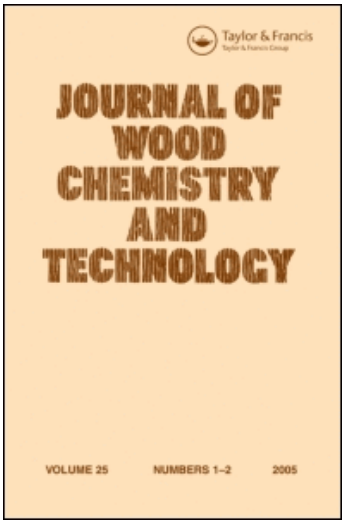

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

Determination of Furfural and Hydroxymethylfurfural Formed From Biomass Under Acidic Conditions

Congcong Chi[ab]; Zeng Zhang[a]; Hou-min Chang[b]; Hasan Jameel[b]
[a] State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, P. R. China [b] Department of Wood and Paper Science, North Carolina State University, Raleigh, North Carolina, USA

Online publication date: 18 November 2009

To cite this Article Chi, Congcong , Zhang, Zeng , Chang, Hou-min and Jameel, Hasan(2009) 'Determination of Furfural and Hydroxymethylfurfural Formed From Biomass Under Acidic Conditions', Journal of Wood Chemistry and Technology, 29: 4, 265 — 276
To link to this Article: DOI: 10.1080/02773810903096025
URL: http://dx.doi.org/10.1080/02773810903096025

Journal of Wood Chemistry and Technology, 29: 265–276, 2009

ISSN: 0277-3813 print / 1532-2319 online
DOI: 10.1080/02773810903096025

Taylor & Francis
Taylor & Francis Group

Determination of Furfural and Hydroxymethylfurfural Formed From Biomass Under Acidic Conditions

Congcong Chi,[1,2] Zeng Zhang,[1] Hou-min Chang,[2] and Hasan Jameel[2]
[1]State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, P. R. China
[2]Department of Wood and Paper Science, North Carolina State University, Raleigh, North Carolina, USA

Abstract: A rapid method for simultaneous determination of furfural and hydroxymethylfurfural (HMF) in the filtrate of an acidic treatment of biomass was developed based on UV spectrophotometer. Interference from acid soluble lignin is alleviated by the use of absorbance difference spectrum before and after reduction with sodium borohydride ($NaBH_4$). The concentrations of furfural and HMF in the filtrate are determined by measuring the absorbance of the difference spectrum at 277 nm and 285 nm, the characteristic absorption maxima for furfural and HMF, respectively. Two simultaneous equations are solved to obtain the concentrations of furfural and HMF in the filtrate. Acid soluble lignin in the solution is determined to cause negligible interference on the analysis.

Keywords: Acid hydrolysis, biomass, furfural, HMF

INTRODUCTION

As we all know, resources shortage and energy crisis have been universal problems for a long time. In recent years, the topic of biorefinery has gained

This work was done by the senior author at North Carolina State University as a part of her doctoral dissertation for her degree requirement at South China University of Technology. The senior author is grateful to the National Natural Science Foundation of China (Project number 30671647) and the China Scholarship Council for the financial support for her study at North Carolina State University. The authors are grateful to Cleantech Partners/DOE for partial financial support of this research through VPP project.

Address correspondence to Concong Chi, State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, 510640, P.R. China. E-mail: congcongchi@163.com

its popularity and the concept of Integrated Forest Biorefinery (IFBR) was brought forward.[1] One of the important approaches in the IFBR mill is to extract some hemicelluloses from raw materials before chemical pulping. This has been known as value prior to pulping and has been a hot research topic during the past few years. Prehydrolysis with hot water or dilute acid is usually used to hydrolyze some hemicelluloses into water- soluble monomers and oligomers of pentoses and hexoses, which can be fermented to produce ethanol. Another hot research project in recent years is the conversion of lignocellulosic biomaterial into biofuel. Regardless of approaches, the bioconversion processes normally involve a pretreatment followed by saccharification to convert most polysaccharides into fermentable sugars. Most of the pretreatments such as steam explosion, dilute acid pretreatment, and autohydrolysis employ high temperature under acidic conditions.

