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Quantitative Analysis of Lignocellulosic Components of Non-Treated and Steam Exploded Barley, Canola, Oat and Wheat Straw Using Fourier Transform Infrared Spectroscopy

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Abstract: Rapid and cost effective quantification of lignocellulosic components (cellulose, hemicelluloses and lignin) of agricultural biomass (barley, canola, oat and wheat) is essential to determine the effect of various pre-treatments (such as steam explosion) on biomass used as feedstock for the biofuel industry. Fourier Transformed Infrared (FTIR) spectroscopy was considered as an option to achieve this objective. Regression equations having R^2 values of 0.89, 0.99 and 0.98 were developed to predict the cellulose, hemicelluloses and lignin compounds of biomass, respectively. The average absolute difference in predicted and measured cellulose, hemicellulose and lignin in agricultural biomass was 7.5%, 2.5%, and 3.8%, respectively.

Key words: Fourier Transformed Infrared (FTIR), photoacoustic spectroscopy, lignin, cellulose, hemicellulose.

1. Introduction

Agricultural (lignocellulosic) biomass residues such as barley, canola, oat and wheat straw have the potential to be used as the feedstock for the biofuel industry [1]. After harvest, the low bulk density straw has to be processed and densified for efficient handling and transportation, and reaps the potential economic benefits [2].

It has been reported by Sokhansanj et al. [3] that densified straw often results in poorly formed pellets or compacts, and are difficult to handle and costly to manufacture. This is primarily due to the lack of complete understanding on the binding characteristics of biomass at the molecular level. The natural binding characteristics of lignocellulosic biomass can be

enhanced by modifying the structure of cellulose-hemicellulose-lignin matrix by application of pre-processing and pre-treatment methods [3]. However, the effect of various pre-processing and pre-treatment methods on the lignocellulosic matrix at the molecular level is not well understood. Applications of pre-processing methods such as size reduction or increasing porosity, and pre-treatment techniques such as steam explosion and pulse electric field on agricultural biomass have demonstrated an improvement in pellet (compact) quality that can be attributed to the changes in the lignocellulosic components and distribution [4-7]. Therefore, it is critical to rapidly quantify the change in cellulose, hemicelluloses and lignin components of biomass due to application of pre-treatment methods.

Infrared spectroscopy has the potential to produce qualitative and quantitative analytical data for samples with minimum or no sample preparation, and at high

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speed and throughput [8-11]. Traditionally, chemical analyses of the individual components (e.g., lignin) of lignocellulosics have been performed by acid hydrolysis followed by gravimetric determination of lignin [12]. These methods can provide highly precise data. However, these methods are laborious, time-consuming, and, consequently, expensive to perform and sample throughput is limited. Hence, there is a need to develop analytical tools that can be used to rapidly and inexpensively measure the chemical composition of biomass [12-14].

One of the early studies on quantitative analysis of component mixtures of acetylsalicylic acid, salicylic acid and filler or binder with varying concentration using Fourier transform infrared (FTIR) photoacoustic spectroscopy (PAS) was performed by Rosenthal et al. [15]. They were able to develop partial least square models with high correlation coefficients. A study by Belton et al. [16] successfully used FTIR spectroscopy for the quantitative analysis of protein and starch mixtures. Similarly, Moh et al. [17] used FTIR spectroscopy to investigate and develop a foundation for the rapid determination of β -carotene content of crude palm oil. They have also developed separate partial least squares calibration models to predict β -carotene based on spectral region from 976 to 926 cm^{-1} for FTIR spectroscopy. The use of infrared spectroscopy in the study of fats and oils has been reviewed by Guillen and Cabo [18]. Van de Voort et al. [19] developed FTIR spectroscopy that operates in the mid infrared region (4,000-400 cm^{-1}) and has been proven to be a powerful tool for quantitative analysis of fats and oils.

Rodrigues et al. [20] have obtained linear correlation with high regression coefficients to estimate lignin content in *Eucalyptus globulus* wood, using bands characteristic for lignin in FTIR spectra and bands characteristic for carbohydrate as reference. Similarly, the estimate of lignin and polysaccharide content in eucalyptus and pine acetosolv pulps was performed by Vazquez et al. [14] using FTIR spectroscopy to obtain

mathematical models. They have employed a STEPWISE regression analysis for the selection of spectra bands that correlate satisfactorily with experimental results.

The characterization and stages of organic municipal solid waste matter decomposition during mechanical and biological treatment was determined using the FTIR spectroscopy [21]. The technique was used to observe the maturity and stability of waste organic matter based on the missing spectral bands that indicate metabolic activities.

