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## **Qualitative and Quantitative Analysis of Lignocellulosic Biomass using Infrared Spectroscopy**

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### **1. ABSTRACT**

In this study, preliminary predictive models are developed that can potentially calculate the quantity of lignocellulosic components (cellulose, hemicelluloses and lignin) of agricultural biomass (barley, canola, oat and wheat straw) by using Fourier transform infrared spectroscopy (FT-IR). In order to achieve the goal set for this study, it was essential to estimate critical parameters through analytical specification of lignocellulosic biomass and consequently the development and validation of a procedure for the qualitative and quantitative analysis of cellulose-hemicellulose-lignin composition.

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**Key Words:** Infrared Spectroscopy, FT-IR, Agricultural Biomass, Agricultural Straw, Quantitative Analysis, Lignocellulose, Lignin, Cellulose, Hemicellulose

## 2. 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 (Liu *et al.* 2005). After harvest, the low bulk density straw has to be processed and densified in order to facilitate efficient handling and transportation, and reap the potential economic benefits (Adapa *et al.* 2009).

It has been reported by Sokhansanj *et al.* (2005) 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 (Sokhansanj *et al.* 2005). 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 (Ade-Omowaye *et al.* 2001; Bagby 1982; Bhazal *et al.* 2003; Focher *et al.* 1998).

Infrared spectroscopy has the potential to produce qualitative and quantitative analytical data for samples with minimum or no sample preparation, and at high speed and throughput (Budevaska 2002; Luypaert *et al.* 2003; Smola and Urleb 2000; Tucker *et al.* 2000). Traditionally, chemical analyses of the individual components (e.g., lignin) of lignocellulosics have been performed by acid hydrolysis followed by gravimetric determination of lignin (Kelley *et al.* 2004). These methods can provide highly precise data; however, are laborious, time-consuming, and, consequently, expensive to perform and sample through-put 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 (Kelley *et al.* 2004).

The literature review of lignocellulosic biomass have indicated that infrared spectromicroscopy could be used successfully to study the chemical structure and spatial distribution of cellulose, hemicellulose and lignin in various agricultural biomasses as applied to food, feed, biocomposite, textile, and paper and pulp industries (Adapa *et al.* 2009).

Smola and Urleb (2000) developed a procedure to estimate critical parameters in the analytical specification of oxytetracycline using near infrared (NIR) spectroscopy. They have developed a procedure in order to satisfy the European guideline for Good Manufacturing Practice (GMP), which requires examination of supplies by pharmaceutical

manufacturers. In another study, Matkovic *et al.* (2005) successfully developed and validated quantification method for ibuprofen tablets using IR spectroscopy.

Kelley *et al.* (2004) analyzed the chemical composition of a wide variety of agricultural biomass using NIR spectroscopy. They established a qualitative procedure to differentiate the samples and accurately predict the chemical composition of agricultural biomass.

Lupaert *et al.* (2003) measured the quality parameters of green tea (*Camellia sinensis* (L.)) using NIR spectroscopy. In addition, they used NIR to build calibration models to predict the content of caffeine, epigallocatechin gallate (EGCG) and epicatechin (EC), and the total antioxidant capacity of green tea.

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 (FT-IR) photoacoustic spectroscopy (PAS) was performed by Rosenthal *et al.* (1988). They were able to develop partial least-square models with high correlation coefficients. Another study by Belton *et al.* (1987) successfully used FT-IR spectroscopy for the quantitative analysis of protein and starch mixtures. Similarly, Moh *et al.* (1999) used FT-IR and NIR spectroscopy to investigate and develop a foundation for the rapid determination of  $\beta$ -carotene content of crude palm oil. They have also developed separate partial least squares calibration models to predict  $\beta$ -carotene based on spectral region from 976 to 926  $\text{cm}^{-1}$  for FT-IR spectroscopy and 546 to 819 nm for NIR spectroscopy.

The use of infrared spectroscopy in the study of fats and oils has been reviewed by Guillen and Cabo (1997). van de Voort *et al.* (1994) developed FT-IR spectroscopy that operates in the mid infrared region (4000-400  $\text{cm}^{-1}$ ) and has been proven to be a powerful tool for quantitative analysis of fats and oils.

Tucker *et al.* (2000) successfully performed the quantitative analysis of glucose, mannose, xylose, and acetic acid using FT-IR spectroscopy on liquors from dilute-acid-pretreated softwood and hardwood slurries.

The authors were unable to find any literature pertaining to quantitative analysis of agricultural (lignocellulosic) biomass using infrared spectroscopy that could be used to determine change in cellulose-hemicellulose-lignin composition prior to and after application of various pre-processing and pre-treatment methods. Therefore, the aim of our work was to estimate critical parameters in analytical specification of lignocellulosic biomass and consequently, to develop and validate a method for the qualitative and quantitative analysis of cellulose-hemicellulose-lignin composition using FT-IR spectroscopy.

### **3. MATERIALS AND METHOD**

#### **3.1 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. All of the straw samples were manually chopped using a pair of scissors and subsequently ground using a precision grinder (Falling Number, Model No. 111739, Huddinge, Sweden)

with a screen size of 1.0 mm. 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 ASAE Standard S358.2 (ASAE, 2008).

### 3.2 Reference Material Preparation

Quantitative analysis of change in cellulose, hemicelluloses and lignin composition of sample material subjected to various pre-processing and pre-treatment methods is critical in order to predict and evaluate natural binding characteristics. Therefore, pure cellulose (microgranular), hemicelluloses (xylan) and lignin (hydrolytic) powders were obtained from Sigma-Aldrich Canada Ltd. (St. Louis, MO, USA), and were subsequently mixed in different proportions (Table 1) to determine the relationship (predictive models) between their respective quantity in the mixture and representative spectra. Due to lack of availability of pure hemicellulose, only one mixture (mixture number 6) having equal proportions of cellulose-hemicellulose-lignin was prepared. Carbon black powder reference spectrum was used to correct for FT-IR wavenumber-dependent instrumental effects.

