

# Near Infrared Spectroscopy as a Screening Tool for Sugar Release and Chemical Composition of Wheat Straw

J. Lindedam<sup>1,2,\*</sup>, S. Bruun<sup>1</sup>, J. DeMartini<sup>3</sup>, H. Jørgensen<sup>2</sup>, C. Felby<sup>2</sup>,  
B. Yang<sup>3,4</sup>, C. E. Wyman<sup>3</sup>, and J. Magid<sup>1</sup>

<sup>1</sup>Plant and Soil Science Laboratory, Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, DK-1871 Frederiksberg C, Denmark

<sup>2</sup>Wood and Biomass Science, Forest and Landscape, Faculty of Life Sciences, University of Copenhagen, DK-1871 Frederiksberg C, Denmark

<sup>3</sup>Center for Environmental Research and Technology, Bourns College of Engineering, University of California Riverside, 1084 Columbia Avenue, Riverside CA 92507, USA

<sup>4</sup>Now at Center for Bioproducts and Bioenergy, Washington State University, 2710 University Drive, Richland, Washington 99354, United States

Bioethanol production would benefit from a rapid screening method to determine the ability of feedstock to be processed into fermentable sugars. The aim of this study was to relate near infrared (NIR) spectra of straw to the release of sugars for ethanol production from cultivars of winter wheat, and to establish a calibration model to quickly determine the content of structural carbohydrates, lignin, and ash. We applied a high-throughput pretreatment and enzymatic hydrolysis (HTPPH) assay, involving hydrothermal pretreatment (180 °C for 17.6 min) and enzymatic hydrolysis, to establish the release of glucose and xylose from 20 cultivars grown in two replicates at two sites; in total 79 samples were measured. The NIR spectra could explain 56% of the variance in sugar release with a root mean square error of cross-validation (RMSECV) of 0.014 g g<sup>-1</sup> dm. NIR calibrations predicting content of structural carbohydrates and lignin could explain only about 25% of total variance, whereas calibrations predicting ash content could explain 94% of total variance. The relatively low percentage of explained variance of sugar release was due mainly to uniformity of samples, which rendered the uncertainty of HTPPH method to be large compared with variance between samples. NIR spectroscopy, therefore, has potential to assess sugar release of wheat straw. Improved prediction of carbohydrates and lignin require better compositional analysis for homogeneous material. Despite successful prediction of ash content, site-specific cross-validation indicated that there might be problems with model transferability from site to site.

## Keywords:

## 1. INTRODUCTION

In recent years it has been increasingly apparent that an important step in making second generation bioethanol production a commercial reality, is to obtain a better understanding of how feedstock affects production.<sup>1</sup> Varying capacity of the feedstock to be processed into fermentable sugars after pretreatment could be related to variability in chemical composition and differences in recalcitrance of the feedstock. High-throughput pretreatment and enzymatic

hydrolysis (HTPPH) methods have been developed in order to analyze a large number of feedstock samples for sugar production following pretreatment.<sup>2,3</sup> Although HTPPH methods dramatically reduce processing times compared to prior approaches, they still take time for grinding biomass, accurately weighing materials, and conducting the pretreatment and hydrolysis steps.

Near Infrared Spectroscopy (NIR) has been suggested as a rapid and non-destructive method to replace reference methods for determination of chemical composition of feedstock and capacity for bioethanol production.<sup>4</sup> Spectroscopy holds an advantage over HTPPH methods in

\* Author to whom correspondence should be addressed.  
Email: lindedam@life.ku.dk

that samples can be analysed much faster and require less preparation. Few feasibility studies on sugar or ethanol yield following pretreatment have been published to date, although spectroscopy has previously been used in calibration models for analytical parameters during hydrolysis and fermentation<sup>5,6</sup> and for assessing chemical composition of maize stover.<sup>7–10</sup> Isci et al.<sup>11</sup> proved NIR spectroscopy to be able to satisfactorily predict ethanol yield from maize stover after pretreatment with aqueous ammonia and simultaneous saccharification and fermentation. For wheat straw, Lomborg et al.<sup>12</sup> found good NIR prediction accuracy for glucan and xylan content in 44 samples of straw. No attempts, however, to develop NIR calibrations for sugar release after pretreatment and enzymatic hydrolysis of wheat straw have been published.

The aim of this study was to establish a validated calibration model between release of fermentable sugars in pretreatment and enzymatic hydrolysis as determined in a HTPPH system and the content of structural carbohydrates, lignin, ash, and NIR spectra of wheat straw.

