



Chemical and thermal properties of precipitates made from hydrolysate of spruce wood chips

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Abstract

Autohydrolysis is a widely used technology for extracting hemicelluloses from wood chips. In addition to hemicelluloses, hydrolysates produced in this process contain lignin. Hemicelluloses and lignin could be isolated from hydrolysates and used for generating value-added products. In this study, hydrolysates were produced via autohydrolysis of spruce wood chips. Then, acidification and ethanol precipitation were employed to isolate lignocelluloses from hydrolysates. Generally, acidification and ethanol treatment led to the precipitation of lignin–carbohydrate complexes, but their removals depended on the hydrolysis conditions. The heat capacity (C_p) of the dried hydrolysates was in the range of 0.41–0.45 J/g °C, while the precipitates of acidification and ethanol treatment had the C_p of 1.18 J/g °C and 1.38 J/g °C, respectively. This increment could be related to their higher lignin content and thus their molecular mobility as these segments had a higher amount of methoxyl groups and more H_α and H_β inter-linkages. The results of this study suggested that the lignocellulosic materials obtained after acid or ethanol treatment of hydrolysates could be considered as additives for heat-resistant biopolymers or hemicellulose-based films, respectively.

Introduction

Lignocellulosic materials have been proposed to be used for manufacturing value-added products or fuel. Lignin can be converted to carbon fibers, phenols, adhesives, flocculants, dispersants and hydrogen, for example (Fatehi et al. 2016; Lora and Glasser 2002; Olivares et al. 1988; Stewart 2008; Vishtal and Kraslawski 2011).

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Hemicelluloses are widely used for alcohol production, while cellulose is vastly used in the pulp and paper industry (Garcia et al. 2001; Pothiraj et al. 2006).

For their use in industry, lignocelluloses need to be isolated from biomass. Autohydrolysis is a cost-effective, efficient, environmentally friendly and widely applied technology for hemicelluloses extraction from biomass (Carvalho et al. 2008; Sixta et al. 2013). The application of autohydrolysis technology mainly leads to the isolation of oligomeric sugars with various degrees of polymerization from biomass (Pu et al. 2013; Sipponen et al. 2014). Along with hemicelluloses, however, this treatment leads to the dissolution of lignin in the hydrolysates generated in this process (Carvalho et al. 2008). In this work, the hydrolysates produced via autohydrolysis of spruce wood chips were considered for further investigation, as spruce species has recently been considered as a wood resource for dissolving pulp production.

The hydrolysates are very dilute in nature, which impairs their direct use in generating value-added materials. Acidification and solvent extraction have been reported to be efficient methods for lignocellulose isolation from prehydrolysis liquor (PHL), black liquor of kraft pulping process, and spent liquor (SL) of neutral sulfite semi-chemical (NSSC) process (Liu et al. 2011a, b; Shi et al. 2011; Tarasov et al. 2015). However, the isolation process influences the properties of extracted materials (Liu et al. 2011a; Tarasov et al. 2017).

The key parameters affecting the industrial application of the extracted materials are molecular weight (MW), charge density, ash content, thermal stability, glass transition temperature (T_g) and heat capacity (C_p) (Aro and Fatehi 2017; Olsson and Salmen 1997; Tarasov et al. 2017). Lignocelluloses with a high or low MW can be applied for the production of flocculants or dispersants, respectively (Aro and Fatehi 2017). The possibilities for lignocellulose utilization as fuel or fuel additives are directly affected by the thermal stability (T_g and C_p) of lignocelluloses (Stelte et al. 2012; Granada et al. 2002; Lumadue et al. 2012). The first objective of this work was to assess the thermal properties of precipitates produced via acidification or ethanol treatment of hydrolysates in order to identify a suitable potential application for the extracted materials.

It was reported that the amounts of phenolic, aliphatic, carboxylic acid and methoxyl groups influence the properties of lignocellulosic materials. Phenolate hydroxyl groups improve lignin's hydrophobicity and structural flexibility (Li et al. 2016; Olsson and Salmen 1997). Methoxyl groups impede the covalent cross-linking in lignin and hence the mobility of lignin moieties (Olsson and Salmen 1997). This leads to a lower glass transition temperature (Olsson and Salmen 1997) and a higher heat capacity of lignin (Li and McDonald 2014). To recognize a feasible application for the precipitates, the functional groups of the generated precipitates were evaluated as the second objective of this research.

Lignin–carbohydrate complexes (LCCs) are composed of chemically bound lignin and carbohydrate moieties (Uraki et al. 2006). The evidence of lignin–carbohydrate complexes (LCC) in native biomass of coniferous, deciduous and non-wood plants was provided in some studies (Dammstrom et al. 2009; Lawoko et al. 2005; Yao et al. 2016; You et al. 2015; Zhang et al. 2016). The presence of LCC was also reported in the black liquor of kraft pulping process (Lawoko et al. 2004, 2005; Tamminen et al. 1995).

Fatehi et al. (2016) confirmed the presence of LCC in hardwood-based PHL and SL of NSSC process. In a previous paper, the existence of LCC in softwood-based hydrolysates was discussed (Tarasov et al. 2018). However, Uraki et al. (2006) suggested the self-aggregation of LCC in water due to the hydrophobic interactions of lignin part of LCC. It was also reported that LCC linkages are decomposed under acidic conditions (Kosikova et al. 1979; Zoia et al. 2008). The third objective of this study was to investigate the presence of LCC in the precipitates generated via acidification and ethanol treatment of hydrolysates.

In the present study, the properties of lignocelluloses precipitated from softwood-based hydrolysates were analyzed. The main purpose of this research was to evaluate the properties of these precipitates for the first time to identify potential applications for them. In addition, the compositions and functional groups of LCC were determined.

Materials and methods

Materials

Ethanol (C₂H₅OH, 95 vol%), sulfuric acid (H₂SO₄, 98 wt%) and sodium nitrate (NaNO₃, 99.9% purity) were purchased from Fisher Scientific and used without purification. Dimethyl sulfoxide-d₆ (DMSO, 99.9% purity), 3-(trimethylsilyl) propionic-2,2,3,3-d₄ acid sodium salt (TSP) for nuclear magnetic resonance (NMR) analysis, xylose, arabinose, rhamnose, glucose, galactose and mannose (all analytical grades) were purchased from Sigma-Aldrich and used for ion chromatography analysis. Spruce wood chips were received from a pulp mill in northern Ontario, Canada, and used without any pretreatment.

