



# An improved method for the hydrolysis of hardwood carbohydrates to monomers

Samar K. Bose<sup>a</sup>, Vincent A. Barber<sup>b</sup>, Ericka F. Alves<sup>a</sup>, David J. Kiemle<sup>b</sup>, Arthur J. Stipanovic<sup>b</sup>, Raymond C. Francis<sup>a,\*</sup>

<sup>a</sup> Department of Paper and Bioprocess Engineering, College of Environmental Science and Forestry, State University of New York, 1 Forestry Drive, Syracuse, NY 13210, USA

<sup>b</sup> Department of Chemistry, College of Environmental Science and Forestry, State University of New York, 1 Forestry Drive, Syracuse, NY 13210, USA

## ARTICLE INFO

### Article history:

Received 26 March 2009

Received in revised form 21 April 2009

Accepted 22 April 2009

Available online 3 May 2009

### Keywords:

Xylan content

Glucan content

Hemicellulose

Sulfuric acid hydrolysis

Autohydrolysis

## ABSTRACT

The range of xylan content reported for sugar maple (*Acer saccharum*) is wider than for most hardwoods. Credible values have been reported that range from less than 16 wt% to more than 19 wt% based on extractive-free wood. Carbohydrate composition of biomass is normally determined by a two stage H<sub>2</sub>SO<sub>4</sub> hydrolysis followed by quantification of the sugar monomers. Acetic and uronic acids are also quantified for xylan-rich materials. In this research, the H<sub>2</sub>SO<sub>4</sub> hydrolysis conditions were modified and an average xylan content of 18.7% was obtained for sugar maple with a standard deviation (SD) of 0.41% for eight analyses. Minor refinements were made and an average of 18.6% (SD = 0.29%) was obtained for another eight analyses. On this occasion the acetyl to xylose mole ratio was 0.71 with a standard deviation of only 0.008. Proton NMR was utilized in quantifying sugar monomers and acetic acid. The modified hydrolysis procedure gave all the expected results for *Betula papyrifera*, a species without much controversy regarding its carbohydrate composition. A high percentage of glucan plus xylan was also obtained for an experimentally grown poplar with a low lignin content of 17.7%. The summative analyses varied from 99.7% to 101.5% for the three species.

© 2009 Elsevier Ltd. All rights reserved.

## 1. Introduction

Novel pulping processes are being investigated in our laboratory and a majority of the research is being performed with sugar maple (*Acer saccharum*) because of its abundance in the northeastern US and eastern Canada (Francis, Bolton, Abdoulmoumine, Lavrykova, & Bose, 2008; Francis & Shin, 2007). A point was reached where it became necessary to accurately determine the carbohydrate composition of this species. A review of the literature showed a wide range of values for the amount of xylose that could be hydrolyzed from this wood. The xylose content is normally determined after the *O*-acetyl-4-*O*-methylglucuronoxylan (AMX) has been hydrolyzed to its monomeric constituents and values are usually expressed as monomeric xylose (Xm) or xylan (Xp). The molecular weight ( $M_w$ ) for an individual unit is 132 in Xp and 150 in Xm due to water addition each time a xylosidic linkage is cleaved. Values of Xp < 17.0% are most common (Kaar & Brink, 1991; Kiemle, Stipanovic, & Mayo, 2004; Vinzant, Ehrman, Adney, Thomas, & Himmel, 1997; Watson et al., 2003). However, Timell (1969) reported a Xm value of 21.7% corresponding to Xp = 19.1% for extractive-free sugar maple. This high value appears to be supported by data from Clermont and Schwartz (1951, 1952) who quantified the acetyl content of eight hardwoods. Based on the data in its entirety

it appears as if almost all of the acetyls were being hydrolyzed from xylan. If 0.7 mole acetyl per mole of xylose were to be assumed for Timell's data then an acetyl content of 4.35% would be obtained. A value of 4.58% was reported for sugar maple (Clermont & Schwartz, 1952). The issue of 0.7 acetyl/xylose mole ratio will be addressed later in the paper.

A deeper search into the literature led us to an issue that we believed has not been adequately addressed for more than 50 years. Most quantification of carbohydrate monomers from biomass use a hydrolysis procedure close to the one first reported by Saeman, Moore, Mitchell, and Millet (1954). However, the Saeman et al. paper described the method of Hagglund (1951) that called for 4 h of primary hydrolysis (PH) in 72% sulfuric acid at room temperature followed by intermediate dilution and standing for 6 h, and finally 6 h of secondary hydrolysis (SH). That description was immediately followed by the proposed new method of only 1 h in 72% H<sub>2</sub>SO<sub>4</sub> at 30 °C followed by dilution to 4% H<sub>2</sub>SO<sub>4</sub> and secondary hydrolysis for 1 h at 121 °C (Saeman et al., 1954). Although the Saeman et al. method is still the most widely used approach and has been accepted as a classical method by Tappi (1985) we could find no detailed discussion regarding the importance of diffusion of viscous 72% H<sub>2</sub>SO<sub>4</sub> into all the wood meal particles during a period of only 1 h. Actually, there is not much detailed discussion in the literature about temperature, time and mixing optimization during the 72% H<sub>2</sub>SO<sub>4</sub> or PH treatment. When wetted with 72% H<sub>2</sub>SO<sub>4</sub> wood particles tend to form mini-flocs and diffusion of H<sub>2</sub>SO<sub>4</sub> into

\* Corresponding author. Tel.: +1 315 470 6525; fax: +1 315 470 6945.  
E-mail address: [francis@sy.edu](mailto:francis@sy.edu) (R.C. Francis).

those flocs may be slow if adequate mixing is not applied. The importance of mixing 30 mesh wood meal into 72% H<sub>2</sub>SO<sub>4</sub> was addressed in the following single sentence by Saeman et al. (1954). “The sample is stirred as required to effect solution of the cellulose”. It was our belief that the quality of the summative analysis would be dependent on mixing protocol, temperature and time in the PH stage.