Tucker et al. [11] successfully performed the analysis of glucose, mannose, xylose, and acetic acid using FTIR spectroscopy in conjunction with high-performance liquid chromatography for the quantitative analysis of liquors from dilute-acid-pretreated softwood and hardwood slurries. Similarly, Bjarnestad and Dahlman [22] employed the FTIR PAS technique in combination with partial least square analysis to accurately predict the contents of carbohydrates in hardwood and softwood pulps. In addition, the analytical procedure developed could be used on a routine basis to quantify pulp constituents with considerably less effort and in shorter time than is possible using chemical analysis. Also, Nuopponen et al. [23] successfully studied the chemical modification of Scots pine wood in thermal treatments in the range of 100-240 °C using the FTIR technique with the assistance of PAS detector. They have established that lignin became partly extractable by acetone at 180 °C and the amount of soluble lignin increased with an increase in temperature up to 220 °C. In addition, degradation of hemicelluloses was also detected from the FTIR spectral data.

Gelbrich et al. [13] characterized the bacterial degradation of waterlogged softwood samples using FTIR spectroscopy. They have established a linear relationship between lignin content and the extent of bacterial degradation in softwood.

The literature review of lignocellulosic biomass have indicated that infrared spectroscopy could be used

successfully to study the chemical structure of cellulose, hemicellulose and lignin in various agricultural biomasses as applied to food, feed, biocomposite, textile, and paper and pulp industries [2]. It is evident from previous studies that FTIR spectroscopy has the potential to perform qualitative and quantitative analysis of agricultural (lignocellulosic) biomass to determine change in cellulose-hemicellulose-lignin composition prior to and after application of various pre-processing and pre-treatment methods. Therefore, the objective of this work was to estimate critical parameters in analytical specification of lignocellulosic biomass and consequently, to develop and validate a rapid method for the qualitative and quantitative analysis of cellulose-hemicellulose-lignin composition of non-treated and steam exploded barley, canola, oat and wheat straw using FTIR PAS.

2. Materials and Method

2.1 Sample Material Preparation

Four types of agricultural biomass (barley, canola, oat and wheat straw) were used for FTIR spectroscopy experiments. The straw samples were acquired in square bale form during the summer of 2008 from the Central Butte area of Saskatchewan, Canada. The moisture contents of ground barley, canola, oat and wheat straw were 6.7, 6.7, 5.3 and 4.0% (w.b.), respectively. The moisture content was determined using ASABE Standard S358.2 [24].

The non-treated straw samples were manually chopped using a pair of scissors and subsequently fine-ground in a precision grinder (Falling Number, Model No. 111739, Huddinge, Sweden) having a screen size of 1.0 mm. The steam explosion of straw was performed at the FPInnovations, Forintek pilot plant continuous steam explosion facility at Quebec City, Quebec. The Andritz (ANDRITZ AG, Graz, Austria) pressurized disc refiner having a plate gap of 0.5 mm, powered by a 160 kW (215 hp) motor with a variable speed drive set to operate at 2000 rpm was

used to steam explode the straw. Similar to non-treated straw, the steam exploded straw was ground in a precision grinder having a screen size of 1.0 mm. Further details on material preparation and the steam explosion process could be found in the research of Adapa et al. [25].

2.2 Reference Material Preparation

Quantitative analysis of cellulose, hemicelluloses and lignin composition of non-treated and steam exploded sample material is critical in order to predict and evaluate the change in natural binding characteristics of straw. Therefore, pure cellulose (microcrystalline powder), hemicelluloses (xylan from birch wood) and lignin (hydrolytic) powders were obtained from Sigma-Aldrich Canada Ltd. (St. Louis, MO), and were subsequently mixed in different proportions (Table 1) to determine the relationship (predictive models) between their respective quantity in the mixture and representative FTIR spectra. Carbon black powder reference spectrum was used to correct for FTIR wavenumber-dependent instrumental effects.

2.3 Lignocellulosic Composition of Agricultural Biomass

It is essential to validate the predicted lignocellulosic

Table 1 Pure cellulose, hemicelluloses and lignin mixtures used to obtain reference spectra.