Hydrolysate production

At first, hydrolysates were produced via flow through autohydrolysis of spruce wood chips under the conditions stated in Table 1. In this study, 300 ± 5 g of wood chips was placed in a 2-L pulping digester (Greenwood, TX) along with a certain amount of distilled water. The heating rates were set at 4.5 °C/min and 2.5 °C/min when the temperature in the vessel of the digester was below and above 100 °C, respectively. The residence time of the hydrolysis treatment was adjusted when the temperature in the vessel achieved the set-point value. The flow rate of liquid circulation was set at 6 L/min in all autohydrolysis reactions. Afterward, hydrolysates were collected and stored at 4 °C. The impacts of processing temperature and residence time on the lignocellulose removals in the autohydrolysis can be considered by a severity factor (S_0) (Overend and Chor-net 1987) via following Eq. (1) (Pedersen and Meyer 2010):

$$S_0 = \log \left(t \times e^{\frac{T-100}{14.75}} \right) \quad (1)$$

where T is the temperature (°C); t is the residence time (min.) of hydrolysis; and 14.75 is the arbitrary constant related to the activation energy and temperature

Table 1 The properties of hydrolysis liquors (Tarasov et al. 2018)

Sample ID	Autohydrolysis conditions				Lignocelluloses composition in hydrolysates (wt% based on wood)				
	Temperature, °C	Residence time, min	L/S ratio ^a	S_0^b	Lignin	Total sugars	Mono-sugars	Acetic acid	Furfural
1	180	15	5	3.5	6.8	5.0	1.3	0.4	N/A
2	180	45	5	4.0	7.1	5.3	1.6	0.4	N/A
3	190	15	5	3.8	10.5	4.5	2.2	1.0	1.6
4	180	15	10	3.5	9.7	5.1	1.3	0.3	N/A

^aLiquid-to-solid ratio^bSeverity factor

(Carvalho et al. 2009; Pedersen and Meyer 2010). The compositions of lignocellulosic material in the hydrolysates are presented in Table 1. The concentrations of sugars in samples 1, 2, 3 and 4 were estimated as 8.9, 9.5, 7.1 and 6.1 g/L, respectively. The concentrations of monosugars in the hydrolysates were 2.4, 2.9, 3.4 and 1.5 g/L in samples 1, 2, 3 and 4, respectively.

Extraction processes

Three sets of experiments were conducted for extracting lignocelluloses from hydrolysates. Figure 1 shows the process of precipitate production. In one set of experiments, the pH of hydrolysates was decreased to 1.5 via using 20 wt% sulfuric acid (Liu et al. 2011a; Shi et al. 2011). Then, acidified hydrolysates were centrifuged at 4000 rpm for 5 min. After centrifugation, the precipitates were collected and placed in an oven at 60 °C for 48 h. In another set of experiments, ethanol was added to hydrolysates at the ratio of 4/1 wt/wt (Liu et al. 2011a). Afterward, hydrolysate/ethanol mixtures were centrifuged, and the precipitates were dried. In another set of experiments, the hydrolysates (25 ± 2 g) were placed in an oven at 60 °C for 48 h. The dried materials were used in subsequent experiments.

The precipitates generated via acidification of samples 1, 2, 3 and 4 (Table 1) were labeled as samples 1-A, 2-A, 3-A and 4-A, respectively. The precipitates produced via ethanol treatment of samples 1, 2, 3 and 4 (Table 1) were denoted as samples 1-E, 2-E, 3-E and 4-E, respectively. The materials obtained via evaporation of samples 1, 2, 3 and 4 were labeled samples 1-D, 2-D, 3-D and 4-D, respectively.

Analysis of lignin and hemicelluloses in the precipitates

For lignin and hemicellulose analysis, dried precipitates were mixed with deionized water and placed in a water bath at 30 °C and 50 rpm for 12 h. Lignin content of these mixtures was evaluated using a UV spectrophotometry (Genesys 10S UV–Vis, Thermo Scientific) at 205 nm in accordance with TAPPI UM 250 (Liu et al. 2011a).