The observed variation in xylan content does not appear to be limited to sugar maple alone and some interesting results for aspen (*Populus tremuloides*) will now be discussed. Grohmann, Torget, and Himmel (1987) reported respective values of 52.1%, 18.8% and 18.8% for the glucan, xylan and lignin content of aspen. Francis, Hanna, Shin, Brown, and Riemenschneider (2006) later reported corresponding values of 50.8%, 18.4% and 18.6% while Mansfield and Weineisen (2007) reported 49.4%, 15.3% and 19.0% for the same species. Although the ranges in glucan and lignin contents were ~5% of their respective averages, the range was 20% of the average for xylan content. Interestingly, all three research groups modified the PH treatment conditions recommended by Saeman et al. (1954). Grohmann et al. (1984, 1987) performed the PH treatment for 2 h at room temperature with 64% H<sub>2</sub>SO<sub>4</sub> followed by dilution to 55% H<sub>2</sub>SO<sub>4</sub> and 12 h of additional retention at room temperature. Francis et al. (2006) used the the Saeman et al. formulation but for 1 h at 40 °C instead of 30 °C while Mansfield and Weineisen (2007) used 72% H<sub>2</sub>SO<sub>4</sub> but for 2 h at 20 °C and with mixing every 10 min.

Coupled with our concern about PH optimization we were obtaining results in another project that appeared to indicate that a significant fraction of sugar maple xylan extracted by hot water (autohydrolysis) was difficult to hydrolyze to xylose monomers. We are now reporting on a new approach for H<sub>2</sub>SO<sub>4</sub> hydrolysis of the carbohydrate fraction of sugar maple and two other hardwoods with emphasis on improved mass transfer in the PH treatment with 72% H<sub>2</sub>SO<sub>4</sub> and harsher reaction conditions in the secondary hydrolysis (SH). Proton NMR was used for the quantification of monomers (Kiemle et al., 2004) in the 20–40 wt% H<sub>2</sub>SO<sub>4</sub> SH solutions without neutralization or other purification.

## 2. Materials and methods

### 2.1. Wood meals and extracts

Wood meals (40 mesh) were prepared from debarked paper birch (*Betula papyrifera* Marsh), sugar maple and a *Populus deltoides* identified as D105. The birch and maple were harvested in Central New York while D105 was harvested from an experimental plot in Arlington, WI (Riemenschneider et al., 2001). Extracts were prepared by treating debarked sugar maple chips with aqueous solution in a M&K digester. The temperature profile was 60 min to 165 °C and 70 min at that temperature. Water alone was used in one case (HW) and in the other case 1.5% acetic acid on chips was added to the water (HAA). The liquor to wood ratio was 4.0 liters/kg and the extract was collected after flowing through a coiled tube placed in an ice bath. The pH of the HW extract was 3.38 while it was 3.11 for HAA.

### 2.2. Hydrolysis of milled wood

Milled wood (40 mesh) was added to a 50 ml centrifuge tubes and placed in a water bath at 25 °C. In one approach 0.50 g of wood meal (OD basis) was used and 16 ml of 72% H<sub>2</sub>SO<sub>4</sub> (specific gravity 1.634) was carefully kneaded into the particles over a period of ~5 min using a glass rod. The slurry was allowed to sit in the water bath at ~25 °C for 2 h with mixing every 15 min. Water (21 ml) was then added and the tubes sealed (now 40 wt% H<sub>2</sub>SO<sub>4</sub>), shaken

and placed in water bath at 80 ± 1.0 °C for 1 h. The tubes were shaken occasionally to homogenize the slurry. The tubes were removed and chilled in an ice bath. They were stored overnight in a refrigerator (4 °C) and lignin precipitation occurred. A recorded mass of the supernatant was taken and the internal standards were added. The solution was then analyzed by <sup>1</sup>H NMR as soon as possible. In an alternate approach 1.00 g of wood meal and 16 ml of 72% H<sub>2</sub>SO<sub>4</sub> (26.1 g) were used in the PH stage. After SH in 40% H<sub>2</sub>SO<sub>4</sub> (26.1 g + 21 g) the centrifuge tube was taken out of the 80 °C water bath and cooled in an ice bath. The sample was then transferred to a larger plastic bottle using 47 g of water for washing and dilution. The slurry (now 20 wt% H<sub>2</sub>SO<sub>4</sub>) was chilled and placed in a refrigerator to cause lignin precipitation. A recorded mass of supernatant was taken and handled as described above.