Under acidic conditions and especially at high temperature, 2-furfuraldehyde (Furfural) is readily produced from pentoses and 5-(hydroxymethyl)-2-furfuraldehyde (HMF) is easily formed from hexoses. Because both furfural and HMF are formed from carbohydrates, they interfere with the accuracy of sugar analysis of any biomass materials. Furthermore, both are harmful to the fermentation of sugars if the concentrations of these compounds exceed thrashold concentration.[2] Thus, quantitative determination of these two compounds is necessary for the accuracy of sugar analysis and for optimization of any pretreatment conditions to minimize their formation.

Different analytical methods were developed in recent years to determine furfural compounds in environmental and food samples.[3–6] UV spectral method is relatively simple and rapid. Martinez et al.[2] reported a UV method to monitor furans (furfural and HMF) produced in dilute acid hydrolysates of biomass. They found that the UV spectra of hemicellulose hydrolysate has a single dominant peak at around 278 nm furfural and that HMF have equal absorbance at 284 nm on a weight basis, which can be used to estimate the total of furans in hydrolysates. However, as recognized by the authors, only approximately two-thirds of this peak was attributed to furan absorbance, the rest being contributed from acid soluble lignin and other phenolic compounds such as ferulic acid. The authors used the absorbance at 320 nm to correct for those interference compounds and derived an empirical equation for the estimate of the sum of furans.[2] Furfural and HMF have the characteristic absorbance at 277 nm and 285 nm, respectively, and thus can be determined accurately by UV-spectral method if only one of them is present in the solution without the presence of other interference contaminants. However, in acidic hydrolysis filtrate of biomass, both are present and their accurate determination is problematic. Furthermore, acid soluble lignin is also present. Lignin, which has characteristic absorption at 280 nm, may also interfere with furfural and HMF determination. In this article, we describe a new UV spectral protocol that allows simultaneous measurement of furfural and HMF while alleviate the interference of acid soluble lignin.

EXPERIMENTAL

Materials

Mixed southern hardwood chips were kindly provided by the Riegelwood mill of International Paper Company, Riegelwood, NC. Loblolly pine chips were produced from a pine log in the pilot plant of North Carolina State University.

Milled wood lignin (MWL) were prepared and purified according to the method of Bjorkman [7,8] from extractive-free wood meal (40–60 mesh) of loblolly pine and mixed southern hardwood. Extraction with 2:1 benzene:ethanol was carried out in a soxhlet over night.

Dioxane used for MWL preparation and for UV spectra was bought from Fisher Scientific. Prior to each use, dioxane was distilled over metallic sodium. Furfural and HMF were purchased from Fisher Scientific. The standard solution of furfural and HMF (0.0079–0.1189 mmol/L) was prepared with deionized water. The sodium borohydride was purchased from Sigma Aldrich.

Methods

For the determination of acid-soluble lignin, furfural, and HMF in Klason lignin filtrate, 0.1 g of extractive-free wood meal (40–60 mesh) was used and carried out according to the Klason procedure.[8] From the filtrate (57.5 mL), three 5 mL aliquots were transferred to three 25 mL volumetric flasks. Small amount of NaOH (17.5%) was added to each flask to neutralize the filtrate to about pH 7. The first flask was filled to 25 mL with deionized water and the UV spectrum was scanned between 200 nm and 400 nm using a UV-Vis spectrophotometer (PerkinElmer Lambda XLS). To the other two flasks, approximately 30 mg of sodium borohydride was added. The reduction was terminated by the addition of small amount of HCl (3%), one after 5 min and the other one after standing overnight. Both flasks were then filled to 25 mL with deionized water and UV spectra were scanned.

For the determination of acid-soluble lignin, furfural, and HMF in the acid pretreatment filtrates, approximately 1 g of wood meal (40–60 mesh) was put into a 45 mL Parr reactor and 4 mL of sulfuric acid solution of the desired concentration were added. The reactor was placed in an aluminum block preheated to 185°C for 20 min. (The inside of the reactor reached 185°C in 5 min.) The hydrolysis was arrested by submerging the reactor in a cold water bath. After hydrolysis, the content of the reactor was carefully transferred to a 50 mL centrifuge tube using 46 mL of deionized water. After centrifugation, 1 mL of the supernatant was transferred to a 25 mL volumetric flask and dilute to 25 mL with deionized water to give the stock solution for UV measurement. From the stock solution, three 5 mL aliquots were transferred to three 25 mL

volumetric flasks and the UV spectra of unreduced and reduced samples were scanned according to the procedure described earlier.

