifuged at 4000 rpm for 10 min and freeze dried. The precipitate yields were determined as the mass of precipitates obtained from each litre hydrolysate (g/L). The recovery yield of the total sugar in the wood chips was also calculated according to the ratio of liquor:solid and the mass percentage of sugars in wood chips (67.65%).

Chemical composition analysis

The Klason lignin (acid-insoluble lignin) and acid-soluble lignin content of raw wood chips were measured according to TAPPI T222 om-02 (2002) and TAPPI UM250 (1991). The total extractives and ash content of raw wood chips were determined by TAPPI T204 om-88 (1988) and TAPPI T211om-93 (1993). The sugar content of wood chips was determined by ion chromatography. The wood chip powder was subjected to two-step hydrolysis with 72 %(w/w) H_2SO_4 for 2 h at 18 °C to 20 °C, then 4%(w/w) H_2SO_4 at 121 °C for 1 h, and the hydrolysate was collected for analysis. To determine the oligosaccharide content in the hydrothermal hydrolysate, an additional acidic hydrolysis was conducted on the samples with 4% (w/w) H_2SO_4 at 121 °C for 1 h. After this acid hydrolysis, the samples contained only monosugars (total sugars). Therefore, the oligosugar content of the samples was calculated based on the differences in the monosugar content of the samples before and after additional acidic hydrolysis. The precipitated hemicelluloses were also

hydrolyzed with 4% (w/w) H_2SO_4 at 121 °C for 1 h to investigate their sugar contents. The sugar analysis was performed with an ion chromatography unit (Dionex-5000) equipped with a CarboPac™ PA20 analytical column (150 × 3 mm), a CarboPac™ PA20 guard column (30 × 3 mm), and an ED5000 electrochemical detector. Deionized water was used as the eluent at a flow rate of 1 mL/min. A 0.2 M NaOH solution was used as the supporting electrolyte at a flow rate of 1 mL/min. The samples were filtered and diluted prior to analysis. For construction of the calibration curve, a mixture solution containing 10 mg/L of each kind of monosaccharide (glucose, xylose, arabinose, mannose, and galactose) was prepared. The saccharide species and content were reported as monosaccharaides. UV spectrophotometry (Varian 300) was used to detect lignin in the precipitated hemicelluloses. The samples were subjected to the treatment described in TAPPI UM250 (1991). The resulting liquor was diluted four times with deionized water prior to scanning, and the lignin content was calculated from the absorbance at 205 nm using an absorption coefficient of 110 L/(g·cm).

Characteristics of precipitated hemicelluloses

Solution state ^{13}C NMR spectra of the precipitates sample were obtained using a Bruker Advance III 400 MHz spectrometer with a 5 mm-PABBO probe head at 25 °C. The ^{13}C-NMR spectra were recorded at 100.6 MHz, acquiring 30000 scans using 60 mg samples dissolved in 1.0 mL D_2O, with 2 to 3 drops NaOD added to dissolve it. Tetramethylsilane (TMS) was used as an internal standard (δ = 0 ppm).

The molecular weight distribution (MWD) and average molecular weight (M_w and M_n) of the hemicelluloses were determined by gel permeation chromatography (GPC). Prior to the GPC analysis, the hemicellulose samples were dissolved in the mobile phase (0.02 M KH_2PO_4) at 65 °C for 7 h under the assistance of ultrasound irradiation. The GPC analyses were carried out using a Waters high performance liquid chromatography system consisting of two TSK-GEL G-4000 PWxl (7.8 × 300 mm) and TSK-GEL G-2500 PWxl (7.8 × 300 mm) columns linked in series and a refractive index (Waters 2414, RI) detector. The mobile phase comprised 0.02 M KH_2PO_4 (pH 6.5; 35 °C) at a flow rate of 0.6 mL/min. The solution was filtered through a 0.45 μm membrane and injected into the GPC system for analysis; glucan was used as a calibration standard.

RESULTS AND DISCUSSION

Chemical Composition of Acacia Wood Chips

The main components of Vietnam acacia wood chips are listed in Table 1. These data confirm previous results of the same raw material and are similar to those of other well-studied hardwoods, such as poplar species (Berlin *et al*. 2006; Yánez *et al.* 2009; Ferreira *et al.* 2011). The dry material contained approximately 43% glucose and 20% xylose, which could be utilized for production of pulp and other bio-based products. Because this species is relatively abundant and grows quickly, it is an appropriate feedstock for the pulp mill-based biorefinery process of lignocellulosic material.