### 2.3. Chemical analyses

Ethanol/toluene extractives in the woodmeals were determined in accordance with Tappi Method T 204 om-88; while Tappi Method T 222 om-88 was used for Klason lignin and Tappi Method UM 250 for acid-soluble lignin (ASL). The Klason filtrate containing the ASL was diluted with 3% H<sub>2</sub>SO<sub>4</sub> until its 205 nm absorbance was <0.7. Dissolution of chip mass into HW and HAA extracts was determined by the conversion of residual chips to pulp with the aid of a disk refiner. Gravimetric pulp yield was determined and materials dissolved were obtained from an overall material balance. The initial chip mass was 800 OD grams. The lignin content of an extract was determined by adding 100 ml of it to 50 ml of H<sub>2</sub>O and 4 ml of 72% H<sub>2</sub>SO<sub>4</sub> then heating the slurry as described in Tappi Method T222 om-88. Acid-soluble lignin (ASL) was determined as previously described.

### 2.4. NMR analysis

In this study we used <sup>1</sup>H NMR spectroscopy to quantify the relative molar concentration of sugars resulting from wood hydrolysis. All spectra were acquired at 10 °C with a Bruker AVANCE 600 spectrometer (600 MHz <sup>1</sup>H frequency) equipped with a 5 mm triple resonance z-gradient probe. Data were acquired and processed in TOPSPIN v1.3 from Bruker BioSpin. Due to the low pH, the water signal (typically at 4.7 ppm) was shifted out of the anomeric proton region which made monomer quantification possible. Furthermore, the dynamic range of the resonances of interest was improved by pre-saturating the residual water peak. To obtain quantitative data the <sup>1</sup>H spectra were acquired with a relaxation delay set to 10 s, 30° pulse angle, 2.7 s acquisition time and spectral widths of 16 ppm.

The hydrolyzed samples were allowed to settle overnight before a known mass equivalent to approximately 1 ml of the clear supernatant was transferred to a NMR tube. To it was then added a known mass (equivalent to 0.1 ml) of standardizing solution containing trimethylamine hydrochloride (TMA) ( $1.82 \times 10^{-5}$  mol/g) and 3-(trimethylsilyl)propionic-2,2,3,3-d<sub>4</sub> acid (TSP) 0.05% in D<sub>2</sub>O. The TMA served as a standard to calibrate the mass of each of the sugars in the sample while the TSP served as a chemical shift reference (0.0 ppm).

A detailed discussion of the quantification algorithm employed was presented elsewhere (Kiemle et al., 2004). To summarize, the <sup>1</sup>H NMR resonances associated with the C1- $\alpha$  and C1- $\beta$  anomeric protons were integrated and summed for each sugar providing a relative molar concentration. For most sugars the ratio of  $\alpha$  to  $\beta$  anomers was well known and would remain constant in solution of equal temperature and pH (Robyt, 1998). The locations of the relevant  $\alpha$  and  $\beta$  peaks in acidic conditions and  $\alpha/\beta$  ratios were presented in Table 1 (Robyt, 1998). For those sugars where both the C1- $\alpha$  and C1- $\beta$  <sup>1</sup>H NMR resonances were not well resolved, we used

**Table 1**

Location and ratio of sugar peaks in the  $^1\text{H}$  NMR spectra of sugars in 40% sulfuric acid after spectrum shift of TSP to 0.0 ppm.

Location of sugar peaks in $^1\text{H}$ NMR spectra of Sugars in 40% sulfuric acid			
	Approximate Peak Location (ppm)		Anomeric Ratio (Robyt (1998))
	$\alpha$	$\beta$	
D-Galactose	5.33	4.67	0.99
D-Glucose	5.30	4.72	0.63
D-Xylose	5.25	4.64	0.62
D-Mannose	5.24	4.98	1.59
L-Arabinose	5.31	4.60	0.47
L-Rhamnose	5.18	4.95	1.31

the integrated intensity of either the C1- $\alpha$  or C1- $\beta$  proton and then used the values of the anomeric ( $\alpha/\beta$ ) ratio in Table 1 to approximate the intensity of the second anomeric proton.

Furfural was typically observed in wood hydrolyzates due to acid over conversion of xylose sugars. As a result, we added the integrated intensity of the 9.46 ppm peak of furfural (see Table 2 for location of non-sugar peaks) to the xylose intensity to correct for the over conversion. The number of moles of each substance in a sample could be calculated from the area ratios of the corresponding components and the area of the protons determined in the TMA standard. Since TMA contains 9 protons per molecule, the peak area for TMA was divided by 9 to get the peak area for each mole of protons. Acetyl groups had peaks for free (acetic acid) and bound groups, which were added to get the total acetyl peak area. The total acetyl peak area values had to be divided by 3 because each component reflects 3 protons per molecule. The mass of each of the sugars or non-sugar wood components was calculated by multiplying the number of moles in the sample by the molecular weight of that substance. The concentration in the sample analyzed was then calculated by dividing the mass of a substance by the mass of the sample analyzed.

### 3. Results and discussion

#### 3.1. Hydrolysis of sugar maple extracts with sulfuric acid

The carbohydrate composition of wood meals was the primary focus of this research. However, it was important to report on our experience in hydrolyzing xylan rich extracts to monomers. The results obtained demonstrated the importance of carbohydrate depolymerization during the primary hydrolysis (PH) stage with 72%  $\text{H}_2\text{SO}_4$  when using the approach of Saeman et al. (1954). The HW extract (23.7% wood dissolution) was  $\text{H}_2\text{SO}_4$ -hydrolyzed using a treatment condition similar to the secondary hydrolysis (SH) stage of Saeman et al. (1954). The extract was adjusted to 4%  $\text{H}_2\text{SO}_4$  in 100 ml stainless steel autoclaves. The autoclaves were then placed in an M&K digester containing recirculating water.