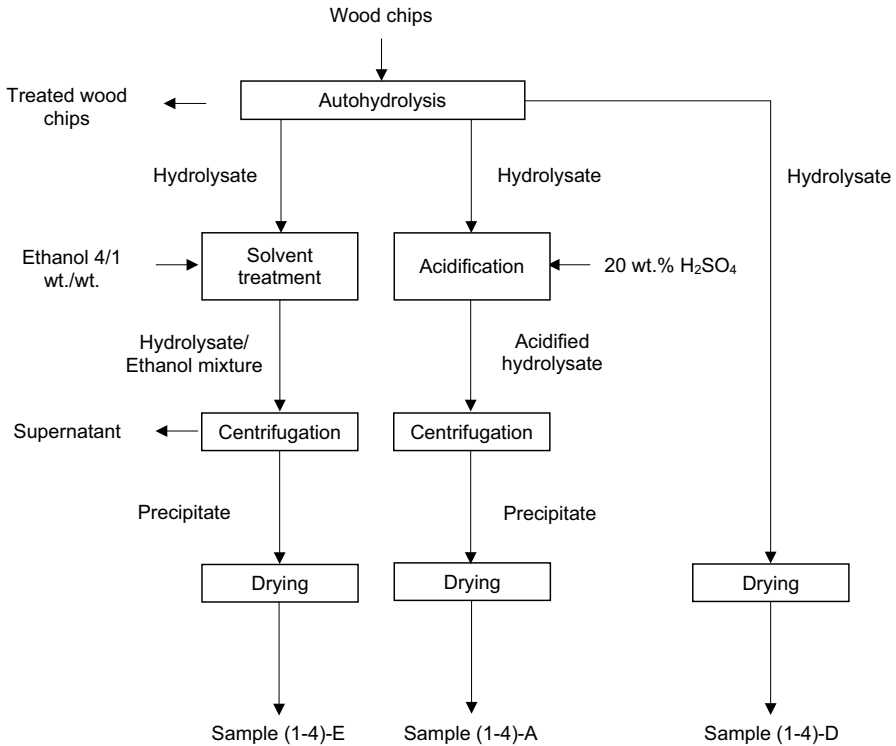


Fig. 1 Procedure for generating precipitates from hydrolysates

Ion chromatography was used for analyzing hemicellulose content of the mixtures. In one set of experiments, 5 mL of the mixtures was used for determining the monosaccharide content of the samples by an ion chromatography (Dionex, ICS 5000, ThermoFisher Scientific), which was equipped with CarboPac™ SA10 column and an electrochemical detector (ED) (Dionex-300, Dionex Corporation, Canada). Ion chromatography was unable to determine polysaccharide concentration. Therefore, the mixtures were treated with 4 wt% sulfuric acid and placed in a reactor at 121 °C for 1 h to convert polysaccharides to monosaccharides (Liu 2008). Then, the concentration of monosaccharides in the mixture was determined by ion chromatography as stated earlier. As all polysaccharides were converted to monosaccharides after this acidulation process, this analysis showed the total sugars of the samples. Lignin and hemicellulose concentrations in the samples were considered for determining the amount of lignin and hemicelluloses in the precipitates as a fraction of that in the dried mass of wood chips.

Molecular weight determination

The precipitates were diluted in 0.1 mol/L NaNO₃ to obtain an approximate concentration of 5 g/L and placed in the water bath at 30 °C and 50 rpm overnight. The

analysis was conducted after turbulent shaking and ultrasonication prior to this test in order to eliminate the possibilities for the physical agglomeration of lignin and carbohydrates in hydrolysates. For molecular weight estimation, the gel permeation chromatography (Malvern GPCmax VE2001 Module+Viscotek TDA305) equipped with multi-detectors and PolyAnalytic PAA206 and PAA203 columns was used. In this experiment, 0.1 mol/L NaNO₃ was used as solvent and eluent. The column temperature was adjusted at 35 °C, and the flow rate in GPC was adjusted at 0.70 mL/min. The molecular weight analysis of lignin was determined using a UV detector (at 280 nm wavelength) and that of hemicelluloses was determined using reflective index (RI) and intrinsic-differential pressure (IV-DP) detectors for determining the molecular weight of LCC (Fatehi et al. 2016).

Thermal properties analysis

The thermal stability of the samples was investigated via a thermogravimetric analysis (TGA), Instrument Specialist, i1000. In TGA analysis, the precipitates were heated from 25 to 700 °C with a 10 °C/min heating rate. Experiments were performed in a nitrogen atmosphere at a 35 mL/min flow rate.

DSC analysis

Glass transition temperature (T_g) and heat capacity (C_p) values of the samples were evaluated by a differential scanning calorimetry (DSC, TA Instruments Q2000). Oven-dried samples (2–5 mg) were placed in DSC pans, and the samples were treated in the temperature range of 0 °C and 200 °C with a heating rate of 3 °C/min (Persson et al. 2012). The samples were heated to 200 °C, then cooled down to 0 °C temperature and reheated again to 200 °C. In the second heating cycle, the T_g and heat capacity of the samples were analyzed (Sammons et al. 2013).

NMR experiments

For ¹H-NMR spectroscopy experiments, the Varian Unity Inova 500 MHz NMR instrument was employed. The samples were prepared in accordance with the procedure described by Nagy et al. (2010). Approximately, 25 mg of the dried samples was dissolved in 0.5 mL of deuterated DMSO-*d*₆. Then, 0.5 wt% of TSP was added to the samples and served as an internal standard. Hydroxyl groups were identified by signals in assigned areas of ¹H-NMR spectrum (Nagy et al. 2010; Li and Lundquist 1994).

Results and discussion

Lignin and hemicellulose extraction

The total solid contents of hydrolysates were 13.5, 16.7, 17.9 and 16.1 wt% for samples 1, 2, 3 and 4, respectively. The higher solid content of samples 2 and 3 is

attributed to the extraction of more lignin from wood chips in the hydrolysates at elevated temperatures and longer residence times (Table 1) (Tarasov et al. 2018). The increases in the autohydrolysis temperature or residence time extension escalate the concentration of acetic acid in the hydrolysates, which leads to a higher acidity and thus elevation in the lignin and hemicellulose extraction (Leppanen et al. 2011; Song et al. 2008).

Figure 2 presents the lignin content of the precipitates. It can be seen that lignin comprises 55 wt% of the solid content of hydrolysates in all samples. Acidification changed the lignin content of the precipitates to 64, 79, 88 and 55 wt% for samples 1, 2, 3 and 4, respectively. The acidification seemed to be ineffective in increasing the lignin content of sample 4. As explained earlier, sample 4 was produced at a high *L/S* ratio of 10 (Table 1). It is possible that the produced lignin was more acid soluble (Tunc 2014). Samples produced from mixing hydrolysates and ethanol had lower lignin content than original hydrolysates.

Figure 3 depicts the total sugar content of the samples. It is seen that hemicelluloses are comprised of 20–25 wt% of dried hydrolysates. Acidification of samples 2 and 3 led to the precipitation of materials with a relatively lower concentration of hemicelluloses compared with dried hydrolysates. It is well known that hemicelluloses are acid soluble, and thus, acidification was ineffective in isolating hemicelluloses. The precipitates obtained via acidification of sample 1 had higher hemicelluloses than did dried hydrolysates. The extraction of hemicelluloses could be related to the extraction of LCC from hydrolysates (Fatehi et al. 2016; Tarasov et al. 2018). The summation of lignin and hemicelluloses after acidification (Figs. 2, 3) seemed to be slightly higher than 100%, which is due to experimental errors.