Reference Mixtures	Lignin (%)	Cellulose (%)	Hemicellulose (%)
C1H0L0*	100	0	0
C0H1L0	0	100	0
C0H0L1	0	0	100
C5H2L2	50	25	25
C2H5L2	25	50	25
C2H2L5	25	25	50
C7H2L0	75	25	0
C2H7L0	25	75	0
C2H0L7	25	0	75
C0H2L7	0	25	75
C3H3L3	33	33	33
C7H0L2	75	0	25
C0H7L2	0	75	25

*: C, H and L represent Cellulose, Hemicellulose and Lignin, respectively.

quantity of sample agricultural straw (section 2.1) using correlation models developed from the analysis of reference material spectra (section 2.2). The experimental lignocellulosic composition of agricultural straw was performed in replicates of three at the Agriculture and Agri-Food Canada, Saskatoon lab facility using the modified NREL LAP method for “Determination of Structural Carbohydrates and Lignin in Biomass” [26]. This procedure uses a two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. During this process, the lignin fractionates into acid insoluble material and acid soluble material, while the polymeric carbohydrates are hydrolyzed into the monomeric forms, which are soluble in the hydrolysis liquid and subsequently measured by HPLC (Waters Acquity UPLC, Waters, MA). Percentage cellulose in the samples was measured by using the percentage glucan content, while percentage hemicelluloses was measured by adding percentage mannose, galactose, xylose and arabinose content in the biomass samples.

2.4 Fourier Transformed Infrared (FTIR) Equipment

Mid-IR beamline (01B1-1, energy range: 4,000 to 400 cm^{-1}) at the Canadian Light Source Inc. (CLS, University of Saskatchewan, Saskatoon SK, Canada) was used to collect IR data of reference compounds and fine-ground sample agricultural straw in replicates of three. The beamline has a MTEC Model 300 photoacoustic cell (MTEC Photoacoustic Inc., Ames, IA) for FTIR photoacoustic spectroscopy (FTIR PAS) of bulk samples. The FTIR spectra of reference and straw samples were recorded using Globar source (silicon carbide rod). The FTIR PAS determines the absorption of radiation by samples via measuring the changes in thermal expansion of gas surrounding the sample using a microphone [27]. The reference and straw samples were filled in the sample cup and purged with dry helium to remove water vapor and CO_2 from the sample chamber. The spectrum for each sample was recorded separately by averaging 32 interferograms collected from wavenumbers of 2,000

to 400 cm^{-1} at a resolution of 4 cm^{-1} .

The OPUS 6.5 (Bruker Optics Inc., Billerica, MA) software was used to record and analyze the FTIR PAS data. The software Origin (version 7.5, OriginLab, Northampton, MA) was used to plot the data.

2.5 Quantitative Analysis Using FTIR Spectra

The quantitative analysis of absorption spectrometry is based on the Bouguer-Beer-Lambert law [28]. According to this law, the absorbance at any frequency for a single compound in a homogenous medium is expressed as:

$$A = abc$$

where A is the measured sample absorbance at the given frequency, a is the molecular absorptivity at the frequency, b is the path length of source beam in the sample, and c is the concentration of the sample. The law implies that the intensities of absorption bands are linearly proportional to the concentration of each component in a homogenous mixture or solution [28].

Therefore, a number of quantification parameters, which included peak height, peak area, and derivatives, were used in quantitative analysis. In this study, the authors used peak height as the quantification parameter since preliminary analysis using peak area did not produce an identifiable trend and agreeable results.

2.5.1 Peak Height Method

The spectral information of pure (100%) cellulose, hemicelluloses and lignin was developed in order to identify characteristic peaks of respective components. The distinguishable characteristic peaks of individual components were chosen to measure respective peak heights. The height of the peak (intensity of maximum absorption) was measured by calculating the difference between the peak intensity ($\overline{\nu_{\max}}$) of the absorption band and the baseline ($\overline{\nu_{\min}}$) (Fig. 1). In this study, the baseline was considered at zero intensity. Therefore, peak height was equal to peak intensity ($\overline{\nu_{\max}}$).

The characteristic wavenumbers identified for pure cellulose, hemicelluloses and lignin were used as the guide to calculate the characteristic peak height for respective components in the reference compound

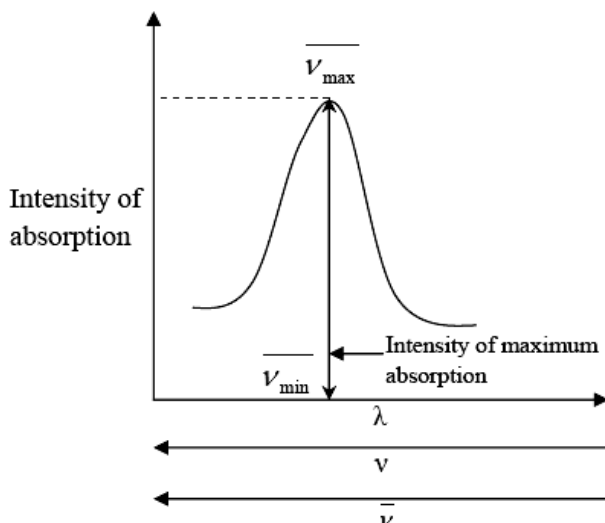


Fig. 1 Schematic of absorption spectra [29] (Note: λ is wavelength, ν is frequency and $\bar{\nu}$ is wavenumber of IR radiation).

mixtures in replicates of three. Subsequently, same characteristic wavenumbers were used to measure the characteristic peak height for cellulose, hemicelluloses and lignin components in the fine-ground non-treated and steam exploded straw sample spectra in replicates of three.