Table 1. Chemical Composition of Vietnam Acacia Wood Chips

Klason Lignin (g)	Acid-soluble Lignin (g)	Ash (g)	Extractives (g)	Glucose (g)	Xylose (g)	Mannose (g)	Galactose (g)	Arabinose (g)
18.64	2.22	0.44	12.33	42.96	19.90	3.20	0.86	0.73

Note: Results based on 100 g oven-dry wood chips

Effect of Acetic Acid on the Saccharides Components in the Hydrolysate

Organic acid catalysts are a promising approach to enhance the selective depolymerisation of carbohydrate polymers. As acidic substances are predominantly generated from the hydrolysis of acetate groups, mainly hemicelluloses, choosing acetic acid does not introduce additional impurities. Two kinds of hydrothermal pretreatment, with and without additional acetic acid, were conducted in Table 2.

Table 2. Content and Proportion of Monosaccharide and Oligosaccharides in Hydrolysate with and without 1 % (w/w) Acetic Acid

Hot water						
Time (min)	25	30	35	40	45	50
Monomers (g/L)	0.288	0.477	1.400	1.974	3.114	3.514
Xylose (%)	31.71	34.36	45.99	55.18	60.12	62.38
Glucose (%)	8.31	6.29	5.33	4.18	3.64	4.36
Mannose (%)	4.29	3.78	7.08	5.10	4.88	5.61
Galactose (%)	15.98	15.18	14.41	12.47	12.23	11.66
Arabinose (%)	39.69	40.37	27.16	23.04	19.12	15.95
Oligomers (g/L)	2.228	3.763	8.679	12.135	15.608	15.760
Xyl-oligomer (%)	52.66	58.45	71.94	74.93	77.73	77.12
Glu-oligomer (%)	13.85	13.40	7.14	7.36	7.47	6.72
Man-oligomer (%)	8.31	7.86	10.24	7.61	6.10	5.04
Gal-oligomer (%)	14.16	12.79	7.97	8.03	7.47	6.62
Ara-oligomer (%)	11.00	7.49	2.69	2.04	1.31	1.04
Total sugars (g/L)	2.516	4.240	10.079	14.109	18.722	19.274
Oligomers/Total sugars (%)	88.54	88.74	86.10	86.00	83.36	81.76
Hot water/ 1% acetic acid						
Time (min)	25	30	35	40	45	50
Monomers (g/L)	0.549	0.896	2.261	3.413	4.717	5.536
Xylose (%)	38.25	44.53	56.61	66.36	68.30	69.54
Glucose (%)	9.28	11.27	6.14	4.62	4.62	4.57
Mannose (%)	4.18	4.35	4.49	3.89	4.20	4.06
Galactose (%)	16.93	13.72	10.30	8.61	9.80	10.26
Arabinose (%)	31.32	26.11	22.02	16.49	13.15	11.56
Oligomers (g/L)	4.346	9.612	13.876	16.63	17.879	18.49
Xyl-oligomer (%)	61.82	73.23	76.78	77.78	79.62	79.92
Glu-oligomer (%)	11.98	9.33	7.06	7.62	7.01	7.04
Man-oligomer (%)	8.90	7.62	7.99	7.66	5.44	5.88
Gal-oligomer (%)	12.14	7.20	6.76	5.94	7.04	6.45
Ara-oligomer (%)	5.13	2.61	1.38	0.98	0.86	0.68
Total sugars (g/L)	4.895	10.508	16.137	20.043	22.596	24.026
Oligomers/Total sugars (%)	88.78	91.47	85.98	82.97	79.12	76.96

Although monomeric saccharides were present in low amounts in hydrolysate, treatment with 1% (w/w) acetic acid increased the monomeric saccharide content from 3.514 g/L to 5.536 g/L at 50 min. Compared with the small change of glucose and mannose

proportion, the xylose increased from 31.78% to 38.25% at 25 min and from 62.38% to 69.54% at 50 min after treatment with 1% (w/w) acetic acid. Arabinose dramatically decreased from 39.69% at 25 min to 15.95% at 50 min. This result is probably due to the structure of the hardwood hemicelluloses, as arabinose is usually branched as a side chain on the linear xylan. These saccharide units were easily removed from the backbone of xylan during hydrolysis (Nabarlatz *et al.* 2004). Thus, the initial arabinose proportion was quite high, and thereafter the depolymerisation of the main chains of other saccharides into oligosaccharides and monosaccharides decreased the arabinose in the hydrolysate.