For autohydrolysis, mixed southern hardwood chips (800 g) were hydrolyzed with 3.2 liters of hot water at the maximum temperature of 160°C in an M/K digester, with time to temperature of 40 min and time at temperature of 1 h. From the filtrate, 1 mL was diluted to 50 mL in a volumetric flask to give the stock solution for UV spectral scan. From the stock solution, three 5 mL aliquots were transferred to three 25 mL volumetric flasks and the UV spectra of unreduced and reduced samples were scanned according to the procedure described earlier.

RESULTS AND DISCUSSION

Spectrum of Furfural and HMF Before and After Reduction with $NaBH_4$

The UV spectra of furfural and HMF before and after reduction with sodium borohydride ($NaBH_4$) at various concentrations are shown in Figures 1 and 2, respectively (the reaction time were 5 minutes for both). As can be seen, furfural and HMF have an absorbance maximum at 277 nm and 285 nm, respectively, before reduction. Both absorption maxima disappeared totally upon reduction with $NaBH_4$. Therefore, the furfural or HMF content can be calculated from the absorbance difference before and after reduction (ΔA_R) at

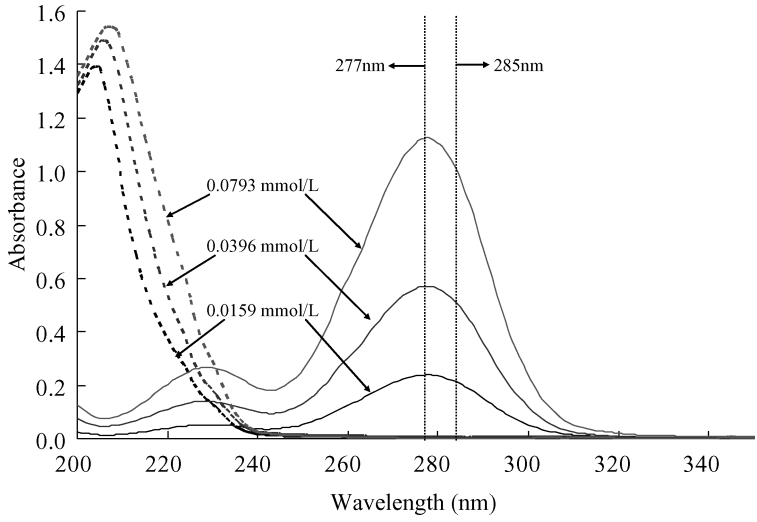

Figure 1. Spectrum of furfural before (solid lines) and after (broken lines) $NaBH_4$ reduction.

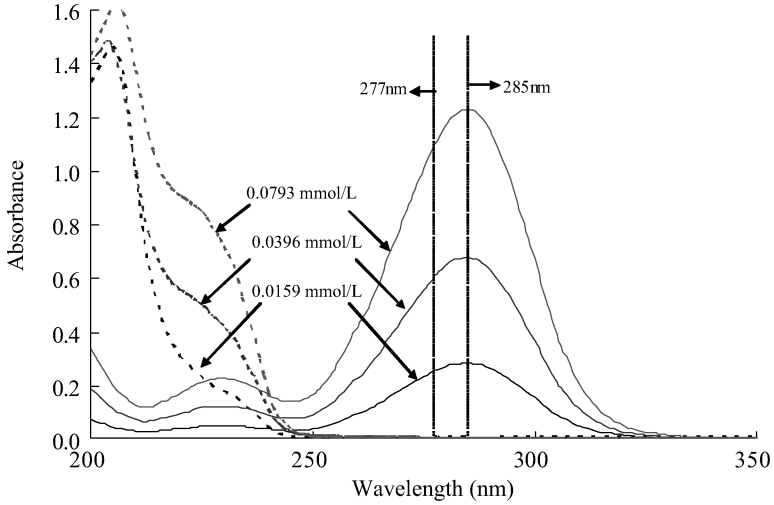

Figure 2. Spectrum of hydroxymethylfurfural before (solid lines) and after (broken lines) $NaBH_4$ reduction.