2.5.2 Data Normalization Procedure

The agricultural biomass samples FTIR spectra intensity data were corrected for any wavenumber-dependent instrumental effects through division by carbon black reference spectrum intensity. This strategy implicitly assumes that the stability of the instrumentation used is adequate to ensure reliable results, even though the sample and reference spectra were acquired at different times [30]. Also, the effect of reference and sample straw bulk density was eliminated by dividing the data with respective mass of the sample contained in the PAS sample cup.

In order to further standardize the methodology, the carbon black and mass normalized FTIR data were normalized to 0 to 1 (intensity) by dividing the intensity spectra of individual biomass samples by corresponding maximum intensity value. Hence the normalization process ensures that the model is adaptable for quantitative analysis of FTIR spectra obtained for any lignocellulosic biomass.

2.6 Statistical Analysis

The trend in variation of peak height of cellulose, hemicellulose and lignin components in the reference mixtures was correlated to their percentage composition. In addition, two replicates out of three were selected at random from the fine-ground biomass sample spectra and were included with reference mixture spectra. Regression models for cellulose, hemicelluloses and lignin were developed that has the capability to predict quantity (percentage composition) of cellulose, hemicelluloses and lignin in agricultural biomass.

The experiments were set up as completely randomized experimental design with 3 replications of FTIR tests on reference samples. The percentage composition of cellulose, hemicelluloses and lignin from chemical analysis was considered as dependent variable, while respective characteristic wavenumbers were treated as independent variables. Statistical analyses were conducted using SAS for Windows (version 9.1) [31]. In order to understand and explain the effect of characteristic wavenumbers and their interactions on percentage cellulosic composition, the SAS general linear model (GLM) for polynomial regression and stepwise analysis were performed [31].

Subsequently, the characteristics peak heights for lignocellulosic composition from the third replicate of biomass sample spectrum was inserted in the developed models to predict percentage composition of lignocellulosic components in the non-treated and steam exploded barley, canola, oat and wheat straw. The average absolute difference between predicted and lab measured lignocellulosic composition was determined.

3. Results and Discussion

3.1 Lignocellulosic Composition of Agricultural Biomass

Table 2 shows the lignocellulosic composition of non-treated and steam exploded barley, canola, oat and wheat straw samples. In general, the cellulose,

Table 2 Lignocellulosic composition of non-treated and steam exploded agricultural straw.