As expected, precipitates produced with ethanol treatment were richer than other samples in hemicelluloses. The precipitates generated via mixing ethanol with samples 1, 2, 3 and 4 had 42.4, 27.9, 33.7 and 41.8 wt% of hemicelluloses, respectively. The variations in hemicelluloses could be related to the difference in the molecular

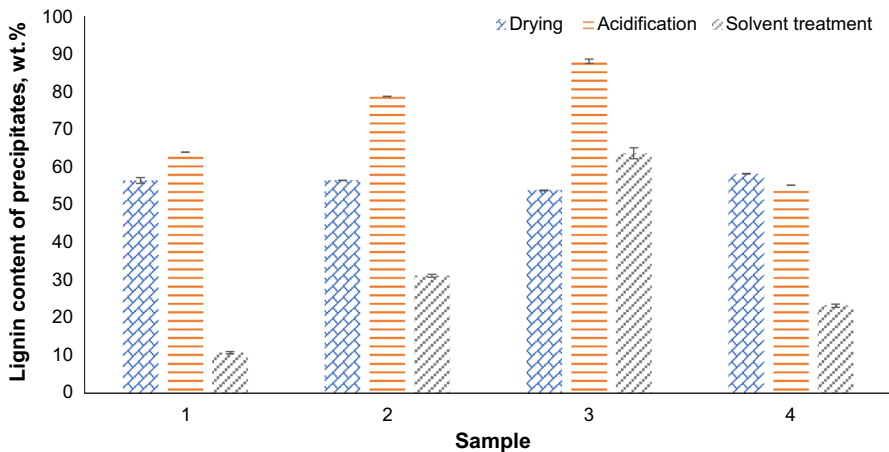


Fig. 2 Lignin content of the precipitates produced via acidification, ethanol treatment or drying

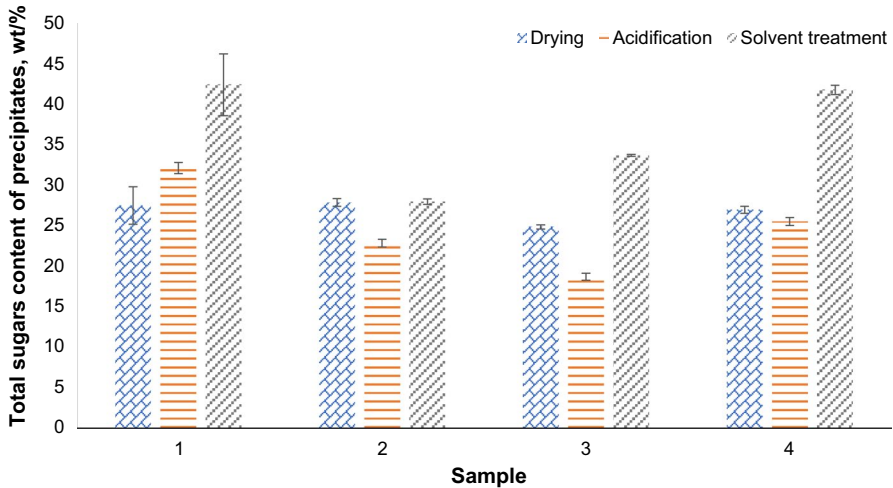


Fig. 3 Total sugar content of the precipitates produced via acidification, ethanol treatment or drying

weight of hemicelluloses in the hydrolysates and their tendency to isolate when treated with ethanol. Recent findings showed that the ethanol treatment of hydrolysates led to the precipitation of hemicelluloses with a high MW (Tarasov et al. 2018).

It is seen in Figs. 2 and 3 that the summation of lignin and sugars in the materials obtained via solvent extraction for samples 1, 2, 3 and 4 was 53, 59, 97 and 65 wt% of the extracted materials, respectively. In a previous study, it was observed that samples 1, 2 and 4 contained a high amount of relatively high molecular weight lignocelluloses, whereas sample 3 was composed of relatively smaller ones (Tarasov et al. 2018). Due to their different molecular weights, the precipitates made of solvent extraction were partially soluble in water after collection and drying. The remaining proportional weights of these samples are most likely the insoluble parts of the precipitates. Du et al. (2013) reported that glucan–lignin fraction of LCC demonstrated a low solubility in comparison with xylan–lignin material.

Table 2 lists the compositions of hemicelluloses in the precipitates. The presence of galactoglucomannan (GGM) and arabinoglucoroxylan (ArXyl) was reported in softwood species (Lawoko et al. 2005; Uraki et al. 2006). Lawoko et al. (2005) also suggested that lignin in softwoods existed in two different structures. One type was surrounded by xylan (Xyl) and the other by glucomannan (GM). These lignin types are bound to carbohydrates, forming four different LCCs, i.e., GGM–lignin, Glu–lignin, GM–lignin–Xyl and Xyl–lignin–GM, which contain 8, 4, 40 and 48% of total lignin in spruce wood species, respectively (Lawoko et al. 2005).

The precipitates generated by acidification of samples 1 and 4 possessed slightly higher glucose, galactose and mannose, as well as arabinose and xylose. Thus, it may be suggested that these precipitates possessed higher GGM and ArXyl compared with dried hydrolysates. This increment could be attributed to hemicellulose precipitation along with lignin in the form of GGM–lignin and ArXyl–lignin

Table 2 Hemicellulose compositions of precipitates

Sample ID	Arabinose	Galactose	Rhaminose	Glucose	Xylose	Mannose	Total
Total sugars/monosugar concentration in precipitates (wt%/wt%)							
1-D	2.69/1.89	5.71/1.56	0.29/0.21	3.67/0.17	3.91/1.69	11.21/0.65	27.48/6.17
2-D	1.15/1.01	6.14/2.14	0.23/0.20	4.83/0.46	3.15/2.20	12.33/1.70	27.85/7.71
3-D	0.76/0.67	5.48/2.68	0.17/0.16	4.72/0.87	2.44/2.27	11.28/2.84	24.85/9.49
4-D	1.57/1.46	6.50/1.56	0.27/0.18	4.06/0.20	3.49/1.58	11.06/0.78	26.95/5.76
Compositions of total/mono saccharides in precipitates obtained via acidification (wt%/wt%)							
1-A	2.26/0.29	6.94/0.42	0.34/0.01	4.56/0.16	4.63/0.69	13.38/0.66	32.11/2.22
2-A	0.83/0.09	4.41/0.29	0.12/0.01	4.00/0.29	2.72/0.42	10.78/0.94	22.86/2.05
3-A	0.51/0.04	4.15/0.16	0.08/ND	3.50/0.19	1.78/0.17	8.67/0.41	18.70/0.98
4-A	1.67/0.13	5.97/0.24	0.25/ND	3.70/0.19	3.41/0.34	10.51/0.54	25.50/1.45
Compositions of total/mono saccharides in precipitates obtained via ethanol treatment (wt%/wt%)							
1-E	0.18/0.12	12.22/0.08	0.10/ND	8.16/0.08	1.99/0.02	19.78/ND	42.42/0.31
2-E	0.56/0.55	6.54/0.97	0.10/0.09	5.28/0.21	1.79/0.94	13.69/0.87	27.95/3.63
3-E	1.00/0.93	6.38/1.95	0.21/0.15	6.26/0.50	3.06/1.59	16.78/1.90	33.68/7.02
4-E	0.18/0.10	11.14/0.08	0.05/ND	7.76/0.01	1.66/0.05	21.00/0.06	41.78/0.30