both wavelengths for both compounds. ΔA_R as a function of various concentrations at both wavelengths for furfural and HMF are shown in Figure 3. As can be seen in both figures, excellent linear relationships exist for both compounds at both wavelengths (ΔA_{R277} and ΔA_{R285}) as expected by the Lambert-Beer Law. The slope of each straight line is the molar absorption coefficient (ΔE_R) of the compound at the wavelength. Thus, furfural has a ΔE_{R277} of 14,100 L·mol^{-1}·cm^{-1} and a ΔE_{R285} of 12,410 L·mol^{-1}·cm^{-1}, whereas the corresponding numbers for HMF are $\Delta E_{R277} = 13{,}600$ L·mol^{-1}·cm^{-1} and $\Delta E_{R285} = 15{,}440$ L·mol^{-1}·cm^{-1}.

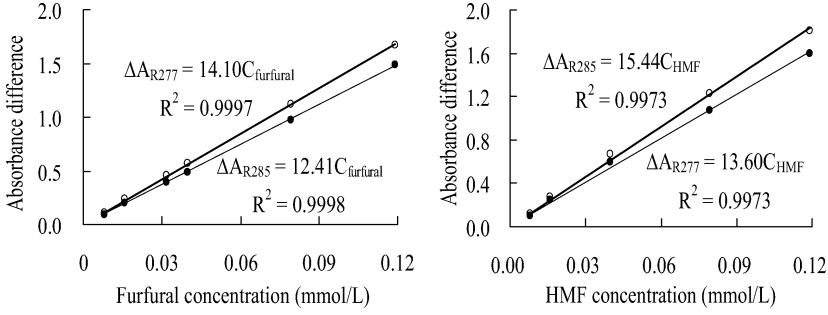

Figure 3. Standard curve of furfural and HMF.

Determination of Furfural and HMF by Dual-Wavelength UV-Spectral Method

In an UV difference spectrum of a solution containing a mixture of furfural and HMF, the contribution to absorption difference at the two wavelengths by both compounds can be deduced based on Lambert-Beer Law as shown below by Eq. (1) and Eq. (2):

$$\Delta A_{R277} = 14,100\, C_{furfural} + 13,600\, C_{HMF} \quad (1)$$

$$\Delta A_{R285} = 12,410\, C_{furfural} + 15,440\, C_{HMF} \quad (2)$$

where ΔA_{R277} and ΔA_{R285} are the absorbance difference before and after reduction at 277 nm and 285 nm, respectively; $C_{furfural}$ and C_{HMF} are concentrations of furfural and HMF in mole per liter in the mixture. The concentration of furfural and HMF can be calculated from the above simultaneous equations as shown in Eq. (3) and Eq. (4).

$$C_{furfural} = 0.0003156\, \Delta A_{R277} - 0.0002780 \Delta A_{R285} \quad (3)$$

$$C_{HMF} = 0.0002882\, \Delta A_{R285} - 0.0002536\, \Delta A_{R277} \quad (4)$$

Alternatively, the concentration of furfural and HMF can be calculated and expressed in millimole per liter using Eqs. (5) and (6), respectively.

$$C_{furfural} = 0.3156\, \Delta A_{R277} - 0.2780\, \Delta A_{R285} \quad (5)$$

$$C_{HMF} = 0.2882\, \Delta A_{R285} - 0.2536\, \Delta A_{R277} \quad (6)$$

Method Evaluation

The evaluation of this method was implemented by the recovery testing experiments, with a set of standard solutions of mixed furfural and HMF. The results are listed in Table 1. As is seen from Table 1, the recovery of furfural is from 99.0% to 100.8%, and it is from 98.5% to 101.0% for hydroxymethylfurfural. This indicates that the present method is justifiable to be applied for simultaneous analysis of furfural and hydroxymethylfurfural.