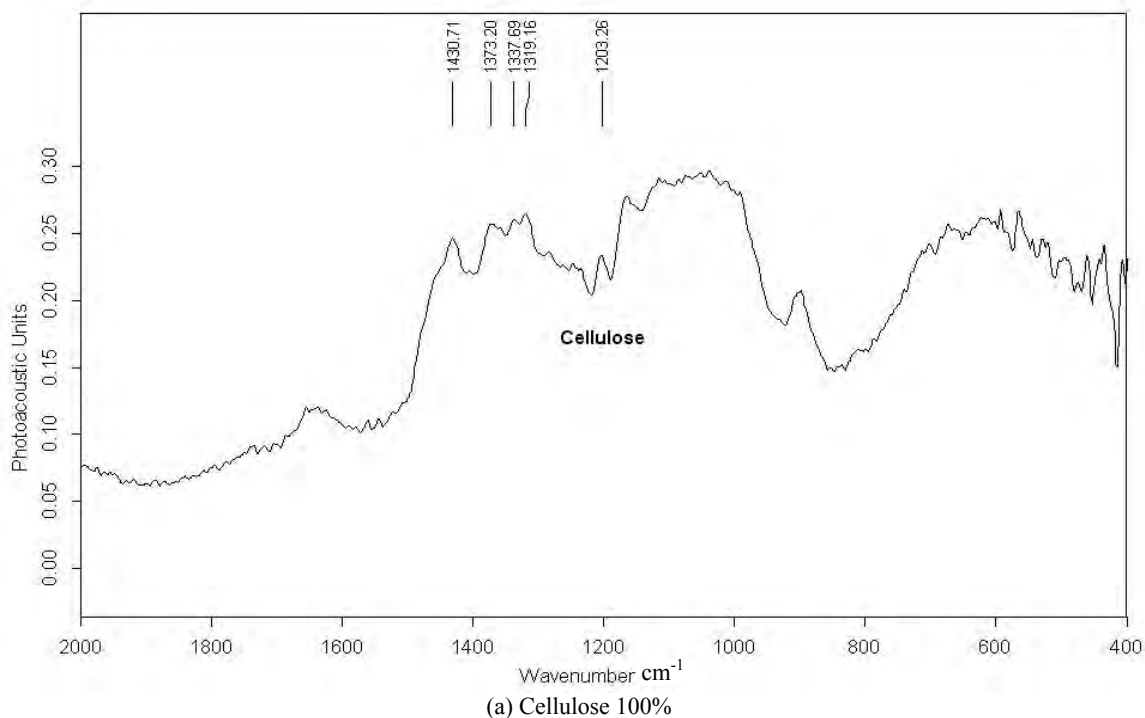
Composition (% DM)	Barley straw		Canola straw		Oat straw		Wheat straw	
	NT	SE	NT	SE	NT	SE	NT	SE
Cellulose ^b	22.7 ± 0.9 ^a	25.3 ± 1.8	22.4 ± 0.8	27.5 ± 1.1	25.4 ± 1.0	27.4 ± 2.4	27.1 ± 1.0	29.9 ± 1.4
Hemicellulose ^c	21.2 ± 0.5	21.0 ± 1.4	16.9 ± 0.5	20.2 ± 0.7	21.7 ± 0.9	18.8 ± 1.2	21.1 ± 0.5	19.7 ± 0.9
Galactose	0.9 ± 0.0	0.7 ± 0.0	1.0 ± 0.0	0.9 ± 0.1	0.8 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.9 ± 0.1
Mannose	1.6 ± 0.2	1.5 ± 0.0	2.3 ± 0.1	1.9 ± 0.4	1.4 ± 0.0	1.7 ± 0.1	1.6 ± 0.1	2.8 ± 0.2
Xylose	14.4 ± 0.3	15.3 ± 1.0	11.5 ± 0.5	14.3 ± 0.2	15.1 ± 0.8	13.3 ± 1.0	14.9 ± 0.4	13.5 ± 0.4
Arabinose	4.4 ± 0.2	3.5 ± 0.5	2.0 ± 0.1	3.2 ± 0.0	4.4 ± 0.2	3.1 ± 0.2	3.9 ± 0.1	2.6 ± 0.2
Total lignin ^d	21.0 ± 0.6	21.6 ± 0.6	19.6 ± 0.6	22.3 ± 0.2	19.5 ± 0.6	23.7 ± 0.2	22.5 ± 0.7	24.2 ± 0.3
Soluble lignin	1.6 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	1.2 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.4 ± 0.0	1.0 ± 0.1
Insoluble lignin	19.4 ± 0.6	20.2 ± 0.6	18.0 ± 0.6	21.1 ± 0.1	17.9 ± 0.7	22.4 ± 0.1	21.0 ± 0.7	23.3 ± 0.4