complexes. Precipitates of samples 2 and 3 obtained after the acidification had slightly lower amounts of sugars than dried hydrolysates. This may be related to the precipitation of carbohydrate-free lignin under strong acidification conditions.

The precipitates generated via mixing samples 1 and 4 with ethanol appeared to have lower lignin (Fig. 2), arabinose and xylan. They also had higher glucose, galactose and mannose. Therefore, it may be claimed that these samples had less ArXyl content, but more GGM than dried hydrolysates. Therefore, it is possible that the ethanol treatment of samples 1 and 4 led to the precipitation of material with high amounts of GGM–lignin and Glu–lignin complexes. The ethanol treatment of samples 2 and 3 slightly increased the relative content of GGM and ArXyl in comparison with that of dried samples.

Among the samples reported in Table 2, four samples were selected for further analysis. Sample 1-E showed a relatively higher hemicellulose content, and sample 3-A had the highest lignin content (Fig. 2). For evaluation of the changes caused by the acid or ethanol supplement, samples 1-D and 3-D were also analyzed as control samples.

Molecular weight analysis

Table 3 lists the molecular weights of lignin and hemicelluloses in the selected precipitates. These materials contained both high MW and low MW lignin. Figure 4 presents the response of the UV, RI and IV-DP detectors of GPC for molecular weight analysis of selected precipitates. Low MW lignin and hemicelluloses show similar retention times in the GPC analysis. Similar retention times of UV, RI and IV-DP responses provide evidence of LCC in the precipitates (Fatehi et al. 2016;

Tunc et al. 2010). However, no RI and IV-DP signals were overlapped indicating that the high MW lignin was probably carbohydrate free (Tunc et al. 2010).

It is seen in Table 3 that sample 1-D does not contain carbohydrate-free lignin, while sample 3-D had carbohydrate-free lignin with the MW of 30,000 g/mol. Further, sample 1-D had only a low MW lignin, whereas sample 1-E had both high MW and low MW lignin. These changes could be related to the precipitation of lignin and LCC by ethanol treatment due to the lower solubility of lignin in ethanol–water mixtures (Wang and Chen 2013).

Overall, it was shown that the molecular weight of LCC present in the precipitates was between 1000 and 3600 g/mol (Table 3). Fatehi et al. (2016) reported that the PHL of kraft-based dissolving pulp production process and the SL of NSSC process contained LCC with the Mw of 2000 g/mol and 1500 g/mol, respectively. It is also noted that the Mw of LCC in samples 1-E and 3-A was significantly larger than Mw of LCC in samples 1-D and 3-D. It was reported in the literature that the addition of ethanol could facilitate the precipitation of LCC (Nonaka et al. 2013; Sun et al. 2013). Acidification also likely led to the isolation of large LCC and hemicellulose.

Thermal properties of the precipitates

The thermal stability of lignocelluloses depends on several factors, such as compositions, inter-unit structures and functional groups (Chen et al. 2016; Tanger et al. 2013). Figure 5 depicts the weight loss and the weight loss rate of the precipitates. Samples 1-D and 3-D show similar behavior with the degradation onset temperature (T_{onset}) of 200 °C and the weight loss of 37% and 40%, respectively, in the 200–300 °C temperature region. In the 300–630 °C region, the weight reduction of 30% and 27% was noted for samples 1-D and 3-D, respectively. These regions probably represent hemicelluloses and lignin decomposition (Carrier et al. 2011). Singh et al. (2005) observed similar trends during the pyrolysis of commercial softwood LCC.

Samples 1-D and 3-D show the peak temperature (T_p) around 230 °C with weight loss rates of 0.45%/°C and 0.61%/°C, respectively (Fig. 5b). A possible reason for the higher mass loss in sample 3 can be related to the degradation of β -O-4 inter-linkages and possible depolymerization of lignin structure (Chen et al. 2016; Long et al. 2014). Li et al. (2007) reported the decrease in β -O-4 substructures in lignin samples with the increment in the severity factor of steam

Table 3 Molecular weight of lignin and hemicelluloses in the precipitates

Sample ID	Weight average MW (g/mol)		
	High MW lignin	Low MW lignin	Hemicelluloses
1-D	ND	1405 ± 130	2003 ± 90
3-D	33,320 ± 3770	1004 ± 55	866 ± 110
1-E	34,825 ± 7700	3619 ± 317	4556 ± 246
3-A	22,839 ± 1670	2319 ± 89	3557 ± 800