Lignin Interference

During the acidic pretreatment of wood, small amount of lignin is known to be dissolved in the solution. Lignin has a characteristic absorption maximum at 280 nm and may cause interference with the determination of furfural

Table 1. Method validations

	Actual concentration (mmol/L)		Measured concentration (mmol/L)		Recovery (%)	
Sample no.	Furfural	HMF	Furfural	HMF	Furfural	HMF
1	0.0396	0.0396	0.0399	0.0400	100.8	101.0
2	0.0198	0.0396	0.0196	0.0392	99.0	99.0
3	0.0396	0.0198	0.0393	0.0195	99.2	98.5
4	0.0159	0.0396	0.0158	0.0399	99.4	100.8
5	0.0079	0.0634	0.0079	0.0640	99.0	100.9

and HMF. The use of absorption difference before and after reduction with sodium borohydride practically eliminates most of the interference caused by the presence of lignin in the solution. Only absorption originated from those lignin structures that can be reduced by sodium borohydride would be included in ΔA_{R277} and ΔA_{R285} and thus would interfere with the quantitative determination of furfural and HMF. These structures are known to be the coniferyl/syringyl aldehydes and α-keto groups, which are commonly known as conjugated carbonyl groups.[9] However, the interference from lignin is expected to be relatively small. The amount of lignin becomes soluble in any acidic aqueous solution is small to start with and the amount of conjugated carbonyl groups in lignin is less than 10% and in most case less than 5%.[10]

UV absorption spectra of milled wood lignin (MWL) isolated from loblolly pine (pine) and mixed southern hardwoods (mixed hardwoods) are shown in Figure 4. Both spectra were determined using similar concentrations; 0.0624 $g \cdot L^{-1}$ for loblolly pine and 0.060 $g \cdot L^{-1}$ for mixed southern hardwood. Yet

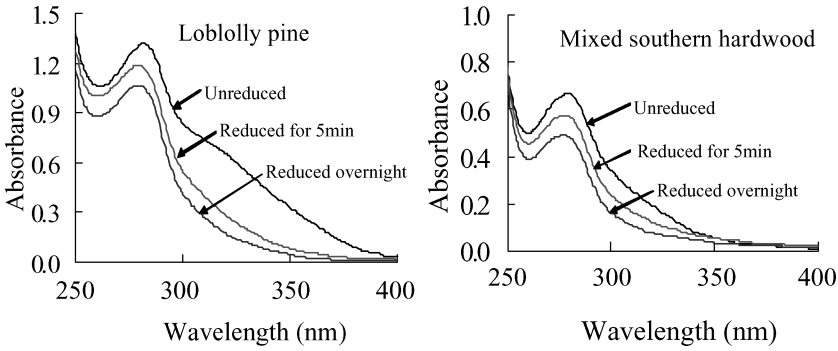

Figure 4. UV spectra of loblolly pine and mixed southern hardwood before and after sodium borohydride reduction.

the absorption at 280 nm of loblolly pine MWL is twice as much as that of mixed southern hardwood MWL. This is expected because hardwood lignin contains, in addition to guaiacylpropane unit, syringylpropane unit, which has only one third of the molar extinction coefficient of guaiacylpropane unit.[11] The reduced MWL has absorbance of 1.06 and 0.47 for pine and mixed hardwood, respectively. The absorption coefficient can be calculated to be 17.0 $L \cdot g^{-1} \cdot cm^{-1}$ for pine and 7.8 $L \cdot g^{-1} \cdot cm^{-1}$ for mixed hardwoods. These values are in good agreement with the published data.[11–13]

The absorbance difference of pine MWL at 280 nm before and after borohydride reduction (Figure 4) is 0.25. Using a molar absorbance coefficient of 10,000 $L \cdot mol^{-1} \cdot cm^{-1}$[10] for conjugated guaiacylpropane units and unit weight of 185 g/C_9 unit[12] for softwood MWL, the concentration of the conjugated carbonyl content can be calculated to be 4.6 $mg \cdot L^{-1}$ out of the 62.4 $mg \cdot L^{-1}$, or about 7%, which is in good agreement with the literature data.[10–11] For mixed hardwood MWL, the absorption difference before and after borohydride reduction is 0.19. Unfortunately, molar absorption coefficient of conjugated syringylpropane unit at 280 nm is not available because syringylpropane model compound with a conjugated α-carbonyl group exhibits an absorption maximum at 300 nm (molar absorption coefficient of 10400 $L \cdot mol^{-1} \cdot cm^{-1}$[11]) rather than at 280 nm. Furthermore, hardwood lignin consists of syringyl- and guaiacyl-propane units, the ratio of which (S/G) varies from species to species.[10–11] In spite of the uncertainty, for hardwood lignin of unknown S/G ratio, an estimated value of 10,000 $L \cdot mol^{-1} \cdot cm \cdot^{-1}$ is probably in the ball park because the absorption maximum of the conjugated syringylpropane is broad. Using this value and a unit weight of 210 g/C_9 for birch MWL[10] as representative of hardwood lignin, the concentration of the conjugated carbonyl content in the mixed hardwood MWL can be calculated to be 4.0 $mg \cdot L^{-1}$ out of the 60.0 $mg \cdot L^{-1}$, or about 7%, which is again in agreement with literature data.[10–11]