The more abundant saccharide compounds were oligosaccharides in the hydrolysate during the hydrothermal treatment. The 1% (w/w) acetic acid addition promoted the depolymerisation of hemicelluloses, as shown by the increased oligosaccharides content from 2.228 g/L to 4.346 g/L at 25 min and 15.760 g/L to 18.49 g/L at 50 min. For both kinds of hydrolysate, the xyl-oligosaccharide was the predominant proportion. Acetic acid addition accelerated the generation of xyl-oligosaccharide, as indicated by the higher proportion during first stage hydrolysis between 25 min and 35 min. However, a similar value was observed with the prolonged hydrolysis time of 50 min. The glu-oligosaccharide did not show obvious differences between the hydrolysate with and without acetic acid addition. Combined with the analysis of monosaccharide content and proportion, this result demonstrated the selectivity of acetic acid with respect to the depolymerisation of carbohydrate compounds. The proportion of arabinosaccharide dramatically decreased in the hydrolysate from 11.0% at 25 min to 1.04% at 50 min. Acetic acid addition enhanced the linkage breaking between arabinose and the attached saccharide backbone, as indicated by the much lower proportion of arabinosaccharide in the hydrolysate obtained at same time, especially at the earlier stage. This behavior was consistent with other lignocellulosic material, such as eucalyptus (Garrote *et al.* 1999; Yang and Wyman 2008). As shown in Table 2, the ratio of oligosaccharides to the total saccharides in the hydrolysate was gradually reduced with the extension of hydrolysis due to the hydrolysis of oligosaccharides to generate monosaccharides, which became more obvious with the acetic acid addition.

Effect of Acetic Acid on the Ethanol Precipitate Yields

Ethanol precipitation is widely used to recover hemicelluloses dissolved in an extraction liquor of lignocellulosic material. To obtain the maximum precipitation yield, the precipitation conditions were optimized by vacuum concentration and a high ratio of ethanol to hydrolysate based on reported methods (Shi *et al.* 2016). Figure 1 shows the ethanol precipitation yields in hydrolysate with and without acetic acid.

The precipitation yield (left vertical axis) did not differ significantly over the earlier stage, from 0.418 g/L at 3 min to 0.536 g/L at 10 min and from 0.384 g/L at 3 min to 0.498 g/L at 10 min for the hydrolysate with and without 1% (w/w) acetic acid, respectively. However, two different trends were observed by prolonging hydrolysis over 10 min. With 1% acetic acid, the precipitation yield increased from 0.536 g/L to 4.116 g/L from 10 min to 25 min, thereafter slowly increased to maximum value of 4.508 g/L at 35 min, and then decreased to 1.268 g/L at 45 min. Without acetic acid, the precipitation yield slowly increased from 0.498 g/L to 1.732 g/L from 10 min to 25 min, then started to quickly increase to the maximum value of 6.002 g/L at 35 min, which was much higher than the value obtained with 1% (w/w) acetic acid. Thereafter, it slowly decreased to 5.636 g/L at 45 min.