**Table 2**

Location of non-sugar peaks in the  $^1\text{H}$  NMR spectra of hydrolyzed samples in 40% sulfuric acid after shift of TSP to 0.0 ppm.

Location of non-sugar peaks and standard solution in $^1\text{H}$ NMR spectra of hydrolyzed wood in 40% sulfuric acid	
	Approximate peak location
Trimethylamine Hydrochloride	2.9
Acetyl groups (free/bound)	2.15/2.25
Furfural	9.45
Hydroxymethylfurfural	9.4
methanol	3.4
TSP	0.0

The water was heated from room temperature to 120 °C over a period of 30 min and maintained at 120  $\pm$  1.0 °C for 45 min. A xylose yield of 8.4% on wood was obtained (7.3% xylose + 0.7% furfural). It was assumed that the furfural came from xylose in an equimolar ratio (Dias, Pillinger, & Valente, 2005).

Wood dissolution corresponded to 23.7% during HW extraction of the sugar maple chips and lignin in the extract was 4.1% on wood (1.5% Klason + 2.6% ASL). It was our estimate that complete hydrolysis of the xylan in the extract should yield at least 10% xylose on wood. The 8.4% xylose yield appears to be lower than other values reported in the literature. However, Tunc and van Heiningen (2008) performed HW (hot water) extraction of wood meal from a mixture of hardwoods from the southern US. An isothermal extraction for 100 min at 160 °C yielded only 6.9% xylose after the exact SH treatment of Saeman et al. (1954), i.e. 60 min in 4%  $\text{H}_2\text{SO}_4$  at 121 °C.

For the HAA extract (27.2% wood dissolution) we started experimenting with other SH hydrolysis conditions and obtained the results in Table 3 for 40 wt%  $\text{H}_2\text{SO}_4$  (60 wt% extract) at lower temperatures. Xylose yields of 15.7% and 15.5% on wood were obtained for 10 min hydrolyzes at 60 °C and 80 °C. It should be noted the xylose yield decreased to  $\sim$ 10.0% on wood when the reaction time was extended to 20 min at both temperatures. Wood dissolution corresponded to 27.2% during HAA extraction and lignin in the extract was 4.7% on wood (1.6% Klason + 3.1% ASL). The total yield of carbohydrate related materials in polymeric form was 19.3% and 20.0%, respectively, for 60 °C and 80 °C (Table 3). If the 4.7% lignin content is added to the 19.7% average carbohydrate value and a total uronic acid content of 3.0% is assumed one arrives at a value of 27.4% on wood of dissolved material. This compares very favorably to the 27.2% determined by mass balance. The average acetyl:xylose mole ratio of 0.61 is also in the expected range. Timell (1969) used 0.7 acetyl/xylose and 0.10 of 4-O-methylglucuronic acid/xylose in converting his xylose content to AMX content. Several research groups have since reported acetyl:xylose ratio of 0.6–0.7 for a wide range of hardwoods (Kaar & Brink, 1991; Lindberg, Rosell, & Svensson, 1973; Maloney, Chapman, & Baker, 1985; Garrote & Parajó, 2002; Tunc & van Heiningen, 2008).

The HW extract was then hydrolyzed in 40%  $\text{H}_2\text{SO}_4$ . The results for 10 and 60 min SH treatment at 60 °C and 80 °C are summarized in Table 4. The xylose content was only 8.8% on wood and the two 10 min values for total carbohydrate related compounds in polymeric form were 11.3% and 11.4% on wood. If the 4.1% lignin content and an assumed uronic acid content of 2.5% on wood are added then the calculated value for total dissolved materials would be 18.0% on wood. Approximately 5.5% on wood of dissolved material is unaccounted for since 23.7% dissolution was obtained by mass balance. It was our tentative conclusion that this extract contained  $\sim$ 5% on wood of AMX that could not be easily hydrolyzed. Also, the much higher recovery of xylose from the HAA extract as

**Table 3**

Carbohydrate related compounds (% on wood) from hydrolysis of HAA extract with 40 wt%  $\text{H}_2\text{SO}_4$ .

	5 min at 60 °C	10 min at 60 °C	10 min at 80 °C
Xylose <sup>a</sup>	13.8	15.7 (13.8) <sup>b</sup>	15.5 (13.6)
Arabinose	0.4	0.9 (0.8)	0.8 (0.7)
Mannose	0.9	0.8 (0.7)	1.2 (1.1)
Glucose	0.6	0.4 (0.4)	1.0 (0.9)
Galactose	0.4	0.6 (0.5)	0.8 (0.7)
Rhamnose	0.4	0.3 (0.3)	0.4 (0.4)
Acetyl ( $\text{CH}_3\text{CO}$ ) <sup>c</sup>	2.5	2.8 (2.8)	2.6 (2.6)
Acetyl:Xylose	0.63	0.62	0.59

<sup>a</sup> Including furfural.

<sup>b</sup> Polymeric yield, i.e.  $M_w$  of 132 instead of 150.

<sup>c</sup> Corrected for the 1.5% added acetic acid.

**Table 4**

Carbohydrate related compounds (% on wood) from hydrolysis of HW extract with 40 wt% H<sub>2</sub>SO<sub>4</sub>.