The potential interference of lignin to the absorbance difference spectra of an acid filtrate can be calculated to be 4.0 ($0.25/62.4 \times 1000$) for pine MWL for every 1 g per liter of lignin present in the filtrate. The corresponding value for hardwood is 3.2. These amounts are relatively small compared with those of furfural and HMF, which give rise to absorbance of 147 ($14{,}100/96$ $g \cdot mol^{-1}$) and 123, respectively, for every one gram per liter of them in the filtrate. Thus, it can be concluded that the interference of lignin for the quantitative determination of furfural and HMF in an acidic filtrate is negligible, unless the analysis is for filtrate sample with very low concentration of these compounds and with relatively high lignin concentration. This scenario is most likely to happen with hardwood hydrolysates, as hardwoods are known to have higher acid-soluble lignin than softwoods. Even in a hypothetical case where there were 4 times higher lignin as furfural or HMF in the hydrolysate, the effect would be overestimate furfural by 9% ($3.2 \times 4/147$) and HMF by 10%.

The interference of lignin can be further decreased by a short time borohydride reduction of the filtrate. While both furfural and HMF are completely

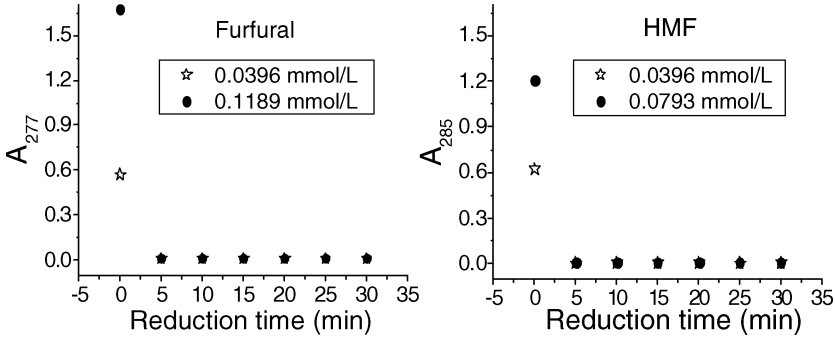

Figure 5. Rate of borohydride reduction of furfural and HMF.

reduced by borohydride in less than 5 min as shown in Figure 5, only one half of the absorbance of the conjugated carbonyl groups is reduced in 5 min (Figure 4). It has been known that conjugated carbonyl groups in lignin with free phenolic hydroxyl group are reduced at a much slower rate than the etherified conjugated carbonyl groups.[12] Thus, if lignin interference is a problem, it can be decreased to about one half by using a short time reduction (5 min).

It is noteworthy that the potential interference from conjugated carbonyl groups of lignin may be even smaller than those obtained from MWL. It is known that conjugated carbonyl groups are formed during ball-milling.[7,13] Thus, the contribution from lignin to absorbance difference of furfural and HMF can be considered the upper limit unless the biomass to be analyzed is subject to some oxidative process prior to analysis.