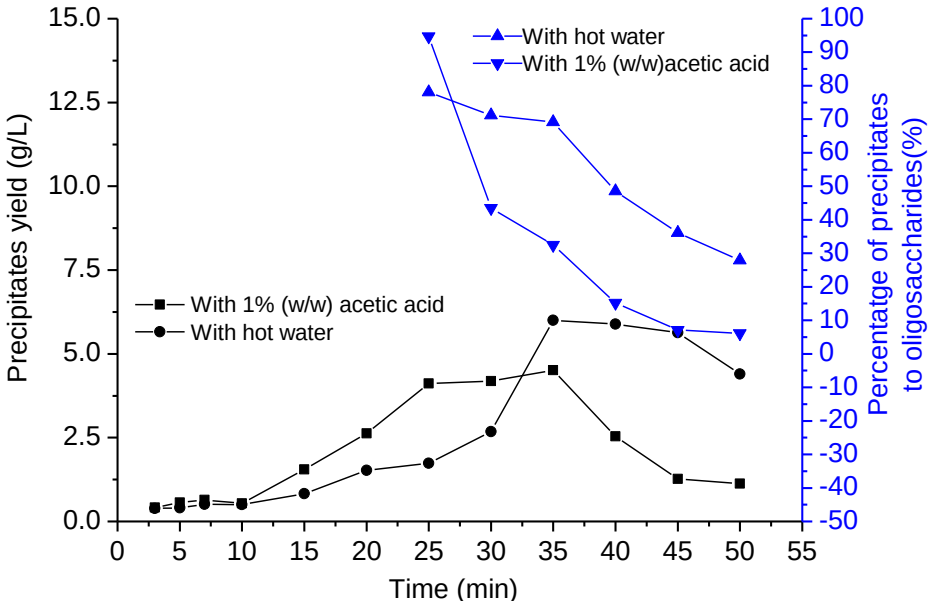


Fig. 1. Precipitate yields and proportion of precipitates to oligosaccharides in hydrolysates obtained with and without 1% (w/w) acetic acid

The ratio of precipitates to oligosaccharides in hydrolysate was also analysed within the range from 25 min to 50 min, as shown in Fig. 1 (right vertical axis). Adding 1% (w/w) acetic acid resulted in a much faster decrease and a lower ratio of precipitate yields to oligosaccharides with the same hydrolysis time compared with the hot water pretreatment; 94.70% of oligosaccharides were precipitated from the hydrolysate at 25 min. However, this value was dramatically reduced to 32.48% with 10 min hydrolysis, and only 6.10% could be precipitated from the hydrolysate at 50 min.

To understand the influence of adding acetic acid, the content of oligosaccharides and total saccharides (oligosaccharides plus monosaccharides) in the hydrolysate with and without acetic acid was measured, as shown in Fig. 2.

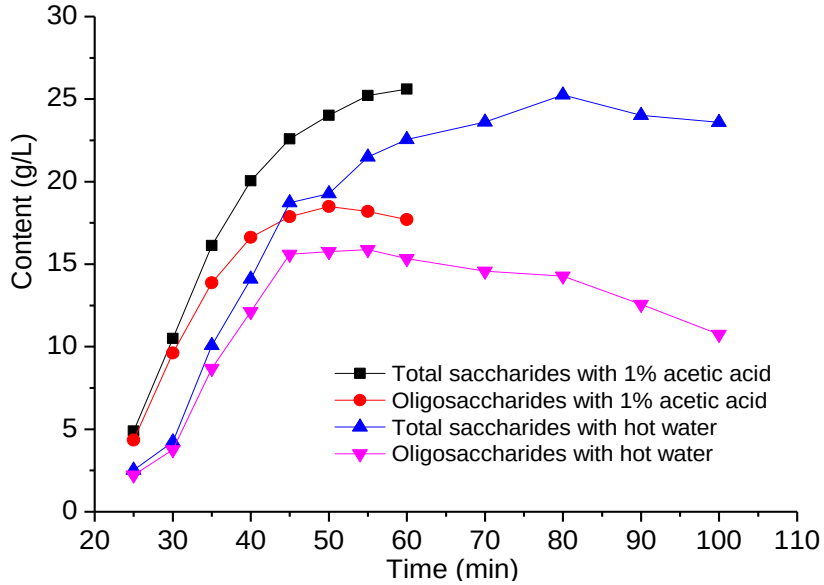


Fig. 2. Content of oligosaccharides and total saccharides in hydrolysate obtained with and without 1% (w/w) acetic acid

Higher contents of both oligosaccharides and total saccharides were present in the hydrolysate with 1% (w/w) acetic acid. This was due to the depolymerisation of hemicelluloses to soluble saccharides, whereas more crystalline celluloses remained inaccessible to the acid catalysis (Stein *et al.* 2011). The highest proportion of oligosaccharides to total saccharides appeared at the early stage despite that the oligosaccharide content being relatively low (Table 1), which is consistent with an earlier study (Yat *et al.* 2008). Further prolonging the hydrolysis time from 25 min resulted in a fast increase in the oligosaccharide content until about 40 min, and then a slow rise to the maximum at 50 min. Surprisingly, a faster increase in the oligosaccharides content did not induce an increase in precipitation yield at a similar rate, especially in the 1% (w/w) acetic acid sample. This phenomenon implied that the oligosaccharides generated during hydrolysis resulted from some different behavior in the treatment of ethanol precipitation. Garrote *et al*. (1999) proposed that high molecular weight (MW) oligosaccharides generated in the early reaction stages are gradually depolymerized to oligosaccharides having lower MW, whereas oligosaccharides with low MW are likely to be converted to monosaccharides in the late hydrolysis stages. As shown in Fig. 2, the monosaccharide content was higher after hydrolysis of 35 min, even more so from the addition of 1% (w/w) acetic acid. Adding 1% (w/w) acetic acid accelerated oligosaccharide formation and their depolymerisation to lower MW oligosaccharides as well as the formation of monosaccharides and non-saccharide substances (Sukhbaatar *et al.* 2014). By adding 1% (w/w) acetic acid, the maximum oligosaccharides content increased, but the amount of precipitation with ethanol decreased compared with no addition of acetic acid.