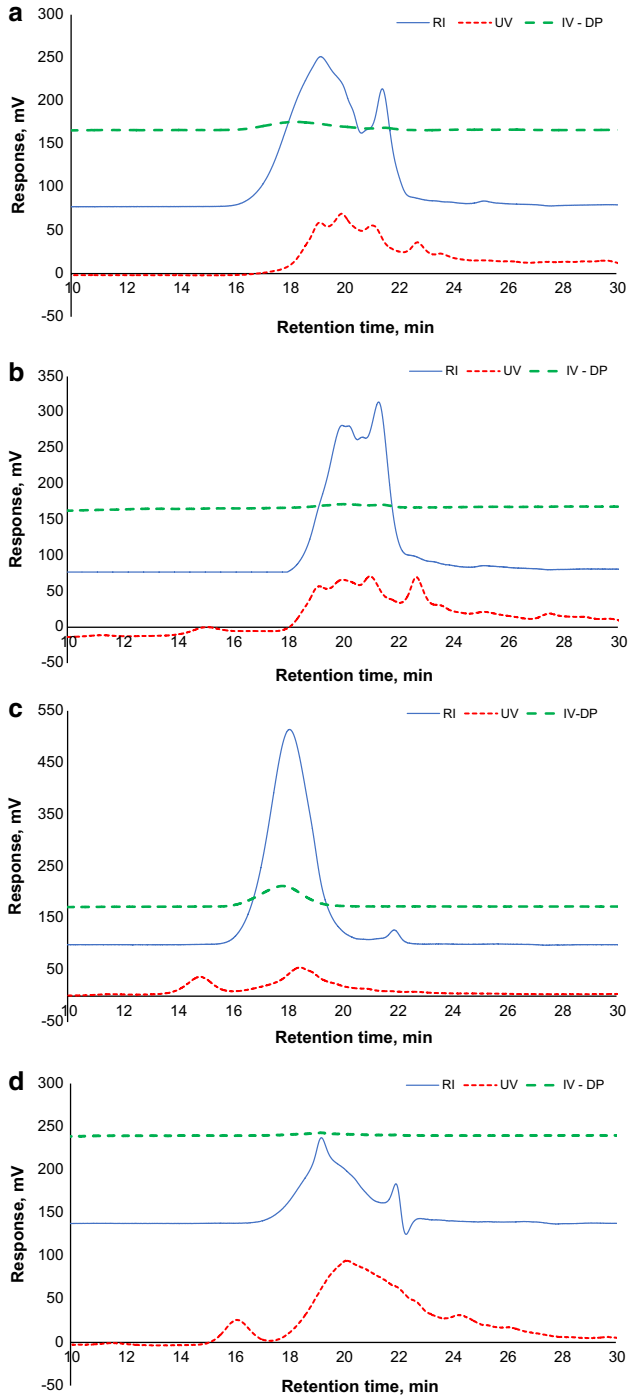


Fig. 4 RI, UV and IV-DP detectors data as a function of retention time for samples **a** 1-D, **b** 3-D, **c** 1-E and **d** 3-A

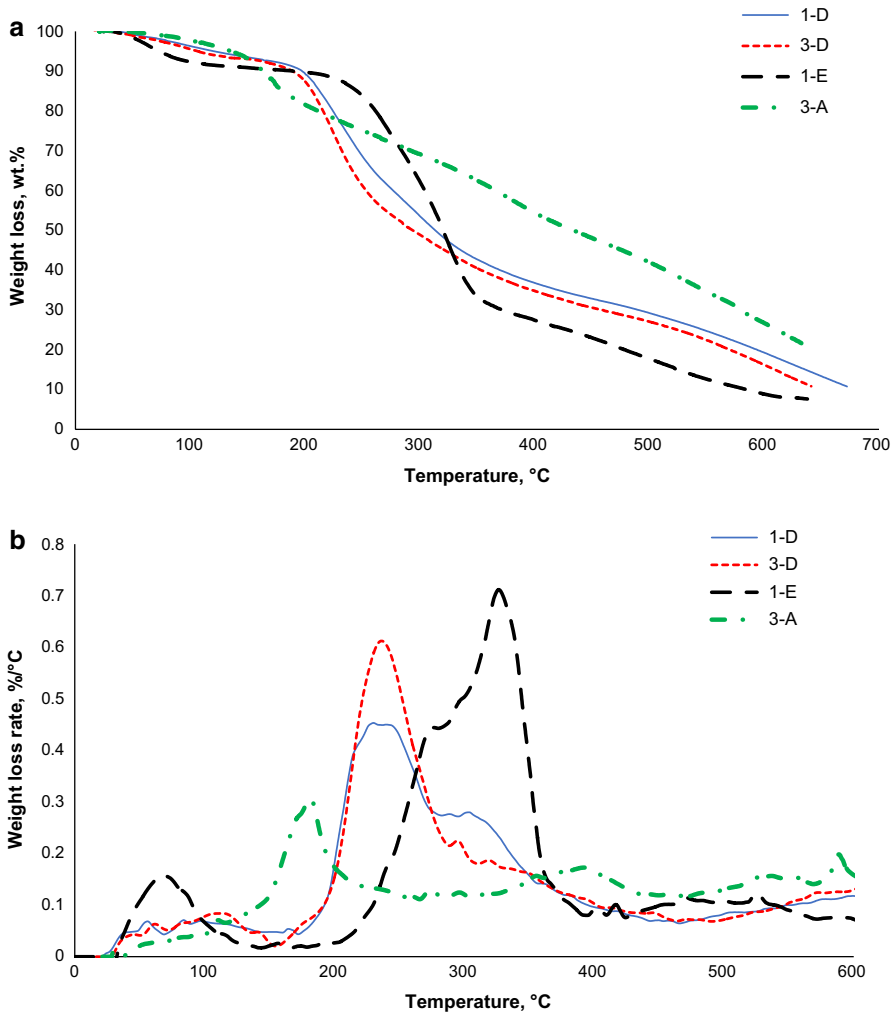


Fig. 5 Weight loss (a) and weight loss rate (b) of the precipitates

hydrolysis from 3.2 to 4.1. It can be seen in Table 1 that samples 1 and 3 were generated at the S_0 of 3.53 and 3.82, respectively.

The thermal stabilities of materials obtained after ethanol or acid supplement are significantly different (Fig. 5a). Sample 1-E shows two shoulders at 230–350 °C and 350–630 °C regions with 54 wt% and 22 wt% weight losses, respectively. The increment in the weight loss at lower temperatures could be related to the higher sugar content of sample 1-E. Sample 3-A demonstrates one degradation region at 160–630 °C. Khazraie et al. (2017) reported a similar thermal behavior of precipitates obtained from hydrolysates via acidification. It was

proposed that the presence of sulfuric acid in precipitates intensified the decomposition of lignin in pyrolysis (Zhou et al. 2013).