Table 1: Pure cellulose-hemicellulose-lignin mixtures used to obtain reference spectra.

Mixture	Lignin (%)	Cellulose (%)	Hemicellulose (%)
1	20	80	-
2	40	60	-
3	50	50	-
4	60	40	-
5	80	20	-
6	33.33	33.33	33.33
7	100*	-	-
8	-	100*	-
9	-	-	100*

\*Note: Spectra for 100% lignin, cellulose and hemicellulose were obtained from Karunakaran *et al.* (2009)

### 3.3 Measured Data

It is essential to validate the calculated cellulose-hemicellulose-lignin quantity of sample agricultural straw (section 3.1) using predictive models developed from the analysis of reference material spectra (section 3.2). The data to validate lignocellulosic components in agricultural straw was obtained using the proximate analysis performed by SunWest Food Laboratory Ltd., Saskatoon, SK, Canada. Protein, fat, lignin, acid detergent fiber (ADF), neutral detergent fiber (NDF) and total ash were determined. The protein content of the biomass was determined using the AOAC standard method (AOAC 2001.11 1990), where

the nitrogen content was multiplied by a factor 6.25. Crude fat was determined using the AOCS standard method Am2-93 (AOCS Am2-93 1999). ADF was determined using the AOAC standard method 973.18 (AOAC 973.18 1990), whereas the NDF was determined using the AOAC standard method 992.16 (AOAC 992.16 1990). The total ash content was determined using AOAC standard method 942.05 (AOAC 942.05 1990). Cellulose percentage is calculated indirectly from acid detergent fiber (ADF) and lignin (ADF minus lignin) (Mani *et al.* 2006). Hemicellulose percentage is calculated indirectly from neutral detergent fiber (NDF) and ADF (NDF minus ADF) (Mani *et al.* 2006).

### 3.4 FT-IR Equipment

Mid-IR beamline (01B1-1, energy range: 4000 to 400  $\text{cm}^{-1}$ ) at the Canadian Light Source Inc. (CLS, University of Saskatchewan, Saskatoon SK, Canada) was used to collect IR data of reference compounds and sample agricultural straw. The beamline has a MTEC Model 300 photoacoustic cell (MTEC Photoacoustic Inc., Ames, IA) for FT-IR photoacoustic spectroscopy (FT-IR PAS) of bulk samples. The FT-IR spectra of reference compounds were recorded using the FT-IR PAS system using Globar source (silicon carbide rod). The FT-IR PAS determines the absorption in the infrared region by measuring the changes in the thermal expansion of the gas surrounding the sample using a microphone (McClelland *et al.* 2002). The reference compounds were filled in the sample cup and purged with dry helium to remove water vapor and  $\text{CO}_2$  from the sample chamber. The spectrum for each sample was recorded separately by averaging 32 interferograms collected from wavenumbers of 4000 to 400  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$  (Karunakaran *et al.* 2009).

The OPUS 4.2 (Bruker Optics Inc., Billerica, MA) software was used to record and analyze the FT-IR PAS data. The software Origin (version 7.5, OriginLab, Northampton, MA, USA) was used to plot the data.

### 3.5 Quantitative Analysis

The quantitative analysis of absorption spectrometry is based on the Bouguer-Beer-Lambert law (Sherman Hsu 1997). According to this law, for a single compound in a homogenous medium, the absorbance at any frequency 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 (Sherman Hsu 1997).

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

### 3.5.1 Peak Height Method

Three types of spectral plots were developed. The first plot has spectral information of pure (100%) cellulose, hemicelluloses and lignin in order to identify characteristic peaks of respective components. One of the distinguishable characteristic peaks of individual components was chosen to measure the peak height. The height of the peak (intensity of maximum absorption) was measured by calculating the difference between the peak intensity of the absorption band and value of intensity at the foot of the curve (Figure 1).

The corresponding wavenumber at peak intensity ( $\bar{\nu}_{\max}$ ) and foot of the absorption band ( $\bar{\nu}_{\min}$ ) was recorded.

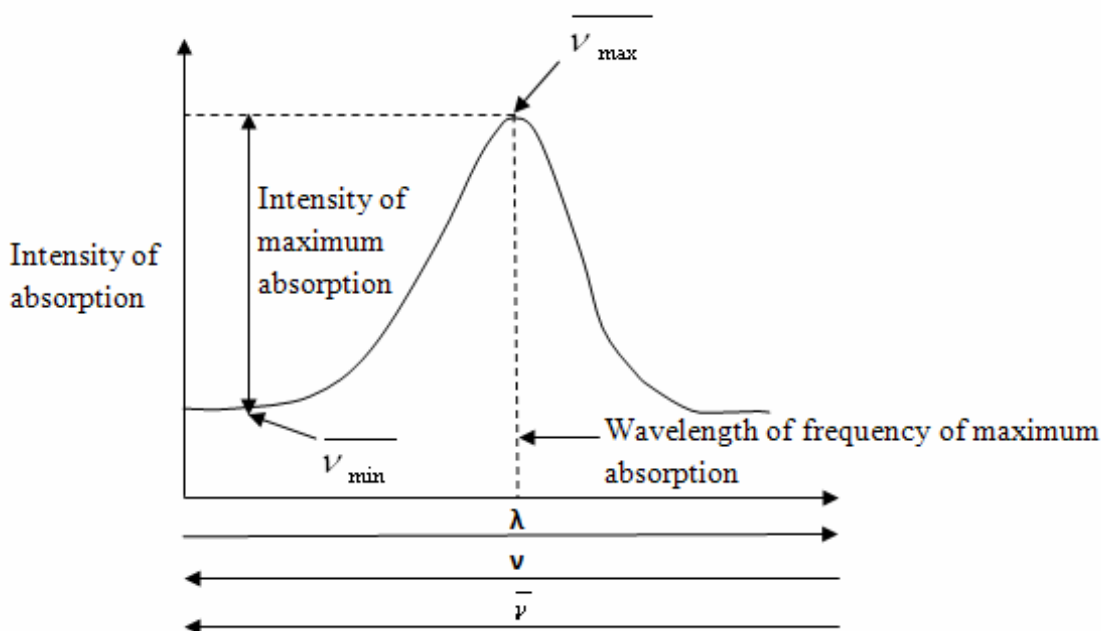


Figure 1: Schematic of absorption spectra (Yadav 2005) (Note:  $\lambda$  is wavelength,  $\nu$  is frequency and  $\bar{\nu}$  is wavenumber of IR radiation).