Figure 3 shows the effects of time and acid concentration on the yield of precipitation from the hydrolysate at 170 °C. The addition of acetic acid resulted in a higher yield of precipitation at the early stage of hydrothermal treatment, and the effect of the higher concentration of the acid was more obvious. However, with the extension of time, precipitation slowed, and the maximum was lower when acetic acid was added. Furthermore, a faster drop of the yield was observed with the higher concentration of acetic acid after the maximum value were achieved.

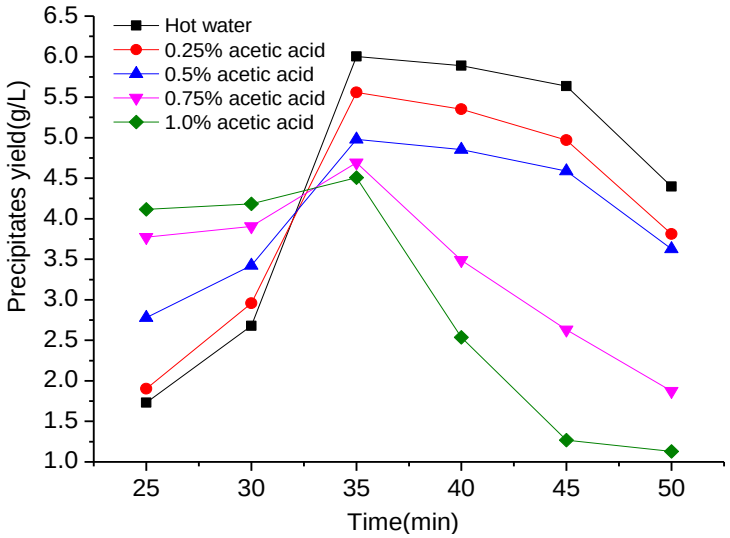


Fig. 3. Precipitate yields of oligosaccharides in the hydrolysate obtained with different utilization of acetic acid at 170 °C

These results implied that both the rate of formation of oligosaccharides from hemicelluloses polymerization in the early stage and further degradation into smaller MW oligosaccharides, and even monosaccharide in the late stage, were increased by acetic acid.

Component Analysis and Characterization of Precipitates

The yield and chemical components of the precipitates are listed in Table 3. Adding acetic acid did not increase the maximum yield of precipitate. The yield decreased from 6.002 g/L to 5.56 g/L with the addition of 0.25% (w/w) acetic acid. With a further increase in the acetic acid to 1% (w/w), the yield gradually decreased to 4.508 g/L. Xylose was the most abundant saccharide in the precipitate (72.73 to 75.23% (w/w)). Its concentration was higher compared with the untreated sample. A higher xylose content was observed in the precipitates with longer hydrolysis time and higher acetic acid addition. All precipitates contained relatively large amounts of glucose and galactose. With increased acetic acid addition, the glucose content decreased from 9.51% (w/w) to 8.77% (w/w), and galactose declined from 6.87% (w/w) to 5.43% (w/w).

Table 3. Yield and Main Chemical Composition of Precipitates from Hydrolysates Obtained with and without acetic acid at 170 °C/35 min

Conditions	Hot water, 170°C			Hot water,170 °C/35 min /acetic acid,% (w/w)			
	30min	35min	40min	35min/0.25%	35min/0.5%	35min/0.75%	35min/1.0%
Precipitate yields ,g/L	2.678	6.002	5.887	5.56	4.982	4.69	4.508
Precipitate yields ,%	1.58	3.54	3.48	3.27	2.94	2.76	2.66
Xylose ,%	69.73	72.36	73.18	72.73	73.21	74.36	75.23
Glucose ,%	9.87	9.44	9.32	9.51	9.76	9.12	8.77
Mannose,%	2.79	2.31	2.53	2.38	3.12	3.43	3.47
Galactose,%	8.42	7.49	7.10	6.87	6.19	5.81	5.43
Arabinose,%	2.95	2.27	1.95	2.22	1.53	0.94	0.64
Lignin,%	2.17	2.36	2.54	2.55	2.78	3.39	3.94
Others,%	4.07	3.77	3.38	3.74	3.41	2.95	2.52