The concentration of lignin in the filtrate can be estimated by using the absorbance at 205 nm of the unreduced spectrum[8] or using the absorbance at 280 nm of the borohydride reduced spectrum. The latter should give good estimation for the softwood lignin using the average extinction coefficient of 16.5 $L \cdot g^{-1} \cdot cm^{-1}$.[11] For hardwood lignin, the extinction coefficient varies according to the S/G ratio.[11–13] Use of absorbance at 205 nm may be better for hardwood lignin.[9] Nevertheless, the absorbance coefficient of most hardwood lignin at 280 nm will fall within 7–10 $L \cdot g^{-1} \cdot cm^{-1}$[11–12] and 8.5 $L \cdot g^{-1} \cdot cm^{-1}$ should be a good number for estimation of lignin concentration. Once the lignin concentration of the filtrate is known, the interference of lignin may be corrected if necessary.

In the dilute acid hydrolysates of non-wood biomass, p-coumaric and ferulic acids are often present in substantial amounts.[2] However, these compounds will not interfere with the furfual and HMF determination as these compounds are not reduced by sodium borohydride under slightly alkaline conditions.[11] Reduction of these conjugated double bonds requires strong electron-withdrawing groups to polarize the double bond.[14] The carboxylic acid and the phenolic hydroxyl groups will be ionized under the alkaline

Table 2. Determination of lignin, furfural, and HMF in acidic filtrates of pine and mixed southern hardwood

Samples[1]	A_{205nm}	ΔA_{277nm}	ΔA_{285nm}	Lignin %[2]	furfural %[2]	HMF %[2]
ASL loblolly pine	0.2753	0.5176	0.4982	0.70	0.67	0.43
ASL hardwood	1.5524	0.8892	0.8864	3.81	0.89	1.02
AuH hardwood	2.0917	0.3049	0.2944	1.90	0.14	0.09
AH hardwood, 0.1%	0.4896	0.1387	0.1283	2.56	0.45	0.13
AH hardwood, 0.5%	0.4149	0.6105	0.5467	2.63	2.72	0.24
AH Loblolly pine, 0.1%	0.1608	0.1663	0.1561	0.87	0.50	0.19
AH Loblolly pine, 0.5%	0.2123	0.5539	0.5265	1.43	2.02	1.05

[1]ASL stands for acid soluble lignin, AuH stands for auto hydrolysis, and AH stands for acid hydrolysis. All hardwood samples are mixed southern hardwood.
[2]Weight percent of wood.

conditions and provide very weak electron-withdrawing power to facilitate the reaction.

Determination of Lignin, Furfural, and HMF in Acidic Filtrates of Pine and Mixed Southern Hardwood

In Klason lignin determination method, acid soluble lignin is determined by absorbance at 205 nm without reduction. Using the same filtrate, furfural and HMF content can be determined using the absorbance difference before and after borohydride reduction. Acid soluble lignin, furfural, and HMF contents as weight percent of wood are given in Table 2 for loblolly pine and mixed southern hardwood. The values for acid soluble lignin content agree with those of the published data (i.e., less than 1% for softwood and around 3–4% for most hardwoods). The total contents of furfural and HMF are low in the filtrate of Klason lignin determination method, slightly over 1% for Scots pine, and about 2% for mixed southern hardwoods.

Also listed in Table 2 are furfural, HMF, and acid-soluble lignin contents in the filtrate of acid pretreatment at two levels of acid concentration for both softwood and hardwood. As can be seen in Table 2, the effect of acid concentration on the formation of furfural and HMF is obvious. While only small amounts of furfural and HMF are formed at 0.1%, substantial amounts are formed at 0.5% concentration for both softwood and hardwood. Because an objective of the acid pretreatment is to convert the hemicelluloses to fermentable sugars and because furfural and HMF are formed from pentosan and hexosan, respectively, through dehydration of two moles of water, their formation has relatively large effect of loss of fermentable sugars. The formation of 2.72% and 0.24%

of furfural and HMF, respectively, from hardwood is equivalent to the loss of 3.74% of pentosan and 0.31% of hexosan from hemicelluloses during the acid pretreatment. The sum accounts for a loss of 14% of hemicelluloses to furfural and HMF during the acid pretreatment, assuming an average hemicellulose content of 30% for most hardwoods. For pine, the equivalent losses are 2.78% for pentosan and 1.35% of hexosan, accounting for about 17% of the average hemicellulose content of 25% in softwood.