## 2. MATERIALS AND METHODS

### 2.1. Wheat Cultivars, Collection and Fractionation

Winter wheat straw was sampled from two sites in Denmark, where field experiments comparing cultivars were conducted. Approximately 80 g of straw from 20 cultivars of mature winter wheat was collected in 2007 from two blocks at each of two sites near the towns of Sejet (55°49'12.43" N and 9°55'21.82" E) and Abed (54°49'40.05" N and 11°19'30.62" E). Collecting straw was done at the same day at the two sites just after normal grain harvest. Growing conditions (fertilizers etc.) were kept the same at the two sites, thus straws represented the natural variation (in climate, soil type etc.) in the biomass feedstock for a Danish ethanol plant. Cultivars were Northern European breeds: Abika, Ambition, Audi, Dinosor, Flair, Florett, Glasgow, Hattrick, Inspiration, Jenga, Oakley, Opus, Penso, Potenzial, Robigus, Samyl, Skalmjeje, Smuggler, Tommi, Tuscan. One sample was lost during harvest and the total set was therefore 79 air-dried samples. Samples were fractionated into anatomical components of ears (flower spike free of grain), leaves (leaves without the leaf sheath), and stem (remaining part). After weighing, anatomical parts were mixed together, milled to <1 mm on a cyclone mill (President, Holbæk, Denmark), and stored at ambient temperature until analysis. A subsample of the milled straw was used for analysis of chemical composition and another subsample for NIR.

### 2.2. Chemical Composition

Chemical compositions of the wheat samples were determined by two-step acid hydrolysis of the carbohydrates, according to the procedure published by NREL.<sup>13</sup> Analyses

were done on air-dried samples containing on average 7.9% weight by weight (w/w) water (standard deviation 0.9%). Dry matter content was determined on a Sartorius MA30 dry weight balance. No extractions have been performed prior to the acid hydrolysis in order to maintain the original composition of the biomass with most resemblance to the biomass used for NIR spectra recording. First, 3 mL 72% (w/w) H<sub>2</sub>SO<sub>4</sub> was added to 300 mg air-dried milled wheat sample and incubated at 30 °C for 1 h. Next, the samples were diluted with 84 mL Millipore water and autoclaved at 121 °C for 1 h (Tuttnauer, 2540 EL). Finally, hydrolyzates were filtered, neutralized with CaCO<sub>3</sub> and diluted with eluent before monomeric sugar concentrations were quantified on a Dionex Summit high performance liquid chromatography (HPLC) system. The separation was performed in a Phenomenex Rezex ROA column at 80 °C with 5 mM H<sub>2</sub>SO<sub>4</sub> as the eluent, running at a flow rate of 0.6 mL min<sup>-1</sup> with a Shimadzu RI-detector. Hemicellulose was calculated as the sum of xylose and arabinose concentrations. Klason lignin content was determined as the weight of the dried filter cake (dried over night at 105 °C) minus the ash content (dried 3 h at 550 °C). All measurements were done in triplicates and results are presented as percentage of dry matter.

### 2.3. Sugar Released from Wheat Straw

To measure sugar release from straw, we utilized the relatively new HTPPH 96-well-plate screening system, developed by Studer et al.<sup>3</sup> The conditions chosen for the experiment were determined by testing different pretreatment conditions and enzyme loadings on a wheat straw cultivar chosen from the data set as a standard. Briefly, all 79 samples were subjected to pretreatment and hydrolysis in the metal well-plate in triplicates. Hydrothermal pretreatment was performed at 1% (w/w) solid loading with indirect steam heating for 17.6 minutes at 180 °C, corresponding to a log severity of 3.6.<sup>14</sup> This was done by loading 2.5 mg dry matter (dm) milled straw to each well and soaking for four hours in de-ionized water (total reaction mass of 250 mg) before heating the well-plate with steam. Following pretreatment, hydrolysis was performed on the entire pretreated slurry by applying a fixed enzyme loading to all wells using a 5:1 (w/w) enzyme mix of cellulase (Celluclast, Novozymes) and cellobiase (Novozyme 188, Novozymes). Enzyme loading for individual cultivars thus ranged from 57.9 to 72.1 FPU g<sup>-1</sup> glucan + xylan in the raw material (standard deviation 3.1 FPU g<sup>-1</sup> glucan + xylan). The well-plate was then placed vertically in an incubation shaker (Multitron InFors, ATR Biotech, MD) at 50 °C and 150 rpm. After 72 hours of hydrolysis, content of each well was transferred to 2 mL centrifuge tubes and centrifuged for 10 minutes at 18,200 g-forces (5415 D, Eppendorf, Hamburg, Germany). Sugar concentrations in the supernatant were analyzed using HPLC