Arabinose was identified in minor quantities in the precipitates. With 1.0%(w/w/) acetic acid addition, only 0.64% (w/w) of arabinose was found in the final precipitates with yield of 4.508 g/L. This could be due to the fact that arabinose attached on the xylan main chain was relatively easier to remove in acidic conditions. This conformed to the results listed in Table 2. As expected, a small amount of lignin was also identified from 2.55% (w/w) to 3.94% (w/w) in all the precipitates obtained with acetic acid. It is generally accepted that free lignin in precipitates hemicelluloses, which are extracted with solvent, such as dioxane, at less than 1.0% (w/w) based on dry precipitates. Tunk and van Heiningen (2011) showed that the free lignin content increased from 0.2% (w/w) to 0.8% (w/w) in the precipitates when temperature increased from 130 °C to 170 °C. During the first 40 min hydrolysis at 160 °C, all lignin-free xylan is removed, and from then on all xylan dissolved is in the form of lignin-carbohydrate complexes (Chen *et al.* 2010). However, due to the strengthening effect of acetic acid and the higher temperatures used in this experiment, LCC-lignin, dominantly produced by the linkage of lignin to xylan, was probably present despite the precipitates that were obtained from the hydrolysate of 35 min. It was apparent that adding acetic acid reduced ash, uronic acid, and other substances.

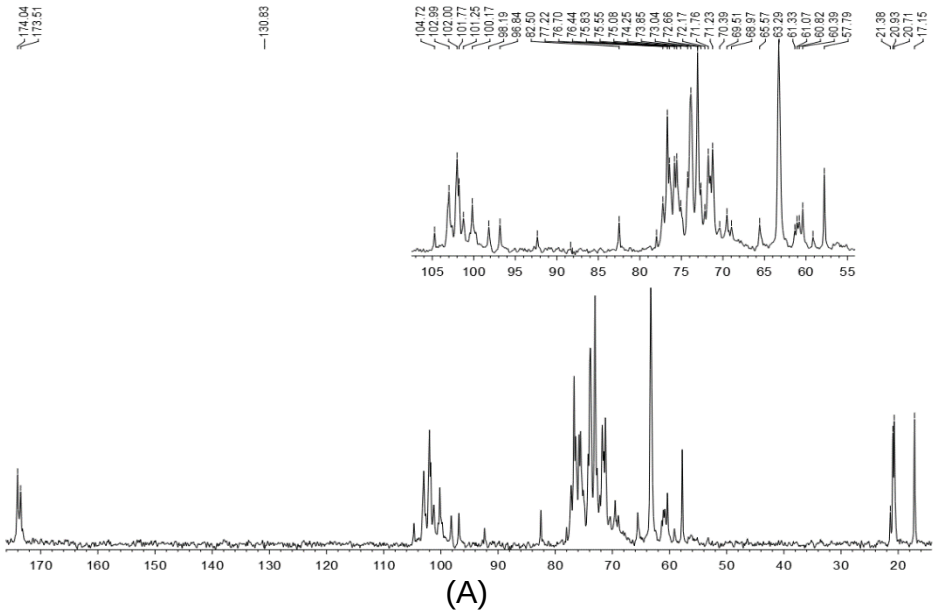
Table 4. The Average M_w of Precipitates Isolated from the Hydrolysate Obtained with and without Acetic Acid at 170 °C

	170 °C/Water				170 °C/ 0.25 % acetic acid				170 °C/ 1.0 % acetic acid			
min	25	30	35	40	25	30	35	40	25	30	35	40
M_w	3050	3091	2497	2115	2409	2860	2384	1978	2327	2680	1996	1902
M_n	2166	2291	1814	1756	1721	2084	1833	1635	1689	1941	1689	1635
M_w/M_n	1.427	1.331	1.376	1.204	1.4	1.372	1.301	1.21	1.377	1.381	1.175	1.163