Sample 1-E shows the degradation rate of 0.7%/°C at T_p (Fig. 5b). Additionally, this precipitate demonstrates a shoulder in the temperature range between 275 and 300 °C with a 0.45%/°C weight loss. Considering the high sugar content of this sample (42.4 wt%) and the fact that T_p of sugar decomposition was reported to be at 290 °C (Gasparovic et al. 2012), it is possible to assume that this region is related to hemicelluloses degradation.

As seen in Fig. 5b, the samples 1-E and 3-A show T_p of 325 °C and 175 °C, respectively. The shift of T_p is most likely attributed to the structure and compositions of samples 1-E and 3-A. The reduction in T_p in case of sample 3-A could be attributed to its higher lignin content (Fig. 2) and its smaller molecular weight (Table 3) (Tong et al. 2017). Another reason for a lower T_p of sample 3-A may be the enhanced decomposition of lignin due to the existence of sulfuric acid moieties as stated above (Zhou et al. 2013).

DSC analysis

Molecular weight, molecular mobility and cross-linking degree of lignin affect the glass transition temperature of lignocellulosic materials (Hatakeyama and Hatakeyama 2010; Guigo et al. 2009; Li and McDonald 2014; Olsson and Salmen 1997). It was reported that T_g of lignin increased with its MW and cross-linking degree (Guigo et al. 2009; Olsson and Salmen 1997). Hydrothermal treatment leads to the depolymerization of lignocelluloses reducing their MW (Chua and Wayman 1979; Li et al. 2007) and T_g (Kong et al. 2017). In addition, the hydrothermal treatment causes water assimilation on the polar sites of the lignin structure. This results in the modification of inter-molecular hydrogen bonds, which increases the mobility of lignin and decreases the T_g of lignin (Guigo et al. 2009). It was reported that T_g of hemicelluloses, lignin and cellulose isolated from wood in aqueous conditions was 40 °C, 50–100 °C and 100 °C, respectively (Kong et al. 2017; Olsson and Salmen 1997). Table 4 lists the T_g and the heat capacity (C_p) values of the examined materials (the heat flows of the precipitates are provided in the Supplementary Material in Figure S1).

Samples 1-D and 3-D had approximately equal glass transition temperatures of 100 °C implying a limited impact of hydrolysis treatment on the T_g of dried hydrolysates. Olsson and Salmen (1997) estimated the softening temperature of wet softwood at 90 °C, which could be considered as T_g of lignin, as the T_g of wood occurs in the same temperature range as lignin (Kong et al. 2017; Olsson and Salmen 1997). Youssefian and Rahbar (2015) reported that the T_g of LCC, which was extracted from bamboo, was 166 °C. The lower T_g value obtained in the current study could be related to lignin plasticization by water (Irvine 1985), which resulted in higher molecular mobility of lignin units and reduction in T_g (Guigo et al. 2009).

The T_g of precipitates obtained via acidification and ethanol treatment was slightly lower than T_g of dried materials, whereas the C_p values of samples 1-E and 3-A were significantly higher. This could be explained by the increment in the

Table 4 Glass transition temperature (T_g) and heat capacity (C_p) of the precipitates

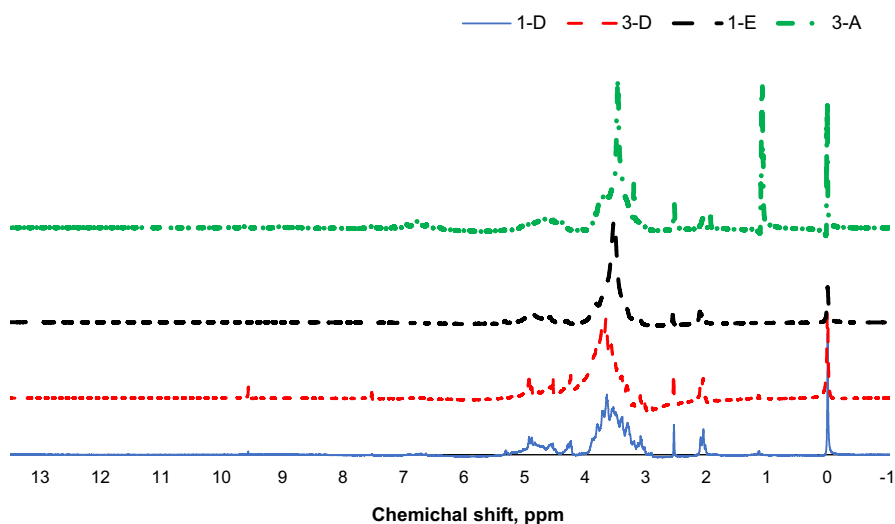
Sample ID	T_g (°C)	C_p (J/g °C)
1-D	101.9	0.45
3-D	98.9	0.41
1-E	87.5	1.38
3-A	85.8	1.18

lignin's molecular mobility in samples 1-E and 3-A (Li and McDonald 2014) due to a greater methoxyl group content (Olsson and Salmen 1997) or cross-linkage density decrease (Hatakeyama and Hatakeyama 2010). These assumptions were confirmed by $^1\text{H-NMR}$ spectroscopy presented later in this study.

Functional group analysis

The $^1\text{H-NMR}$ spectra of examined samples are presented in Fig. 6. Generally, $^1\text{H-NMR}$ spectrum was partitioned into three main sections (Mainka et al. 2015). Signals in the regions of 8.00–6.00, 6.00–4.05 and 2.25–0.00 ppm are related to aromatic groups, side chain and aliphatic groups, respectively (Nagy et al. 2010).

Samples 1-D, 3-D and 3-A show signals at 6.7 ppm, which is associated with guaiacyl units of lignin (Foston et al. 2015). The absence of this signal in the spectrum of sample 1-E could be related to its relatively low lignin content (Fig. 2) (Du et al. 2014). The presence of conjugated carbonyl group was indicated by signals at 9.57 ppm (Tong et al. 2017). The higher intensity of this signal in samples 3-D and 3-A could be related to the degradation of quinoids in lignin due to the high

**Fig. 6** $^1\text{H-NMR}$ spectra of the precipitates

temperature and/or acidification, which led to the formation of carbonyl functional groups (Tong et al. 2017). The quantitative analysis of the functional groups present in the precipitates based on $^1\text{H-NMR}$ spectroscopy is shown in Table 5.