with an Alliance 2695 system (Waters, Milford, MA), an Aminex HPX-87H column (BioRad, Hercules, CA) heated to 65 °C and using 5 mM H<sub>2</sub>SO<sub>4</sub> as eluent in an isocratic mode. Detection was done by a refractive index detector (2414, Waters). Sugar release of each cultivar was calculated as release of glucose (Glu), xylose (Xyl) and glucose plus xylose (total sugar, TS) in gram per gram dry matter of raw biomass (g g<sup>-1</sup> dm).

## 2.4. NIR Spectroscopy

Two NIR spectra were recorded for milled straw samples: One for air-dried sample and one for oven-dried sample (80 °C). NIR spectra were recorded on a NIR system 6500 (FOSS Tecator, Copenhagen, Denmark) spectrometer running in reflectance mode with a spectral range from 400 nm to 2500 nm at 2 nm intervals. The samples are not physically the same samples as used in the biochemical composition analyses. Approximately 1 g of milled straw material was scanned in a 36 mm Ø spinning cup, where 16 spectra from different sections of the cup were averaged. With *R* being the ratio of the reflectance of the sample to a reference standard absorbance was calculated by the equation:  $A = \log_{10} (R^{-1})$ .

## 2.5. NIR Calibration

Partial Least Square (PLS) regressions were performed in LatentX 2.00 (Latent5, Denmark, <http://www.latentx.com>) predicting the sugar release, chemical and anatomical composition from the NIR spectra. The data set on sugar release and chemical composition going into the regressions are the average values of triplicate measurements. Different methods for transforming NIR spectra were tested including multiplicative signal correction,<sup>15</sup> standard normal variate,<sup>16</sup> and second order Savitzky-Golay derivatives.<sup>17</sup> Transforming with finite differences (employing a smoothing window in a second-order polynomial of five segments and a gap of three) was found to give the highest percentage of explained variances in most calibrations and was chosen as the preferred transformation. All PLS models were validated using two different cross-validation schemes: repeated random cross-validation (RRCV) using five segments and 20 drawings, and site-segmented cross-validation (SSCV) using two segments defined by the two growing sites. RRCV thus used the data set four times with 20 random samples left out at a time and SSCV used the data of one site to predict the data of the other site. Validation was used to determine the optimal number of components to be included in the PLS calibration. The advantage of RRCV is that the dataset is used extensively to achieve relatively precise estimates of the performance of the calibrations. Developed calibrations, however, may be prone to problems with transferability and SSCV was therefore applied to test that the developed model could be transferred from one site

to another. The performance of PLS calibrations in the cross-validations are reported as percentage of explained variance of the validated *Y* matrix (*R*<sup>2</sup>) and the root mean square error of cross-validation (RMSECV) is defined as:

$$R^2 = 1 - \frac{\sum (Y_i - Y_{i(\text{pred})})^2}{\sum (Y_i - \bar{Y})^2} * 100\%$$

$$\text{RMSECV} = \sqrt{\frac{1}{n} \sum_{i=1}^n (Y_{i(\text{pred})} - Y_i)^2}$$

where *i* is the individual sample and *i*(pred) is the individual validated predicted sample out of the data set of *n* samples (*n* = 79).

For evaluation of NIR calibrations, RMSECV can be compared with the standard deviation of the laboratory method (SDL) based on the laboratory replicates in the reference method

$$\text{SDL} = \sqrt{\frac{\sum_{i=1}^n \sum_{j=1}^m (X_{ij} - \bar{X}_j)^2}{n * m - 1}}$$

where *i* is the individual laboratory replicate out of *n* replications (*n* = 3) and *j* is the individual sample out of *m* samples (*m* = 79). When the ratio of RMSECV to SDL is 1 it indicates that NIR calibration is as good a predictor as the actual measurements of the reference method itself, i.e., that the uncertainty of NIR prediction is equal to the uncertainty of the reference method.

## 3. RESULTS AND DISCUSSION

### 3.1. Calibrations and Predictions of Sugar Release

The raw data for chemical composition, sugar release after pretreatment and hydrolysis, and anatomical distribution of 79 wheat samples are listed in Table I.