The hemicelluloses precipitated from hydrolysate could also be modified and applied as an additive in paper industry, in addition to its role as a substrate for ethanol production by yeast fermentation. The molecular weight distribution of the hemicelluloses plays a significant role in the extent that paper strength is improved. Megaton *et al.* (2011) had reported that higher molecular weight hemicelluloses are more effective than low molecular weight hemicelluloses as strength additives. The average molecular weight (M_w) of the precipitates obtained with and without acetic acid addition were detected with GPC techniques. The results in Table 4 clearly show that the M_w of the precipitates first increased and then decreased. This trend was generally consistent with the change in the yield of precipitates found by the earlier study in which the M_w of the extracted component decreased with time extension (Ma *et al.* 2014). However, compared with the hot water extraction, a relatively lower polymerization degree was found in the precipitates when acetic acid was used. Under the conditions 170 °C/25 min, the M_w of precipitate was dramatically reduced from 3050 to 2327 with 1% (w/w) utilization of acetic acid. In contrast, a precipitate yield of 4.112 g/L was achieved, quite close to the maximum value of 4.508 g/L obtained at 35 min (Fig. 3). These results implied that the strengthening hydrolysis of wood chips was achieved by acetic acid addition in the earlier stage of hydrolysis, along with the dissolution of hemicelluloses and depolymerisation of dissolved polysaccharides to smaller resultants. However, with the time extension, more significant depolymerisation occurred with acetic acid addition that could be observed by the decrease of M_w from 2680 at 30 min to 1996 at 35 min with the presence of 1% acetic acid. Interestingly, compared with the results without or 0.25% (w/w) acetic acid addition, the precipitates obtained at 40 min remained at a similar M_w of 1902, with the results of 1996 obtained at 35 min. It was also clear from Figs. 1 and 2 that although the concentration of polysaccharides increased from 13.876 g/L at 35 min to 16.63 g/L at 40 min, the precipitate yields decreased quickly from 4.508 g/L to 2.536 g/L. This result implied that further acid hydrolysis extracted more carbohydrates from wood chips but resulted in a relatively low degree of polymerization converted from the extracted polysaccharides, which remain soluble upon ethanol precipitation. Generally, polysaccharides with degree of polymerization (DP) lower than 10 are completely soluble in water. The hydrolysate from hot water hydrolysis of corn stover at 200 °C for 10 min showed that DP of the dissolved hemicelluloses ranged from 1 to 30 by analysis of IC chromatogram (Yang and Wyman 2008). The M_w of hemicelluloses directly precipitated from corn stalk hydrolysate of 180 °C was 2182 (Egüés *et al.* 2012). Based on these findings and the results listed in Table 4, it may be inferred that the M_w of polysaccharides isolated by ethanol precipitation is limited to a certain value, and in this study, the value was not less than 1900.

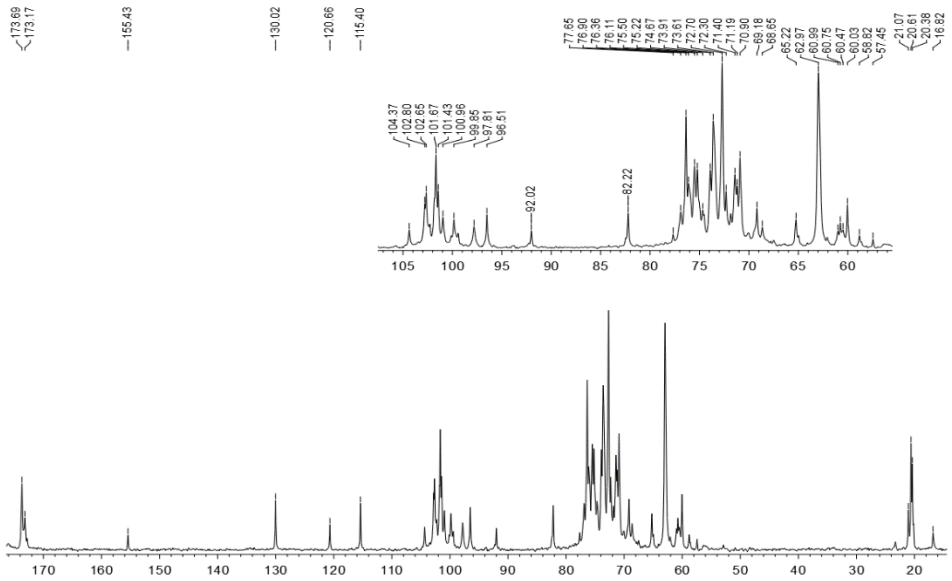
As shown in Fig. 4, at the range of 55 ppm to 110 ppm, the ^{13}C NMR spectra of precipitates obtained with and without 1% (w/w) acetic acid were quite similar. The dominant five signals were observed at 102.99(A) or 102.65(B), 73.85(A) or 73.61(B),