	10 min @ 60 °C	60 min @ 60 °C	10 min @ 80 °C	60 min @ 80 °C
Xylose <sup>a</sup>	8.8 (7.7) <sup>b</sup>	8.6	8.8 (7.7)	7.5
Arabinose	0.3 (0.3)	0.3	0.5 (0.4)	0.4
Mannose	0.7 (0.6)	0.8	0.8 (0.7)	0.7
Glucose	0.5 (0.5)	0.4	0.4 (0.4)	0.2
Galactose	0.3 (0.3)	0.2	0.3 (0.3)	0.2
Rhamnose	0.2 (0.2)	0.2	0.3 (0.3)	0.2
Acetyl	1.7 (1.7)	1.6	1.6 (1.6)	1.4
Acetyl:Xylose	0.67	0.65	0.63	0.65

<sup>a</sup> Including furfural.

<sup>b</sup> Polymeric yield.

compared to the HW extract would indicate that the degree of partial polysaccharide depolymerization prior to SH is critically important.

### 3.2. Wood hydrolysis and carbohydrate composition

An accurate estimate of the xylan content of sugar maple was the primary focus of this research. Two other hardwoods were also included in the investigation as a means of confirming the credibility of any new approaches that would be developed for sugar maple. The first, paper birch, is well known for its high xylan content while the second was an experimental poplar with a lignin content of only 17.7% (Bose, Francis, Govender, Bush, & Spark, 2009). It was of interest to see if a significantly higher carbohydrate content was obtained for this species as compared to sugar maple with a lignin content of 25.0%. It was tentatively concluded from the HAA and HW extracts that the intensity of the acidic treatment during wood dissolution was critical to the success of secondary hydrolysis with 40% H<sub>2</sub>SO<sub>4</sub>. Our initial and only approach to date was to dissolve the woodmeal in 72% H<sub>2</sub>SO<sub>4</sub> and allow reaction for 2 h at 25 °C instead of 1 h at 30 °C. This was followed by SH (40% H<sub>2</sub>SO<sub>4</sub>, 80 °C) for varying reaction times.

### 3.3. Initial results for maple and birch

The first experiment was performed with 2 h of primary hydrolysis followed by SH for 20, 40 and 60 min. Sixteen milliliters of 72% H<sub>2</sub>SO<sub>4</sub> was used to dissolve 0.50 g of wood meal then 21 ml of H<sub>2</sub>O was added to achieve 40 wt% H<sub>2</sub>SO<sub>4</sub> for secondary hydrolysis. The reaction products did not undergo any further dilution prior to NMR analysis and all experiments were performed in triplicate. Steady increases in glucose and xylose yields were observed with time and the 60 min results appeared to be close to the expected maxima for both species. Glucan and xylan contents were calculated from the yields of the monomeric sugars. The analyses were repeated but on this occasion SH retention was for 60, 90 and 120 min. Once again the 60 min results appeared to be representative of the expected carbohydrate composition for both species. When SH was increased from 60 to 120 min there was a minor decrease in glucose yield (<5% of the average for SH = 60) and a moderate decrease in xylose yield (5–10% of the average for SH = 60). The experiment was repeated yet again but with SH retention of 50, 60 and 70 min.

Three samples of birch were hydrolyzed for each of the three retention times and their was no statistical difference between the results for 60 and 70 min which yielded higher glucan values than 50 min of hydrolysis. The average glucan content for five of the six samples hydrolyzed for 60 and 70 min was 42.2% with a standard deviation of 0.71%. The protocol was repeated for sugar maple and the average glucan content for five of the six samples

**Table 5**

Chemical composition of extractive-free (ethanol/toluene) D105 poplar after H<sub>2</sub>SO<sub>4</sub> hydrolysis and <sup>1</sup>H NMR analysis.<sup>a</sup>

	SH = 50 min	60 min	70 min
Glucan <sup>b</sup>	45.3, 49.3, 49.2	49.6, 49.7, 49.9	51.1, 51.1, 50.7
Xylan	17.3, 17.1, 17.1	17.5, 17.4, 17.9	17.3, 17.2, 17.5
Mannan	2.6, 3.0, 3.8	2.6, 3.0, 3.3	4.3, 3.0, 3.5
Others <sup>c</sup>	0.3, 0.3, 0.3	0.3, 0.3, 0.5	0.3, 0.3, 0.3
Acetyl	3.6, 3.7, 3.9	3.8, 3.9, 3.9	3.6, 3.6, 3.9
Acetyl:Xylose	0.65, 0.67, 0.69	0.67, 0.70, 0.66	0.65, 0.66, 0.68

<sup>a</sup> Extractives content = 1.5% on wood; 17.7% lignin on extractive-free wood.

<sup>b</sup> Polymeric yield, % on wood.

<sup>c</sup> Arabinan, galactan and rhamnan.

hydrolyzed for 60 and 70 min was 44.5% with a standard deviation of 0.69%. Although the standard deviation was low for both birch and maple, one of the six samples for each species had to be discarded because it was clearly lower than the other five. It was suspected that poor mixing of the 72% H<sub>2</sub>SO<sub>4</sub> into the wood particles was the likely cause. There was a moderate amount of scatter in the xylan data for birch with one of the nine samples (including SH = 50 min) being >26.0% and one being <24.5%. Somewhat surprisingly, eight of the nine analyses for maple fell in a narrower range of 18.0–19.2% with an average of 18.7% and standard deviation of 0.41%.