It is seen in Table 5 that only a trace amount of formyl groups was found in the precipitates, and no carboxylic acid was present. Sample 3-A had slightly more phenolic groups compared with other precipitates. Acidification resulted in the cleavage of aryl–ether bonds, which led to the formation of phenolic groups (Cateto et al. 2008; Santos et al. 2013). The highest amount of methoxyl groups was found in sample 1-E. Samples 1-E and 3-A contained higher amount of aliphatic groups compared to samples 1-D and 3-D. This increment could be related to the liberation of α -hydroxyl groups due to the lignin–carbohydrate bond disintegration (Sun et al. 2014). The decomposition of LCC linkages in sample 3-A is related to acidification (Kosikova et al. 1979; Zoia et al. 2008). It was reported that the presence of organic solvents promotes the cleavage of α -ether linkages in lignin (Azadi et al. 2013). Ether and ester linkages in LCC are generated at the α -carbon position in phenyl propane units (Saggi et al. 2016). Therefore, it is possible to assume that ethanol supplement causes the partial degradation of lignin–carbohydrate linkages presented in sample 1-D, which results in the increment of aliphatic groups in sample 1-E. In addition, the increase in the aliphatic groups in sample 1-E possibly relates to a relatively high presence of carbohydrates (Menezes et al. 2016). It is shown in Table 5 that the amounts of H_α and H_β inter-units in samples 1-E and 3-A were significantly lower than that of samples 1-D and 3-D. This behavior may be attributed to the partial decomposition of lignin inter-unit and lignin–carbohydrate linkages in sample 3-A due to the acidification (Kosikova et al. 1979; Leschinsky et al. 2008; Monot et al. 2017; Zoia et al. 2008). Lower amounts of inter-units in sample 1-E could be related to its relatively low (10 wt%) lignin content. The deficiency of aromatic and vinyl groups in sample 1-E (Table 5; Fig. 6) supports this assumption. Furthermore, the decomposition of α -ether linkages in lignin due to the presence of ethanol (Azadi et al. 2013) could cause the reduction in α and H_β inter-units content in sample 1-E.

Impact of acidification on the precipitate properties

Acidification of hydrolysate resulted in precipitation of LCC material (Fig. 4d) with the prevalence of lignin (Fig. 2). The obtained material demonstrated a higher heat capacity than original lignocelluloses presented in untreated hydrolysate (Table 4). This increment could be explained by the presence of a higher amount of methoxyl groups (Olsson and Salmen 1997) in sample 3-A (Table 5). In addition, it is possible to assume that acidification led to precipitation of more hydrophobic material due to the higher presence of phenolic groups in sample 3-A than sample 3-D (Table 5). It was reported that application of lignin as an additive to polylactic acid bioplastic composite enhanced thermal stability, strength and hydrophobicity of this biopolymer (Gordobil et al. 2015; Leschinsky et al. 2008; Spiridon et al. 2015). Consequently, inclusion of sample 3-A material may improve hydrophobicity and thermal stability of the biopolymers in composites.

Table 5 Functional groups in the precipitates based on the ¹H-NMR spectroscopy

Sample ID	Lignin's functional groups (ppm) (mmol/g)							
	Carboxylic acid (13.5–10.5)	Formyl groups (10.1–9.35)	Phenolic groups (9.35–8.0)	Aromatic and vinyl (8.0–6.0)	H _α and H _β inter-unit link- ages (6.0–4.05)	Methoxyl groups (4.05–3.45)	Aliphatic groups (2.25–0.00)	
1-D	ND	0.05	0.22	0.71	4.95	9.43	0.21	
3-D	ND	0.02	0.24	0.62	5.32	10.44	0.44	
1-E	ND	ND	ND	ND	1.54	17.95	0.68	
3-A	ND	0.02	0.47	0.59	0.96	12.90	2.55	

Impact of ethanol treatment on precipitate properties

The precipitates generated via ethanol supplement contained LCCs (Fig. 4c) with the abundance of hemicelluloses (Fig. 3). The increment in the heat capacity (Table 4) is most probably related to a high amount of methoxyl groups present in sample 1-E (Table 5). In the past, hemicellulose-based films were modified (Gao et al. 2018) or mixed with various additives, such as potassium acetate (Bahcegul et al. 2014) and phyllosilicate (Gröndahl et al. 2013) to improve their thermal stability, mechanical properties and moisture resistance of films. Therefore, an application for these precipitates could be in the production of heat-resistant hemicellulose-based films, for instance.

Conclusion

Generally, acidification resulted in the precipitation of materials with the highest lignin content, whereas precipitates generated by ethanol supplement showed the highest concentration of hemicelluloses, but the presence of hemicelluloses and lignin was observed in all precipitates implying the removal of LCC compounds from hydrolysates, which was confirmed by GPC analysis. The TG/DTG analysis suggested the partial decomposition of lignin–carbohydrate linkages in the precipitates generated by acid or ethanol supplement. The $^1\text{H-NMR}$ analysis revealed that sample 3-A contained significantly higher aliphatic groups than did sample 3-D, which is possibly related to the degradation of LCC linkages. The precipitates formed after acid supplement also demonstrated an increment in phenolic groups amount, which may positively affect the hydrophobicity of the material. The reduction of H_α and H_β lignin inter-linkages was also noted in samples 1-E and 3-A. Acidification and ethanol supplement increased the molecular mobility of lignin units in precipitates due to their higher content of methoxyl groups. Additionally, acidification and ethanol supplement increased the heat capacity of the precipitates from 0.41 to 1.18 J/g °C and from 0.45 to 1.38 J/g °C, respectively. Due to the improved heat capacity, the precipitates made via acidification could be considered as an additive in composite production. A possible application of materials generated via ethanol supplement of hydrolysate can be in hemicellulose-based film manufacturing for heat-resistant quality enhancement.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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