While acid soluble lignin has a relatively small effect on the determination of furfural and HMF at 277–285 nm, the estimate of acid soluble lignin content by absorbance 205 nm is affected to a larger extent by the presence of furfural and HMF. The absorptivities of furfural and HMF can be calculated to be 11.7 and 21.3 $L \cdot mol^{-1} \cdot cm^{-1}$, respectively, as compared with 110 $L \cdot mol^{-1} \cdot cm^{-1}$ for that of lignin. Thus, for the presence of an equal amount of lignin furfural and HMF in the hydrlysate, the acid soluble lignin content could be overestimated by 11% due to the presence of furfural and by an additional 19% due to the presence of HMF. Therefore, for those hydrolysate with relatively high contents of furfural and HMF, it is probably more accurate to estimate the acid soluble lignin by the absorbance of the reduced hydrolysate at 280 nm.

CONCLUSIONS

A dual-wavelength UV-spectrophotometric method using absorbance difference spectrum before and after reduction with $NaBH_4$ was developed for simultaneous determination of furfural and hydroxymethylfurfural in the acidic filtrate with minimal interference from lignin. This method is simple, rapid, and accurate, which allows one to access the formation of these inhibitory compounds under acidic pretreatment conditions for any biorefinery study and allows correction of carbohydrate losses in the acidic filtrate due to their formation.

REFERENCES

1. van Heiningen, A. Converting a kraft pulp mill into an integrated forest products biorefinery. Pulp & Paper Canada **2006**, *107* (6), 38–43.
2. Martinez, A.; Rodriguez, M.E.; York, S.W.; Preston, J.F.; Ingram, L.O. Use of UV absorbance to monitor furans in dilute acid hydrolysis of biomass. Biotechnol. Prog. **2000**, *16*, 637–641.
3. Tu, D.; Xue, S.; Meng, C.; Mansilla, A.E.; Pena, A.M.D.A.; Lopez, F.S. Simultaneous determination of 2-furfuraldehyde and 5-hydroxymethyl-2-furfuraldehyde by Derivative Spectrophotometry. J. Agric. Food Chem. **1992**, *40* (6), 1022–1025.
4. Rao, C.N.R. *Ultra-Violet and Visible Spectroscopy: Chemical Applications;* Butterworth: London, 1961.

5. Silverstein, R.M.; Bassler, G.C.; Morrill, T.C. *Spectrometric Identification of Organic Compounds*; John Wiley & Sons: New York, 1974.
6. Marcy, J.E.; Rouseff, R.L. High-performance liquid chromatographic determination of furfural in orange juice. J. Agric. Food Chem. **1984**, *32* (5), 979–981.
7. Bjorkman, A. Studies on finely divided wood. Part I. Extraction of lignin with neutral solvents. Svensk Papperstin. **1956**, *59*, 477–485.
8. Lundquist, K. Isolation and purification of lignin in wood. In *Methods in Lignin Chemistry,* Lin, S.Y., Dence, C. W., Eds.; Spring-Verlag: Berlin, 1992; 65–66.
9. Dence, C.W. Determination of lignin. In *Methods in Lignin Chemistry,* Lin, S.Y., Dence, C. W., Eds.; Spring-Verlag: Berlin, **1992**; 34–39.
10. Lai, Y.Z.; Sarkanen, K.V. Isolation and structural studies. In *Lignins: Occurrence, Formation, Structure and Reactions*; Sarkanen, K.V.; Ludwig, C. H., Eds.; Wiley Interscience, New York, **1971**; 226–228.
11. Goldschmid, O. Ultraviolet spectra. In *Lignins: Occurrence, Formation, Structure and Reactions*; Sarkanen, K.V.; Ludwig, C.H., Eds.; Wiley Interscience, New York, **1971**; 241–263.
12. Sarkanen, K.V.; Chang, H.-M.; Allan, G.G. Species variation in lignins III. Hardwood lignins. TAPPI J. **1967**, *50* (12), 587–590.
13. Chang, H.-M.; Cowling, E.B.; Brown, W.; Adler, E.; Miksche, G. Comparative studies on cellulolytic enzyme lignin and milled wood lignin of sweetgum and spruce. Holzforschung **1975**, *29* (5), 153–159.
14. Kadin, S.B. Reduction of conjugated double bonds with sodium borohydride. J. Org. Chem. **1966**, *31*, 620–622.