### 3.4. Analysis of the D105 poplar

Before the D105 poplar was analyzed a switch was made from flat bottom glass bottles to centrifuge tubes in an attempt to achieve better mixing of the 72% H<sub>2</sub>SO<sub>4</sub> into the wood particles. A glass rod could more easily be used to concentrate solid particles into the conical bottom of a centrifuge tube. The Tappi Method (1985) recommended the use of centrifuge tubes but without explanation. The precision of the D105 results was superior to those of birch and maple with regards to glucan and xylan contents and are documented in Table 5. These results appear to indicate a gradual increase in cellulose content as retention is increased from 50 to 70 min. All nine xylan contents fell in a narrow range of 17.1–17.9 and all the acetyl:xylose ratios fell within a range of 0.65–0.70.

As the amount of collected data became substantial there were indications of a minor fluctuation in glucose and xylose contents depending on how long the 40% H<sub>2</sub>SO<sub>4</sub> hydrolyzates sat at room temperature before NMR analysis. The normal protocol was to add the internal standards to an aliquot of the sugar solution and deliver it to another building for NMR analysis where it could sit from 1 to 100 h. It appeared as if glucose and xylose slowly converted to other compounds when the samples were kept at room temperature for an extended period of time. In an attempt to minimize monomer losses and fluctuation in glucose and xylose yields due to the time variation between sample preparation and analysis, all three species were analyzed starting with 1.00 g of wood meal instead of 0.50 g in 16 ml of 72% H<sub>2</sub>SO<sub>4</sub>. After SH treatment

**Table 6**

Chemical composition of extractive-free paper birch after H<sub>2</sub>SO<sub>4</sub> Hydrolysis and <sup>1</sup>H NMR analysis.<sup>a</sup>

	SH = 50 min	60 min	70 min
Glucan	39.6, 40.7, 41.1	40.7, 40.0, 41.4	41.4, 40.8, 41.5
Xylan	24.5, 24.8, 24.9	24.5, 24.2, 24.7	24.5, 24.3, 24.6
Mannan	1.4, 2.0, 1.6	1.9, 1.9, 2.4	1.9, 2.0, 1.6
Others	0.7, 0.8, 1.3	0.9, 0.6, 1.0	0.8, 0.8, 0.5
Acetyl	5.0, 4.9, 5.1	5.0, 5.1, 5.2	5.1, 5.0, 5.1
Acetyl:Xylose	0.63, 0.60, 0.64	0.63, 0.64, 0.65	0.64, 0.64, 0.64

<sup>a</sup> Extractives content = 2.5% and lignin content of 21.9%.



**Table 7**Chemical composition of extractive-free sugar maple after H<sub>2</sub>SO<sub>4</sub> Hydrolysis and <sup>1</sup>H NMR analysis<sup>a</sup>.

	50 min	60 min	70 min
Glucan	43.9, 42.9, 43.4	44.6, 45.1, 45.4	44.9, 44.1
Xylan	18.9, 18.3, 18.6	18.4, 18.5, 19.0	18.9, 18.3
Mannan	3.2, 2.8, 2.9	2.9, 2.3, 2.8	2.8, 2.8
Others	1.3, 0.4, 0.8	0.5, 0.6, 0.7	0.5, 0.6
Acetyl	4.4, 4.2, 4.3	4.3, 4.4, 4.4	4.4, 4.3
Acetyl:Xylose	0.71, 0.70, 0.72	0.72, 0.72, 0.71	0.72, 0.72

<sup>a</sup> Extractives content = 2.1% and lignin content = 25.0%.

the samples were cooled and diluted to 20% H<sub>2</sub>SO<sub>4</sub> (Section 2). It was our hope that the sugar solutions could be kept for long periods of time in 20% H<sub>2</sub>SO<sub>4</sub> at room temperature without any monomer losses. On our first attempt, the results for D105 did not reproduce those in Table 5 while the results for birch and maple (Table 6 and 7) reproduced the earlier trends quite nicely. The three values for glucan content of D105 after SH = 60 min were 49.2%, 43.5% and 44.2% with corresponding xylan content of 16.9%, 13.5% and 15.0%. After 70 min of SH the three glucan contents were 47.8%, 40.2% and 47.0% with corresponding xylan contents of 16.2%, 13.4% and 16.1%.

Almost all of the values in Tables 6 and 7 reproduce literature results quite well. The 41.0% glucan content (average for 60 and 70 min) for birch is close to other values in the literature (Clermont & Schwartz, 1951; Springer & Zoch, 1968; Sjostrom, 1993). The xylan content is similarly close to other literature values (Springer & Zoch, 1968; Sjostrom, 1993; Maloney et al., 1985). An acetyl content of 5.06% was obtained in the present study and this is close the 4.94% reported by Clermont and Schwartz (1951) and 5.03% reported by Maloney et al. (1985).

Regarding sugar maple, the glucan content in Table 7 is similar to that of Kaar and Brink (1991), Vinzant et al. (1997), and Watson et al. (2003). The xylan content in Table 7 is higher than in the three references above but close to the 19.1% reported by Timell (1969). It should be noted that the eight xylan contents in Table 7 fell in a narrow range of 18.3–19.0% with an average of 18.6% and a standard deviation of only 0.29%. All of the acetyl:xylose ratios fell in a narrow range of 0.70–0.72. Clermont and Schwartz (1952) reported an acetyl content of 4.58% for sugar maple while the eight values in Table 7 averaged 4.34%.