The second plot has spectral information of lignocellulosic mixtures as indicated in Table 1. The wavenumbers recorded for cellulose, hemicelluloses and lignin from the first spectral plot was used as the guide to calculate the characteristic peak height for respective component in the lignocellulosic component mixtures. Thereafter, the trend in variation of peak height of cellulose, hemicelluloses and lignin components in the mixture was correlated to their percentage composition. Subsequently, predictive models for cellulose, hemicelluloses and lignin were developed that has the capability to predict quantity (percentage composition) of cellulose, hemicelluloses and lignin in lignocellulosic biomass.

The third plot provided spectral information of sample biomass materials, that were used to extract qualitative information of various chemical components. In addition, wavenumbers recorded from the first plot were used to measure the characteristic peak height for

cellulose, hemicelluloses and lignin component in the sample spectra. The peak height values obtained from plot three were inserted in the developed models to predict percentage composition of lignocellulosic components in the barley, canola, oat and wheat straw.

### **3.5.2 Data Normalization Procedure**

The agricultural biomass samples FT-IR 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 are acquired at different times (Michaelian, 2005).

In order to further standardize the methodology, the carbon black normalized FT-IR 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 FT-IR spectra obtained for any lignocellulosic biomass.

## **4. RESULTS AND DISCUSSION**

### **4.1 Measured Data**

Table 2 shows the chemical composition of barley, canola, oat and wheat straw samples. Among the tested samples, canola straw had the highest protein content (6.53%); barley straw had the highest level of fat (1.91%) and lignin (17.13%), while wheat straw showed the highest levels of starch (2.58%) and ash (2.36%) contents. Canola and wheat straw showed highest level of cellulose (42.39%) and hemicelluloses (23.68), respectively. Among the chemical components, the presence of protein, starch and lignin may enhance the pelleting property of ground biomass (Mani *et al.* 2006; Kaliyan and Morey 2006). Table 2 also shows that the percentage of starch is significantly lower as compared to protein and lignin compounds in the four straw samples, except for wheat straw. Therefore, protein and lignin could be the deciding factors to enhance the binding characteristics of densified pellets. Protein plasticizes under heat and acts as a binder, which assists in increasing the strength of pelletized product (Winowiski 1988; Briggs *et al.* 1999). In the presence of heat and moisture, gelatinization of starch occurs, which results in binding of ground biomass (Wood 1987; Thomas *et al.* 1998). In addition, mechanical shearing during densification process also improves starch gelatinization (Kaliyan and Morey 2006). At high elevated temperatures and pressures, lignin softens and helps the binding process. Lignin has thermosetting properties and a low melting point of about 140°C (van Dam *et al.* 2004).

Table 2: Lignocellulosic composition of agricultural straw

Composition (% DM <sup>a</sup> )	Barley Straw	Canola Straw	Oat Straw	Wheat Straw
Protein Content	3.62	6.53	5.34	2.33
Fat Content	1.91	0.69	1.65	1.59
Lignin	17.13	14.15	12.85	13.88
Cellulose <sup>b</sup>	33.25	42.39	37.60	34.20
Hemicellulose <sup>c</sup>	20.36	16.41	23.34	23.68
Ash	2.18	2.10	2.19	2.36

<sup>a</sup>DM – Dry Matter

<sup>b</sup>Cellulose percentage is calculated indirectly from acid detergent fiber (ADF) and lignin (ADF-lignin) (Mani *et al.* 2006).

<sup>c</sup>Hemicellulose percentage is calculated indirectly from neutral detergent fiber (NDF) and ADF (NDF-ADF) (Mani *et al.* 2006)

## 4.2 Reference Material Spectra

Figure 2 represents the FT-IR PAS spectra of pure cellulose, hemicellulose and lignin. The characteristic/prominent peaks and small peaks or shoulders for cellulose, hemicellulose and lignin, and their peak assignments are provided in Table 3. The cellulose spectrum has three distinct peaks at wavenumbers of 1634, 1427 and 899  $\text{cm}^{-1}$ . Small peaks or shoulders were present at wavenumbers of 1367, 1319, 1337, 1284, 1203, 1161, 1119, 1114 and 999  $\text{cm}^{-1}$ . Similarly, hemicellulose (Xylan) had prominent peaks at wavenumbers of 1646, 1563, 1044 and 899  $\text{cm}^{-1}$  and small peaks or shoulders at wavenumbers of 1508, 1461, 1420, 1252, 1212, 1164 and 990  $\text{cm}^{-1}$ . The lignin spectrum showed characteristic peaks at wavenumber of 1697, 1603, 1514 and 837  $\text{cm}^{-1}$ . A few small peaks were observed at wavenumbers of 1457, 1423, 1327, 1281, 1121 and 1034  $\text{cm}^{-1}$ .