75.55(A) or 75.22(B), 76.70(A) or 76.36(B), and 63.29(A) or 62.97(B), corresponding to C-1, C-2, C-3, C-4, and C-5 positions of (1-4)-linked β-D xylan chain that is referred to in the results from a standard xyl-oligomer reagent and hemicelluloses, as reported by Sun *et al.* (2005). Two weak signals at 77.99(A) or 77.65(B) and 61.33(A) or 60.99(B) were probably assigned to the C-3 and C-5 positions of α-L-Araf residues (1-3) linked to β-D xylan. A much weaker signal was found from spectrum B than spectrum A. This is probably due to the chemical bond between α-L-Araf and xylan chain, which is unstable in acidic conditions. Signals observed at 173.51(A) or 173.17(B), 100.17(A) or 99.85(B), 71.23(A) or 70.90(B), 72.17(A) or 73.30(B), 82.50(A) or 82.22(B), 72.66(A) or 72.70(B), and 60.39(A) or 60.03(B) were attributed to C-6, C-1, C-2, C-3, C-4, C-5, and methoxyl groups of 4-O-methyl-D-glucuronic acid residues (Sun *et al.* 2011). The small but obvious signal at 104.72(A) or 104.37(B) was assigned to C-1 of β–glucans, and its intensity was slightly stronger than previously reported (Sun *et al.* 2001; Peng *et al.* 2012). The presence of mannan was possibly indicated by the signal at 102.99(A) or 102.65(B) and 74.25(A) or 73.91(B), corresponding to C-1 and C-3 of the mannan residues. The signals at 100.17(A) or 99.85(B) and 61.07 or 60.75(B) were attributed to galactose from the hardwood hemicelluloses. However, from 110 ppm to 160 ppm, four small peaks appeared at 155.43, 130.02, 120.66, and 115.40 ppm in spectrum B that were absent in the spectrum A. The signal at 120.66 was probably assigned to C-6 in guaiacyl lignins. The signal at 130.02 was possibly due to the position of C-1 in the structure of diferulates (5-5'/β-O-4 dehydrodiferulates), whereas no signal appeared at 168.45(C-9) and 57.5(*Ome*) as reported in the literature. The presence of p-coumarate ester was also characterized with the signal at 115.40 ppm that is consistent with earlier research (Sun *et al.* 2005). Based on this result, it may be implied that adding acetic acid could result in the generation of hemicelluloses linked with some structure of lignin known as LCC.



(A)

Fig. 4(A). ^{13}C NMR spectra of precipitates isolated from the hydrolysate obtained with hydrothermal treatment (A) and catalysed by 1%(w/w) acetic acid (B) at 170 °C/35 min



(B)

Fig. 4(B). ^{13}C NMR spectra of precipitates isolated from the hydrolysate obtained with hydrothermal treatment (A) and catalysed by 1%(w/w) acetic acid (B) at 170 °C/35 min

CONCLUSIONS

1. Acetic acid accelerated the hydrolysis of hemicellulose in biomass, but the monosaccharide and oligosaccharide contents were higher without adding acetic acid. An oligosaccharide content of 9.612 g/L was achieved by catalysing with 1% (w/w) acetic acid in a relative short hydrolysis time of 25 min. This was much higher than the result of 3.763 g/L from hydrothermal treatment without acetic acid.
2. The maximum precipitate yield of 4.508 g/L obtained at conditions of 170 °C/35 min/1% (w/w) acetic acid was lower than the yield of 6.002 g/L obtained without adding acetic acid. The precipitate yield of 4.12 g/L was reached at 170 °C/25 min/1% (w/w) acetic acid, which was quite higher than the yield of 1.732 g/L obtained without acetic acid.
3. Acetic acid degraded side chains such as arabinose linked on the xylan main chain, and the dissolution of hemicellulose bonds with lignin (LCC) was increased. A molecular weight of not less than 1900 is suggested when ethanol is used to precipitate oligosaccharides from hydrolysate.

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