It should be noted that this method gave very low values for arabinan, galactan and rhamnan for these samples. When the summative analyses are discussed we will assume a value of 2.0% for the total of the three. The 2.0% value was reported for both a poplar (Chum et al., 1988) and a birch (Alen, 2008).

The approach of using 1.00 g of wood meal in 16 ml of 72% H<sub>2</sub>SO<sub>4</sub> gave very good results for birch and maple and actually gave results close to those in Table 5 when the D105 analysis was repeated. However, we abandoned that approach based on the initially poor results obtained for D105. As previously discussed, glucan contents of 49.2%, 43.5% and 44.2% were initially obtained for SH = 60 min and 47.8%, 40.2% and 47.0% for SH = 70 min. We attribute those low and variable values (compared to Table 5) to

**Table 8**

Effect of room temperature storage time on monomer yields for SH = 70 min hydrolyzate from D105.

Retention time, h <sup>a</sup>	1–4	21–24	69–72	117–120
Glucan, % on wood <sup>b</sup>	51.0	50.5	49.3	49.0
Xylan, % on wood	17.5	17.6	17.1	17.1

<sup>a</sup> Time between addition of internal standard and NMR analysis.<sup>b</sup> Calculate from glucose concentrations.**Table 9**

Summative analyses for the three extractive-free hardwoods.

	D105	Birch	Maple
Lignin (%)	17.7	21.9	25.0
Glucan (%)	51.0	41.0	44.8
Xylan (%)	17.5	24.6	18.6
Mannan (%)	3.6	2.0	2.8
Acetyl (%)	3.8	5.1	4.3
Other sugars (%) <sup>a</sup>	2.0	2.0	2.0
Me-GluU (%) <sup>b</sup>	2.5	3.5	2.7
Other Uronics (%) <sup>c</sup>	0.7	0.7	0.7
Ash (%)	0.9	0.3	0.6
Total (%)	99.7	101.1	101.5

<sup>a</sup> An estimate from the literature (Chum et al., 1988; Alen, 2008).<sup>b</sup> 4-O-methylglucuronic acid residue (*M<sub>w</sub>* = 190); 0.10 mole ratio to xylose (Timell, 1969).<sup>c</sup> Other uronic acid residues (Kaar & Brink, 1991).

poor mixing of the increased amount of wood meal in 16 ml of 72% H<sub>2</sub>SO<sub>4</sub>. However, there was no obvious sign of any differences between the six samples (SH = 60 and 70 min above). We returned to the 0.50 g in 16 ml H<sub>2</sub>SO<sub>4</sub> approach and quantified the loss of monomers (in 40% H<sub>2</sub>SO<sub>4</sub>) with sample storage time at room temperature. This was conducted with SH = 70 min only and the results in Table 8 suggest that the rates of monomer losses are quite low. Our suggested approach for hardwoods is to conduct SH in 40% H<sub>2</sub>SO<sub>4</sub> for 70 min then chill the sample to cause lignin precipitation. Beyond this point it is suggested that the sample be kept in a refrigerator as much as possible before monomer quantification by NMR or another technique.

### 3.5. Summative analyses

The lignin contents obtained for birch and maple are typical of other reported data and the value for D105 was based on multiple analyses (Bose et al., 2009). If the lignin, glucan and xylan contents are all accurate then decent summative analyses shown be obtained and that is demonstrated in Table 9. The values ranged from 99.7% to 101.5%.

## 4. Conclusions

An investigation was performed in order to resolve the issue of the wide range of reported values for the xylan content of sugar maple. Results obtained with xylo-oligomers in solution indicated that secondary hydrolysis at 80 °C with a H<sub>2</sub>SO<sub>4</sub> concentration of 40 wt% was quite effective at generating high monomer concentrations. When extractive-free woodmeal from maple, birch and poplar were depolymerized by two stages of H<sub>2</sub>SO<sub>4</sub> hydrolysis it appeared as if the initial mixing of the 72% H<sub>2</sub>SO<sub>4</sub> into the wood particles was critical to high glucose and xylose yields. A new hydrolysis protocol was developed that appeared to give excellent results for the three hardwoods. It is recommended that in the development of any subsequent hydrolysis protocol centrifuge tubes with conical bottoms be used for the primary hydrolysis with concentrated H<sub>2</sub>SO<sub>4</sub> as they allow for better mixing as compared to flat bottom containers. Also, the liquid to wood ratio should be as high as possible in that initial treatment.

## Acknowledgements

We thank Dr. Don E. Riemenschneider of the US Forest Service for the donation of the D105 poplar. We are most grateful to Andritz Inc., the member companies of the Empire State Paper Research Associates (ESPRA), and the CAPES/Fulbright Doctoral Program (Ericka Alves) for their financial support.