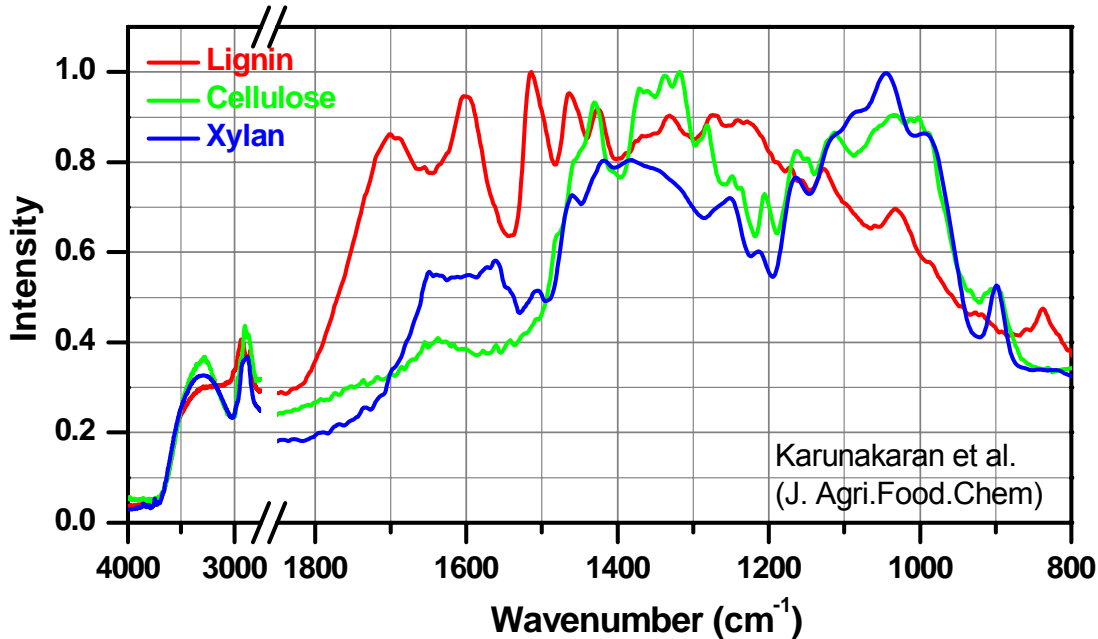


Figure 2: FT-IR PAS spectra of pure cellulose, hemicellulose (xylan) and lignin (Karunakaran *et al.* 2009)

Table 3: Characteristic/prominent peaks and small peaks or shoulders of pure cellulose, hemicellulose and lignin, and their peak assignments (Adapa *et al.* 2009; Karunakaran *et al.* 2009)

Wavenumbers (cm <sup>-1</sup> )	Pure Lignin	Pure Cellulose	Pure Hemicellulose	Peak Assignment
1700-1650	1697	--	--	
1650-1600	1603	1634	1646	1600 – quadrant ring stretching (aromatic lignin) (Colthup <i>et al.</i> 1990; Yu <i>et al.</i> 2007); 1600-1610 – aromatic skeletal vibration (Pandey 1999; Yu <i>et al.</i> 2007); 1635 – carbonyl stretching conjugate with aromatic rings (Cyran 2007)
1600-1550	--	--	1563	1595 – very strong aromatic ring stretch, aromatic C-O stretch (Revol 1982; Stewart <i>et al.</i> 1995); 1595 – phenylpropanoid polymer (Himmelsbach <i>et al.</i> 1998); 1595 – aromatic skeletal vibrations plus C=O stretch (Lin and Dence 1992)
1550-1500	1514	--	1508	1510 – semicircle ring stretching (aromatic lignin) (Colthup <i>et al.</i> 1990; Yu <i>et al.</i> 2007; Yu 2005), 1510 – phenylpropanoid polymer (Himmelsbach <i>et al.</i> 1998); 1510 – very strong aromatic ring stretch, aromatic C-O stretch (Revol 1982; Stewart <i>et al.</i> 1995); 1513 – aromatic C=C stretch (Sun <i>et al.</i> 2005); 1514 – semi-circle stretch of para-substitute benzene rings (Lin-Vein <i>et al.</i> 1991; Budevskas 2002); 1550 – protein (Budevskas 2002)

1500-1450	1457	--	1461	1462 – C-H deformation (methyl and methylene) (Pandey 1999)
1450-1400	1423	1427	1420	1420 – weak C-O stretching (Yu <i>et al.</i> 2007; Wetzel <i>et al.</i> 1998); 1430 – CH <sub>2</sub> in-plane bending vibrations (Schulz and Baranska 2007; Wilson <i>et al.</i> 2000); 1433 – aromatic C=C stretch (Sun <i>et al.</i> 2005)
1400-1350	--	1367	--	1370 – weak C-O stretching (Yu <i>et al.</i> 2007; Wetzel <i>et al.</i> 1998); 1380 – C-H symmetric and asymmetric deformation (Sun <i>et al.</i> 2005); 1382 – C-O stretch (Xu <i>et al.</i> 2007)
1350-1300	1327	1319, 1337	--	1335 – weak C-O stretching (Yu <i>et al.</i> 2007; Wetzel <i>et al.</i> 1998); 1336 – C-H ring in-plane bending vibrations (Schulz and Baranska 2007; Wilson <i>et al.</i> 2000)
1300-1250	1281	1284	--	
1250-1200	--	1203	1252, 1212	1246 – weak C-O stretching (Yu <i>et al.</i> 2007; Wetzel <i>et al.</i> 1998); 1246 – Hemicellulose (Budevskva 2002; Yu <i>et al.</i> 2007); 1250 – Acetylated Hemicellulose (Himmelsbach <i>et al.</i> 1998); 1250 – acetylated hemicelluloses (Budevskva 2002)
1200-1150	--	1161	1164	1160 – glycosidic linkage (Robert 2005); 1162 – C-O-C ring vibrational stretching (Schulz and Baranska 2007; Wilson <i>et al.</i> 2000)
1150-1100	1121	1119	--	1125/1110 – C-O and C-C ring vibrational stretching (Schulz and Baranska 2007; Wilson <i>et al.</i> 2000)
1100-1050	--	1114	--	1078 – β(1-3) polysaccharide (Szeghalmi <i>et al.</i> 2007); 1098 – weak absorbance (Stewart <i>et al.</i> 1995)
1050-1000	1034	--	1044	1035 – C-O, C=C and C-C-O vibrational stretching (Schulz and Baranska 2007; Wilson <i>et al.</i> 2000); 1045 – C-OH bending (Wetzel <i>et al.</i> 2003; Cyran 2007; Robert 2005); 1018 – galactomannans (Szeghalmi <i>et al.</i> 2007); 1025 – non-structural CHO (Yu <i>et al.</i> 2007)
1000-950	--	999	990	985 – OCH <sub>3</sub> (Schulz and Baranska 2007; Wilson <i>et al.</i> 2000); 991 – β-glucan (Robert 2005)
950-900	--	--	--	
900-850	--	899	899	895 – β-1-4 linkage (Robert 2005); 900 – anti-symmetric out-of-plane ring stretch of amorphous cellulose; C-O stretching (Stewart <i>et al.</i> 1995)
850-800	837	--	--	