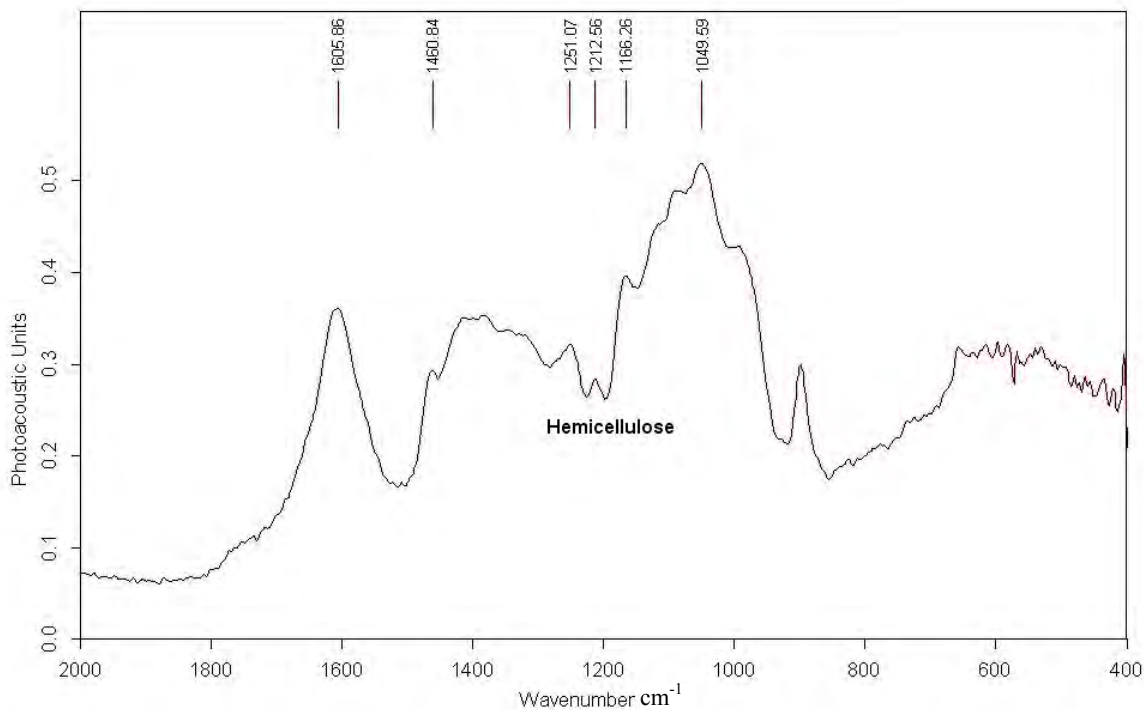
DM-Dry Matter; NT-Non-Treated; SE-Steam Exploded; ^a Average and standard deviation of 3 replicates at 95% confidence interval; ^b %Cellulose = % glucan; ^c %Hemicellulose = % (mannose + galactose + xylose + arabinose); ^d %Total Lignin = % (soluble lignin + insoluble lignin).

hemicelluloses and lignin content of steam exploded straw was higher than non-treated straw. This may be due to other components (soluble lignin, loosely-bound sugars) being washed away during steam explosion, thereby leaving the proportion of insoluble lignin, cellulose and hemicellulose in the resulting dried steam exploded sample higher than the non-treated samples (i.e. higher % of dry mass).

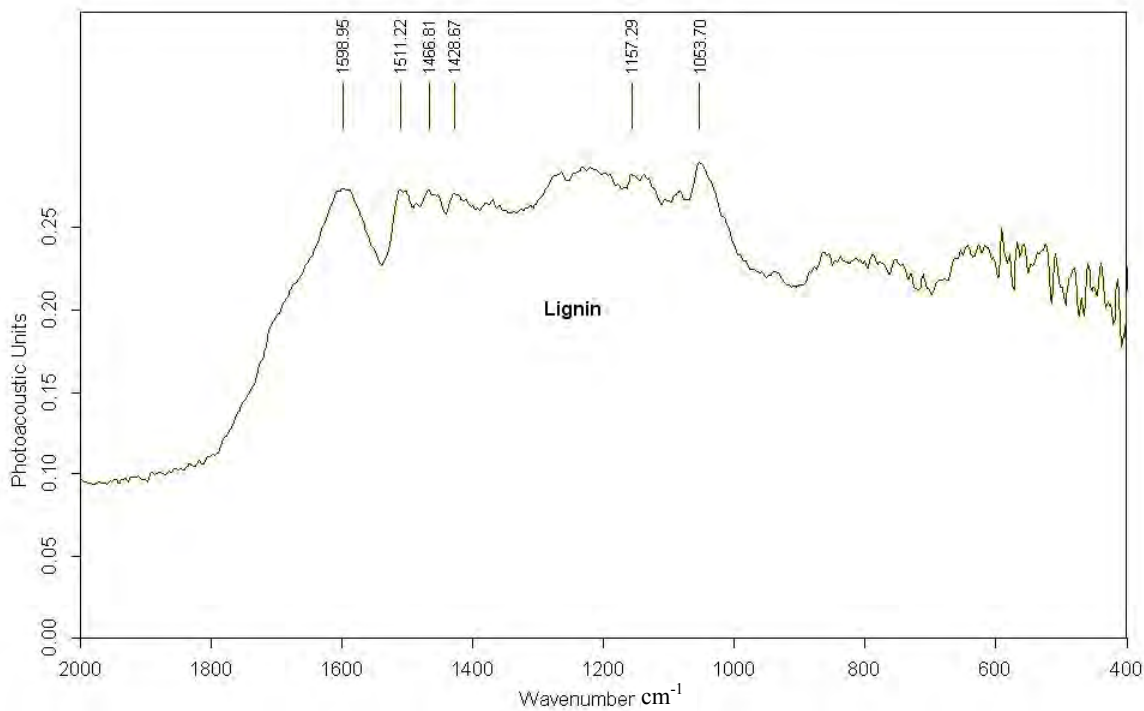
3.2 Reference Material Spectra

Fig. 2 represents the FTIR PAS spectra of pure cellulose, hemicellulose and lignin powder in the range of 2,000 to 400 cm^{-1} . The characteristic/prominent peaks for cellulose, hemicellulose and lignin, and their peak assignments are provided in Table 3. The cellulose spectrum had five distinct peaks at wavenumbers of