## References

- Alen, R. (2008). Practical aspect on biorefinery systems integrated to kraft pulping. *Proceedings of the 2nd annual conference biorefining for the pulp paper industry*. Helsinki, December 2–3.
- Bose, S. K., Francis, R. C., Govender, M., Bush, T., & Spark, A. (2009). Lignin content versus syringyl to guaiacyl ratio amongst poplars. *Bioresource Technology*, 100, 1628–1633.
- Chum, H. L., Johnson, D. K., Black, S., Baker, J., Grohmann, K., & Sarkanen, K. V. (1988). Organosolv pretreatment for enzymatic hydrolysis of poplars: I. Enzyme hydrolysis of cellulosic residues. *Biotechnology and Bioengineering*, 31, 643–649.
- Clermont, L. P., & Schwartz, H. (1951). The chemical composition of Canadian woods. *Pulp and Paper Magazine of Canada*, 52(13), 103–105.
- Clermont, L. P., & Schwartz, H. (1952). The chemical composition of Canadian woods II. *Pulp and Paper Magazine of Canada*, 53(6), 142–143.
- Dias, A. S., Pillinger, M., & Valente, A. A. (2005). Dehydration of xylose into furfural over micro-mesoporous sulfonic acid catalysts. *Journal of Catalysis*, 229, 414–423.
- Francis, R. C., Hanna, R. B., Shin, S.-J., Brown, A. F., & Riemenschneider, D. E. (2006). Papermaking characteristics of three Populus clones grown in the north-central U.S. *Biomass & Bioenergy*, 30, 803–808.
- Francis, R. C., & Shin, N.-H. (2007). Methods for carbonate pretreatment and pulping of cellulosic material. *US Patent Office*, Appl. 2007/0284068 (December 13, 2007).
- Francis, R. C., Bolton, T. S., Abdoulmoumine, N., Lavrykova, N., & Bose, S. K. (2008). Positive and negative aspects of soda/anthraquinone pulping of hardwoods. *Bioresource Technology*, 99, 8453–8457.
- Garrote, G., & Parajó, J. C. (2002). Non-isothermal autohydrolysis of *Eucalyptus* wood. *Wood Science and Technology*, 36, 111–123.
- Grohmann, K., Himmel, M., Rivard, C., Tucker, M., Baker, J., Torget, R., et al. (1984). Chemical-mechanical methods for the enhanced utilization of straw. *Biotechnology and Bioengineering Symposium*, 14, 137–151.
- Grohmann, K., Torget, R., & Himmel, M. (1987). Dilute acid pretreatment of biomass at high solids concentrations. *Biotechnology and Bioengineering Symposium*, 17, 135–151.
- Hagglund, E. (1951). *The chemistry of wood*. New York: Academic Press.
- Kaar, W. E., & Brink, D. L. (1991). Summative analysis of nine common North American woods. *Journal of Wood Chemistry and Technology*, 11, 479–494.
- Kiemle, D. J., Stipanovic, A. J., & Mayo, K. E. (2004). Proton NMR methods in compositional characterization of polysaccharides. *ACS Symposium Series*, 864, 122–139.
- Lindberg, B., Rosell, K. G., & Svensson, S. (1973). Positions of the O-acetyl groups in birch xylan. *Svensk Papperstidning*, 76, 30–32.
- Maloney, M. T., Chapman, T. W., & Baker, A. J. (1985). Diluted acid hydrolysis of paper birch: Kinetics studies of xylan and acetyl-group hydrolysis. *Biotechnology and Bioengineering*, 27, 355–361.
- Mansfield, S. D., & Weineisen, H. (2007). Wood fiber quality and kraft pulping efficiencies of trembling aspen (*Populus tremuloides* Michx.) Clones. *Journal of Wood Chemistry and Technology*, 27, 135–151.
- Riemenschneider, Don E., Berguson, W. E., Dickmann, D. I., Hall, R. B., Isebrands, J. G., Mohn, C. A., et al. (2001). Poplar breeding and testing strategies in the north-central U.S.: Demonstration of potential yield and consideration of future research needs. *The Forestry Chronicle*, 77(24), 5–253.
- Robyt, J. F. (1998). Essential of carbohydrate chemistry. In C. R. Cantow (Ed.) (pp. 49). New York: Springer-Verlag.
- Saeman, J. S., Moore, W. E., Mitchell, R. L., & Millet, M. A. (1954). Techniques for the determination of pulp constituents by quantitative paper chromatography. *Tappi Journal*, 37, 336–343.
- Sjostrom, E. (1993). *Wood chemistry—fundamentals and application* (2nd ed.). New York: Academic Press.
- Springer, E. L., & Zoch, L. L. (1968). Hydrolysis of Xylan in different species of hardwoods. *Tappi Journal*, 51(5), 214–218.
- Tappi (1985). *Carbohydrate composition of extractive-free wood and wood pulp by gas-liquid chromatography*. Tappi Method T 249 cm-85. Atlanta Georgia: Tappi Press.
- Timell, T. E. (1969). The chemical composition of tension wood. *Svensk Papperstidning*, 72, 173–181.
- Tunc, M. S., & van Heiningen, A. R. P. (2008). Hydrothermal dissolution of mixed southern hardwoods. *Holzforschung*, 62, 539–545.
- Vinzant, T. B., Ehrman, C. I., Adney, W. S., Thomas, S. R., & Himmel, M. E. (1997). Simultaneous saccharification and fermentation of pretreated hardwoods. *Applied Biochemistry and Biotechnology*, 62, 99–104.
- Watson, P., Hussein, A., Reath, S., Gee, W., Hatton, J., & Drummond, J. (2003). The kraft pulping properties of Canadian red and sugar maple. *Tappi Journal*, 2, 26–32.