### 4.3 Quantitative Analysis

For the purpose of quantitative analysis, the authors decided to choose one of the characteristic peaks for pure cellulose, hemicellulose and lignin from Figure 2, which has distinguishable peak and base. The characteristic peaks at wavenumbers of 1427, 899 and 1514 cm<sup>-1</sup> were chosen for cellulose, hemicellulose and lignin, respectively. The heights of individual characteristic peaks were determined using the procedure described

in materials and methods (section 3.5.1). Table 4 shows the wavenumbers at peak intensity ( $V_{\max}$ ) and foot of the absorption band ( $V_{\min}$ ) (Figure 1).

Table 4: Wavenumbers used to determine the height of characteristic peaks for cellulose, hemicellulose and lignin

Component	Wavenumber ( $\text{cm}^{-1}$ )	
	$V_{\min}$	$V_{\max}$
Cellulose	1444	1427
Hemicellulose	1145	1044
Lignin	1544	1514

Figure 3 shows the FT-IR PAS spectra for reference mixtures of cellulose, hemicelluloses and lignin (Table 1). The characteristic wavenumbers of cellulose, hemicelluloses and lignin from Table 4 were used to determine the peak height for individual components in the reference mixtures and are provided in Table 5. No further quantitative analysis of hemicelluloses was performed due to lack of information to develop a correlation between peak intensity and percentage hemicellulose in component mixtures.

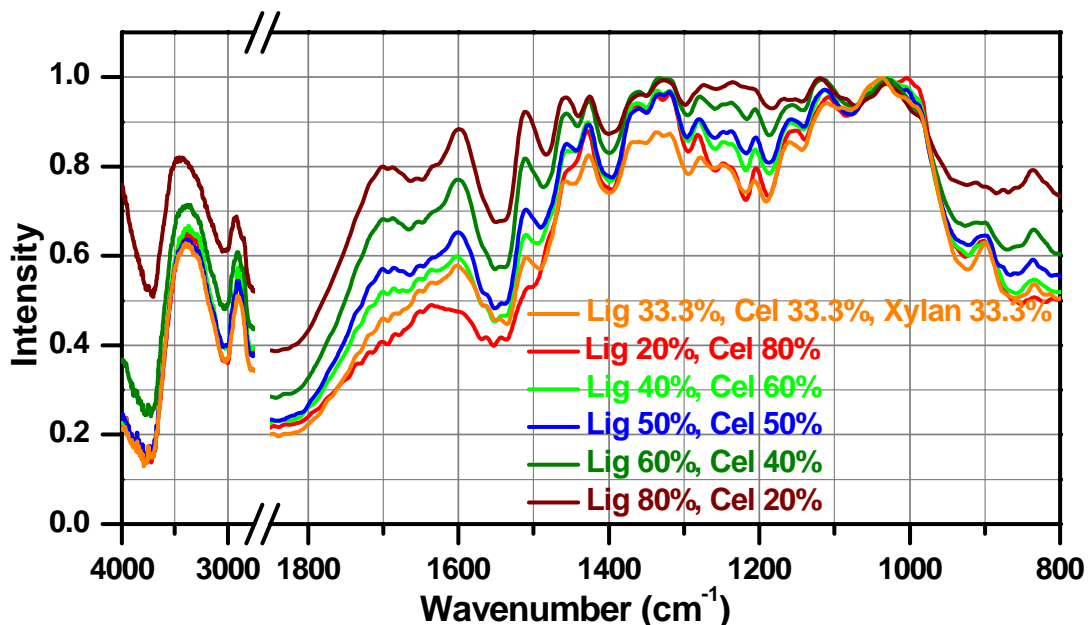


Figure 3: FT-IR PAS spectra of reference cellulose, hemicellulose and lignin mixtures

Table 5: The peak height and corresponding percentage proportion of cellulose and lignin in reference mixtures

Quantity (%)	Cellulose (peak wavenumber 1427 cm <sup>-1</sup> )	Lignin (peak wavenumber 1514 cm <sup>-1</sup> )
20	0.039	0.089
33	0.069	0.128
40	0.059	0.181
50	0.067	0.208
60	0.074	0.241
80	0.076	0.244
100	0.088	0.364

The data presented in Table 5 was plotted in individual graphs (Figures 4 and 5) and correlation models (equations) were obtained by fitting appropriate curves with highest R<sup>2</sup> values. The peak height data obtained for cellulose and lignin at 33% mixture quantity was considered as an outlier and excluded from further analysis since it was producing lower R<sup>2</sup> values. In addition, apart from 33% mixture, hemicellulose was not added in any other mixtures; therefore, the authors deemed it appropriate to exclude its data from statistical analysis. One of the reasons for having an outlier data could be due to improper mixing of pure hemicellulose with cellulose and lignin powders.