(b) Hemicellulose 100%



(c) Lignin 100%

Fig. 2 FTIR PAS spectra of pure cellulose (microcrystalline powder), hemicelluloses (xylan from birch wood) and lignin (hydrolytic) powders showing the prominent peaks at characteristic wavenumbers.

1,431, 1,373, 1,338, 1,319 and 1,203 cm^{-1} (Fig. 2a). Similarly, hemicellulose (xylan) had prominent peaks at wavenumbers of 1,606, 1,461, 1,251, 1,213, 1,166 and

1,050 cm^{-1} (Fig. 2b). The lignin spectrum showed characteristic peaks at wavenumber of 1,599, 1,511, 1,467, 1,429, 1,157 and 1,054 cm^{-1} (Fig. 2c).

Table 3 Characteristic/ prominent peaks of pure cellulose, hemicellulose and lignin, and their peak assignments [2]

Wavenumbers (cm ⁻¹)	Cellulose 100%	Hemicellulose 100%	Lignin 100%	Peak Assignment
1650-1600	--	1,606	--	1,600-quadrant ring stretching (aromatic lignin) [32, 33]; 1,600-1,610-aromatic skeletal vibration [33, 34]; 1,635-carbonyl stretching conjugate with aromatic rings [35]
1600-1550	--	--	1,599	1595-very strong aromatic ring stretch, aromatic C-O stretch [36, 37]; 1,595-phenylpropanoid polymer [38]; 1,595-aromatic skeletal vibrations plus C = O stretch [39]
1550-1500	--	--	1,511	1,510-semicircle ring stretching (aromatic lignin) [32, 33, 40], 1,510-phenylpropanoid polymer [38]; 1,510-very strong aromatic ring stretch, aromatic C-O stretch [36, 37]; 1,513-aromatic C = C stretch [41]; 1,514-semi-circle stretch of para-substitute benzene rings [8, 42]; 1,550-protein [8]
1500-1450	--	1,461	1,467	1,462-C-H deformation (methyl and methylene) [34]
1450-1400	1,431	--	1,429	1,420-weak C-O stretching [33, 43]; 1,430-CH ₂ in-plane bending vibrations [44, 45]; 1,433-aromatic C = C stretch [41]
1400-1350	1,373	--	--	1,370-weak C-O stretching [33, 43]; 1,380-C-H symmetric and asymmetric deformation [41]; 1,382-C-O stretch [46]
1350-1300	1,319, 1,338	--	--	1,335-weak C-O stretching [33, 43]; 1,336-C-H ring in-plane bending vibrations [44, 45]
1300-1250	--	1,251	--	1,250-Acetylated Hemicellulose [38]
1250-1200	1,203	1,213	--	1,246-weak C-O stretching [33, 43]; 1,246-Hemicellulose [8, 33]; 1,250-Acetylated Hemicellulose [38]; 1,250-acetylated hemicelluloses [8]
1200-1150	--	1,166	1,157	1,160-glycosidic linkage [47]; 1,162-C-O-C ring vibrational stretching [44, 45]
1100-1050	--	--	1,054	1,078-β(1-3) polysaccharide [48]; 1,098-weak absorbance [37]
1050-1000	--	1,050	--	1,035-C-O, C = C and C-C-O vibrational stretching [44, 45]; 1,045-C-OH bending [35, 47, 49]; 1,018-galactomannans [48]; 1,025-non-structural CHO [33]

3.3 Quantitative Analysis

After following the data normalization process described in section 2.5.2, the effect of characteristic wavenumbers and their interaction on percentage composition of cellulose, hemicelluloses and lignin were analyzed using SAS GLM procedure and was considered significant when the value of $t \leq 0.05$. In addition, polynomial regression models having highest R^2 values were obtained (Table 4). Also, the stepwise method in the regression procedure was used to determine wavenumbers and interactions having significant affect on lignocellulosic composition of biomass in decreasing order and accordingly organized in the regression equations (Table 4).