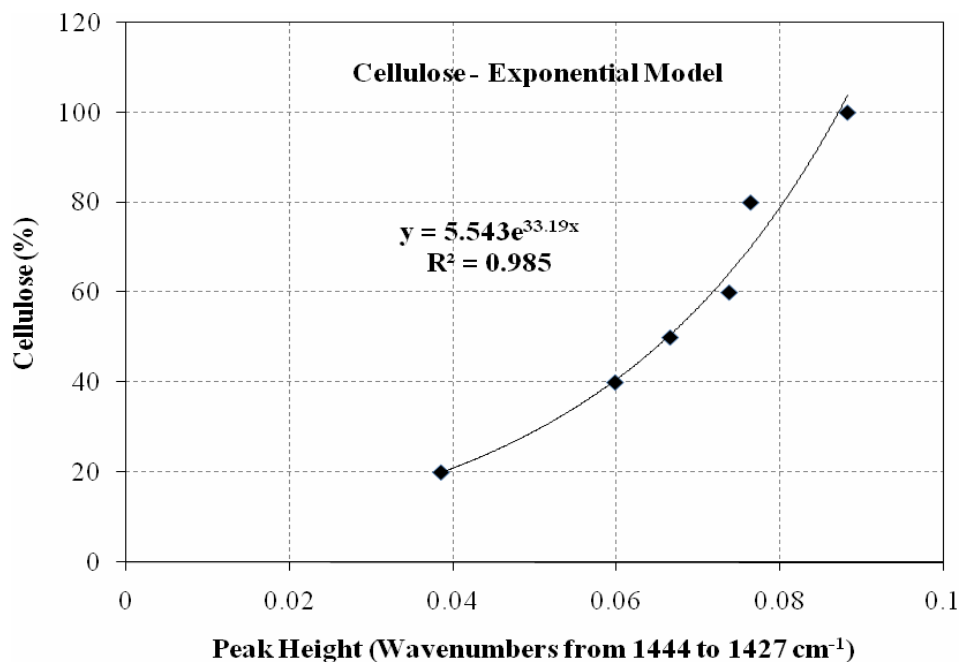


Figure 4: Correlation between peak height and quantity of cellulose in the reference mixtures.

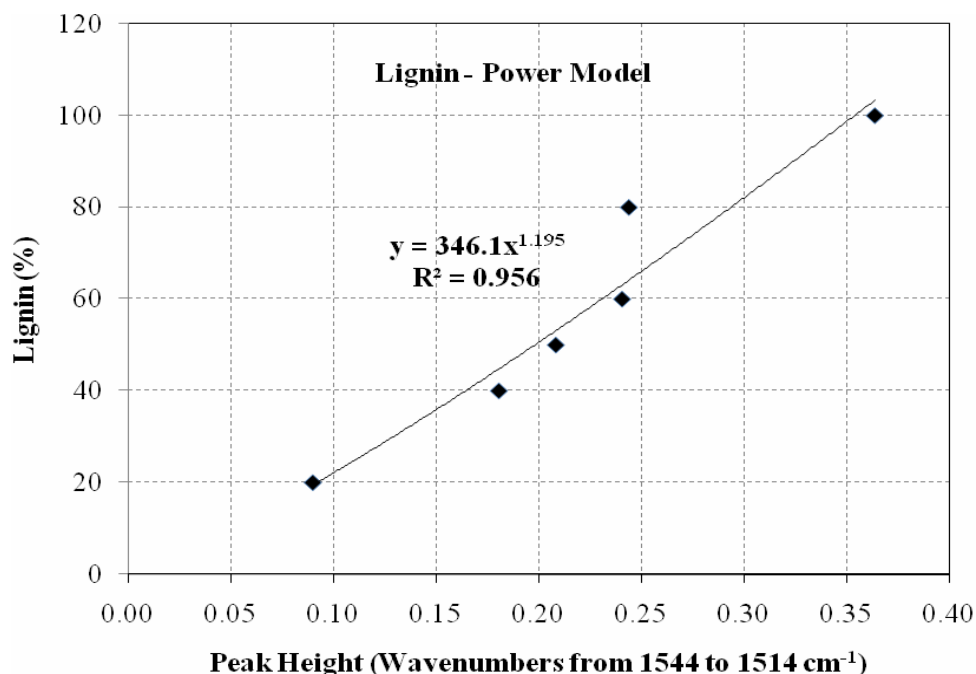


Figure 5: Correlation between peak height and quantity of lignin in the reference mixtures.

#### 4.4 Sample Material Spectra

Figure 6 shows FT-IR PAS spectra of sample agricultural straw. The characteristic peak heights of cellulose and lignin for 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 3.5.1). The peak height values were subsequently used to predict the percentage of cellulose and lignin in the corresponding agricultural straw samples using the predictive models derived from Figures 4 and 5. The predicted values and measured quantity of components (cellulose and lignin) of agricultural straw by proximate analysis (Table 2) were plotted in Figures 7 and 8. Figure 7 shows a close trend in variation in predicted values for cellulose with respect to measured data. However, the predicted values were statistically different from measured values. This could be attributed to the presence of moisture, difference in sample density and larger grind size of sample material as compared to reference material (Agarwal and Kawai 2003). Sherman Hsu (1997) 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 (Sherman Hsu 1997).

Figure 8 shows a close trend in variation in predicted values for lignin content in barley, canola and oat straw; however, the results for wheat straw showed a significantly large variation in values. This could again be attributed to the presence of moisture, difference in sample density and larger grind size of sample material as compared to reference material (Agarwal and Kawai 2003). The intensity of sample spectra could decreased in the C-H

stretch region, which could be largely due to the fact that the aliphatic- and water- hydroxyl groups have non-fundamental vibration transitions. Therefore, it is best to choose a band that is not in the C-H stretch region of the spectrum (Agarwal and Kawai, 2003).

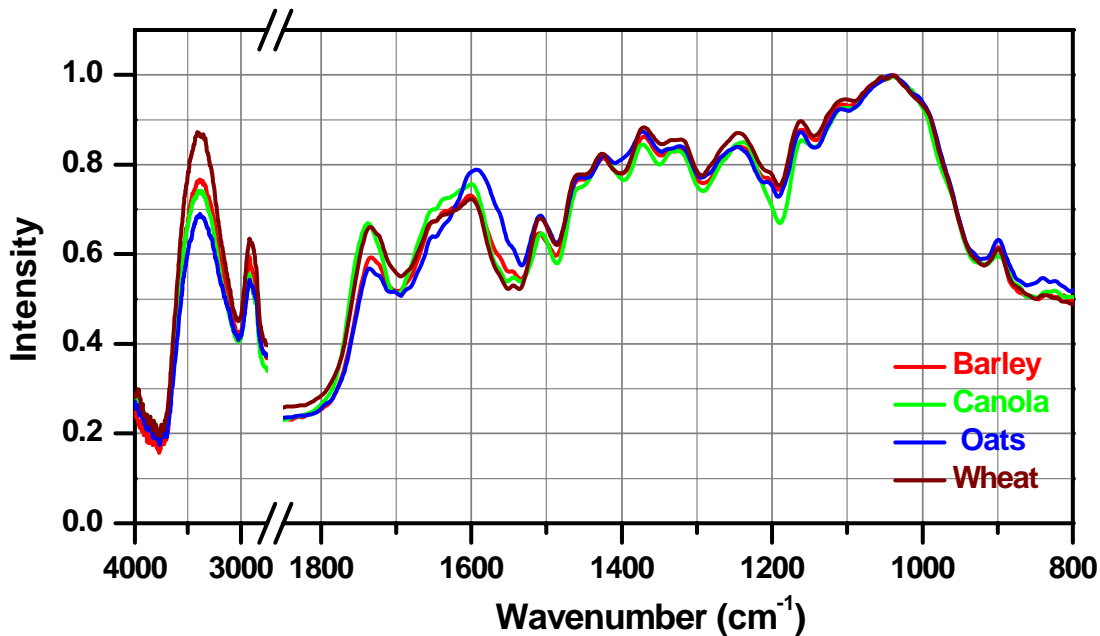


Figure 6: FT-IR PAS spectra of biomass straw samples

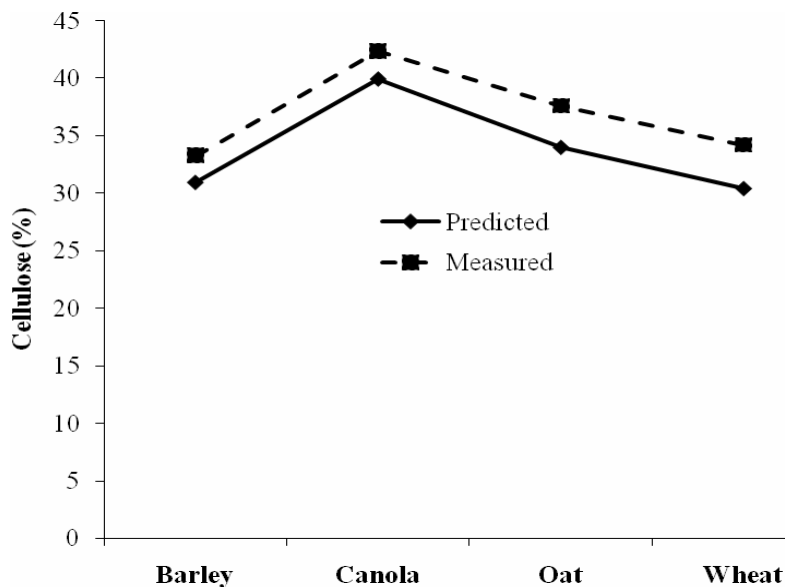


Figure 7: Trend in variation of predicted values for cellulose with respect to measured data for four straw samples.

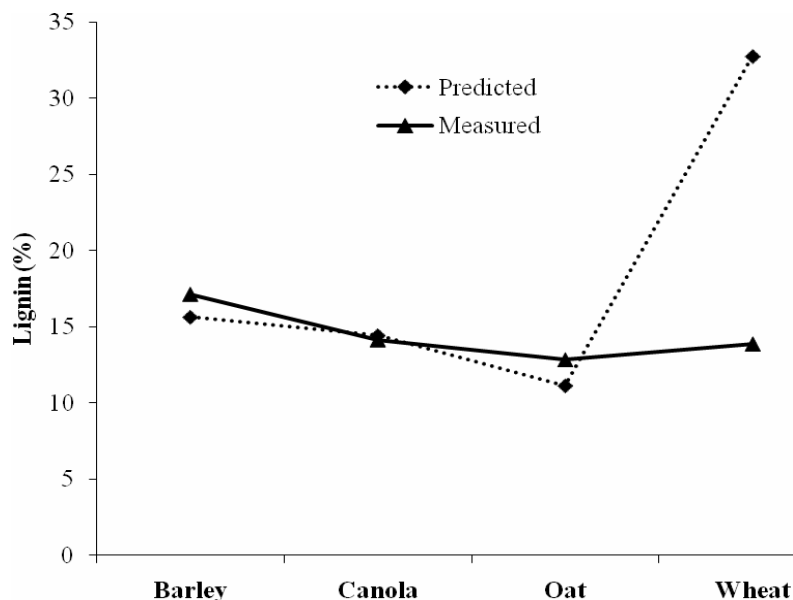


Figure 8: Trend in variation of predicted values for lignin with respect to measured data for four straw samples.

#### 4.5 Alternate Lignin Model

In order to get a better estimate of predicted values for lignin (away from C-H stretch region), the authors decided to concentrate their analysis on another characteristic peak at the wavenumber  $837\text{ cm}^{-1}$  (excluding wavenumbers of  $1697$  and  $1603\text{ cm}^{-1}$ ). Following the procedures established in quantitative analysis (section 4.3), the wavenumbers at peak intensity ( $V_{\max}$ ) and at the foot of the absorption band ( $V_{\min}$ ) for pure lignin (Figure 2), were  $837$  and  $854\text{ cm}^{-1}$ , respectively. The peak height of lignin in the reference mixtures spectra (Figure 3) was plotted in a graph (Figure 9) and correlation model (equation) was obtained by fitting appropriate curves with highest  $R^2$  value.