3.4 Sample Material Spectra

The characteristic peak heights of cellulose, hemicellulose and lignin for the third replicate of barley, canola, oat and wheat straw samples were determined by using the wavenumbers provided in Table 4 and

following the procedure described in the materials and methods (section 2.5.1). The peak height values were subsequently used to predict the percentage of lignocellulosic composition in the corresponding agricultural straw samples using the SAS GLM procedure (Table 4). The average absolute difference in predicted and lab based measured percentage of cellulose, hemicellulose and lignin in agricultural biomass was 7.5%, 2.5%, and 3.8%, respectively (Table 4). The error in prediction could be attributed to larger grind size of sample material as compared to reference material [50]. Sherman Hsu [28] indicated that the deviation from the Bouguer-Beer-Lambert law often occurs in infrared spectroscopy. These deviations stem from both instrumental and sample effects. Assuming negligible instrumental effect, the sample effect will include chemical reactions and molecular interactions such as hydrogen bonding [28]. In addition, the reference materials (pure samples and their mixtures) used in the present experiments were obtained

Table 4 Summary of statistical analysis using the Proc GLM method for polynomial regression analysis.

Significant wavenumbers and interactions	Estimated coefficients	R ² value	Rootmean square error	Mean absolute deviation
%Cellulose = -135.10 + 781.35(PH₁₃₁₉) - 795.57(PH₁₄₃₁) - 135.26(PH₁₂₀₃) + 436.11(PH₁₃₃₈) - 94.24(PH₁₃₇₃)				
Intercept	-135.10			
1319	781.35			
1431	-795.57	0.89	8.39	7.5
1203	-135.26			
1338	436.11			
1373	-94.24			
%Hemicellulose				
= 1638.72 - 2581.71(PH₁₂₅₁ × PH₁₄₆₁) - 1260.90(PH₁₂₁₃) - 2518.05(PH₁₁₆₆) + 1573.69(PH₁₂₁₃ × PH₁₂₅₁) + 118.74(PH₁₀₅₀) + 3128.51(PH₁₁₆₆ × PH₁₂₅₁) + 2179.65(PH₁₄₆₁) + 92.36(PH₁₆₀₆) - 2294.15(PH₁₂₅₁) - 59.29(PH₁₄₆₁ × PH₁₆₀₆)				
Intercept	1,638.72			
1251*1461	-2,581.71			
1213	-1,260.90			
1166	-2,518.05			
1213*1251	1,573.69	0.99	2.76	2.5
1050	118.74			
1166*1251	3,128.51			
1461	2,179.65			
1606	92.36			
1251	-2,294.15			
1461*1606	-59.29			
%Lignin = 7110.87 + 388.32(PH₁₅₁₁ × PH₁₅₉₉) - 16440.93(PH₁₄₆₇) + 447.36(PH₁₅₉₉)² + 19572.82(PH₁₁₅₇ × PH₁₄₆₇) + 18374.36(PH₁₁₅₇) + 15659.98(PH₁₀₅₄ × PH₁₄₂₉) - 4952.80(PH₁₁₅₇ × PH₁₅₉₉) + 800.20(PH₁₅₁₁) - 3032.75(PH₁₄₂₉)² - 11269.16(PH₁₄₂₉) - 948.04(PH₁₅₁₁)² + 3444.69(PH₁₅₉₉) - 12344.90(PH₁₀₅₄) - 16689.44(PH₁₁₅₇)²				
Intercept	7,110.87			
1511*1599	388.32			
1467	-16,440.93			
1599*1599	447.36			
1157*1467	19,572.82	0.98	3.89	3.8
1157	18,374.36			
1054*1429	15,659.98			
1157*1599	-4,952.80			
1511	800.20			
1429*1429	-3,032.75			
1429	-11,269.16			
1511*1511	-948.04			
1599	3,444.69			
1054	-12,344.90			
1157*1157	-16,689.44			

PH represents characteristic maximum Peak Height (Photoacoustic Units) at respective wavenumbers.

commercially, which were derived from different biomass sources. Therefore, pure cellulose, hemicellulose and lignin derived from agricultural straw should be considered for future tests, which may further reduce the percentage difference in measured

and predicted values.

4. Conclusion

The authors successfully developed a novel procedure to quantitatively predict lignocellulosic

components of non-treated and steam exploded barley, canola, oat and wheat straw, which could be easily extended for any form of lignocellulosic biomass using FTIR spectroscopy. The FTIR quantitative analysis of mixtures of pure cellulose, hemicelluloses and lignin, and measured lignocellulosic composition of agricultural biomass samples resulted in predictive regression equations having R^2 values of 0.89, 0.99 and 0.98, respectively. The average absolute difference in predicted and measure cellulose, hemicellulose and lignin in agricultural biomass were 7.5%, 2.5%, and 3.8%, respectively.

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