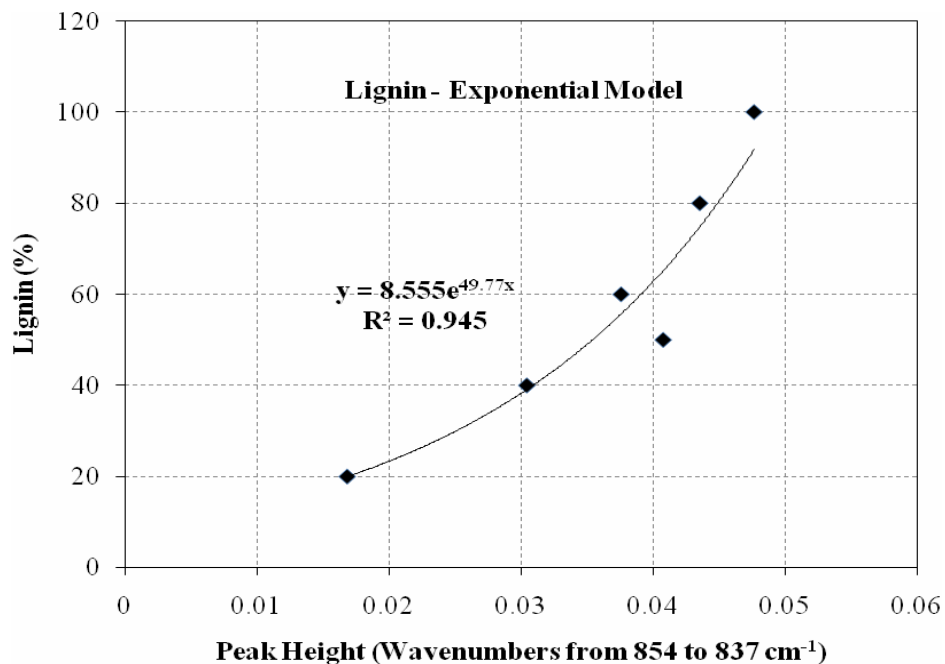


Figure 9: Correlation between peak height and percentage of lignin in the reference mixtures.

The characteristic peak heights of lignin for barley, canola, oat and wheat straw spectra (Figure 6) were determined between wavenumbers of 837 and 854 cm<sup>-1</sup>. The peak height values were subsequently used to predict the percentage of lignin in corresponding agricultural straw using predictive models derived from Figures 9.

The predicted values of lignin at wavenumbers 837 and 1514 cm<sup>-1</sup>, and measured agricultural straw data using proximate analysis (Table 2) for lignin were plotted in Figure 10.



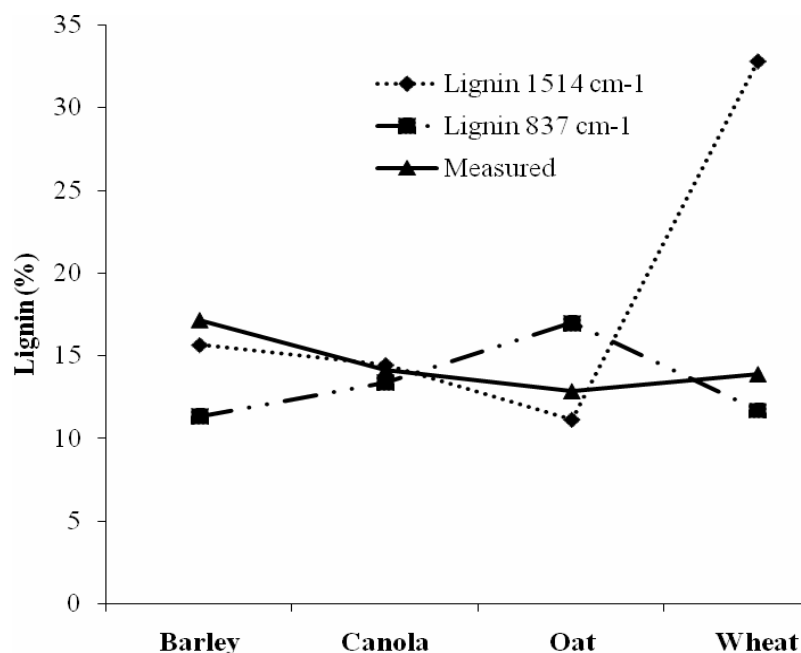


Figure 10: Variation of predicted values for lignin at wavenumbers of 1514 and 837  $\text{cm}^{-1}$  with respect to the measured data

Figure 10 shows that the predictive model developed for lignin using peak height of reference mixtures (Figure 3) at wavenumber of 837  $\text{cm}^{-1}$  produced better results as compared to peak height used at wavenumber of 1514  $\text{cm}^{-1}$ , even though, the new exponential model has  $R^2$  value of 0.945. An improvement in  $R^2$  value could provide a better and close fit trend that could be used as a standard equation.

Considering the above analysis, the authors would like to acknowledge the fact that further standardization of the quantification procedure is desired to obtain accurate results apart from having an agreeable trend in variation of predicted and measure data of sample agricultural biomass. There are various factors that should be taken into account for future studies, which may include variation in sample moisture content, difference in sample density and effect of sample grind size as compared to reference materials. In addition, for all of the experiments the spectrum for each sample was recorded separately by averaging 32 interferograms collected from wavenumbers of 4000 to 400  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$ . However, no replication of the sample spectrum was performed. Therefore, it is recommended for future studies to determine the effect of replication on predictive model as well as increasing the sampling resolution to 2  $\text{cm}^{-1}$ .

The hemicellulose was excluded from almost all of the reference mixtures used to develop predictive models, primarily due to lack of availability of pure hemicellulose. Therefore, future studies should include hemicellulose as one of the components of the reference mixtures to understand its effect on the FT-IR spectra as well as develop predictive model for hemicellulose.

## 5. CONCLUSION

The authors successfully developed a procedure to quantitatively predict lignocellulosic components of barley, canola, oat and wheat straw, which could be easily extended for any form of lignocellulosic biomass using FT-IR spectroscopy. However, there is a need to further standardize the procedure by accounting for the variation of sample moisture content, sample grind size and density, number of replications of sample spectrum and spectral resolution. In addition, pure hemicellulose should be included as one of the components of the reference mixtures.

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