The visible range of the NIR spectra from 400 nm to 1100 nm appeared noisy and using the full NIR spectra resulted in negative values of explained variance with SSCV validations. Thus the spectral range from 1100 nm to 2498 nm was used for all calibrations. During the development of the PLS regressions, six samples which were extremes in cellulose content (four samples above 41% cellulose and two samples below 34% cellulose), resulted in calibrations of small or negative value in explained variance of predicted cellulose for both validations. As omitting these six samples slightly improved the outcome of other calibrations as well, we chose to remove them from the final calibration set. The NIR calibrations predicting the sugar release, chemical components and anatomical fractions are shown in Table II. One calibration model using the chemical components and anatomical distributions to predict the total sugar release was included.

Predictions of total sugar release from air-dried spectra explained 56% of the variance when validated by RRCV

**Table I.** Average chemical composition, average release of total sugar, and average anatomical distribution of 20 wheat straw cultivars grown at two sites in duplicates. Stdev = Standard deviations ( $n = 40$  for Abed site and  $n = 39$  for Sejet site). Minimum and maximum values for the averages of the cultivars are given. Cell = Cellulose, Hemi = Hemicellulose, TS  $\text{g g}^{-1}$  dm = release of total sugar (glucose plus xylose) in gram per gram dry matter biomass.

		Cell %	Hemi %	Lignin %	Ash %	TS $\text{g g}^{-1}$ dm	Leaves %	Ears %	Stem %
Abed	Average	36.6	25.6	19.5	6.6	0.39	10.5	6.9	82.6
	Stdev	1.6	1.3	0.6	0.9	0.02	2.4	2.3	3.7
	Min	34.2	23.2	18.2	5.4	0.36	6.0	2.2	77.2
	Max	40.8	28.6	20.4	8.8	0.43	13.3	10.7	89.6
Sejet	Average	37.0	25.4	19.1	6.3	0.40	8.4	4.6	87.1
	Stdev	1.4	0.9	0.5	0.7	0.02	1.6	1.8	2.5
	Min	34.6	23.6	18.2	5.1	0.36	4.6	2.2	83.2
	Max	38.8	26.7	20.9	7.6	0.42	11.5	7.5	92.9

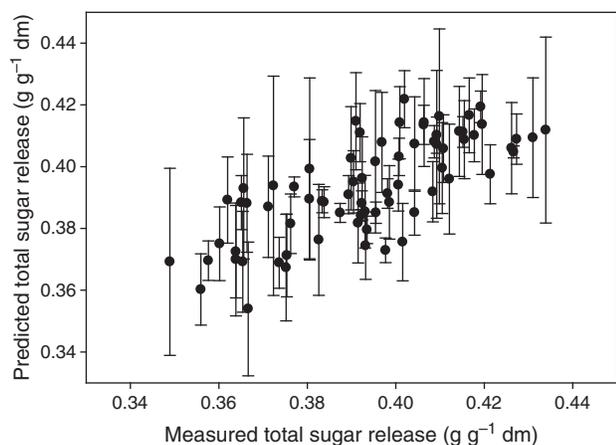
and 46% of the variance when validated by SSCV, using three PLS components models (Table II). When sugar release was predicted from the oven-dried sample spectra, the calibration resulting from the RRCV was more complex (six PLS components), but with an ability to explain 62% of the variance. Oven-drying the samples was done, because we suspected that variation in moisture content of native samples would result in variations in NIR spectra, which would be unrelated to sugar release and thus lower the prediction capacity. The SSCV on oven-dried samples, however, only had an ability to explain 40% of the variance in sugar release with a three-component calibration. Such a change in prediction capacity when using site-segmented validation suggest that the six-component model developed with RRCV was over-fitted and therefore not very robust or transferable to other sites.

The calibration selected as the best for sugar release was therefore the RRCV version predicting 56% of variance in sugar release from air-dried samples (Fig. 1). The RMSECV of this model is  $0.014 \text{ g g}^{-1}$  dm, which

has to be compared with the SDL of the HTPPH assay of  $0.0129 \text{ g g}^{-1}$  dm. Uncertainty of the NIR estimates (RMSECV) were thus 1.09 times greater than the reference method and better predictions cannot be expected. Range of sugar releases in this study was rather narrow (averages of triplicates ranged from  $0.36$  to  $0.43 \text{ g g}^{-1}$  dm, with a mean value of  $0.39 \text{ g g}^{-1}$  dm and a mean standard deviation of  $0.02 \text{ g g}^{-1}$  dm, Table I) with small differences to detect using the HTPPH assay, which has previously been demonstrated to detect differences in yield of over 5%.<sup>3</sup> As illustrated in Figure 1 the standard deviations for the mean measured sugar releases, analyzed in triplicates with HTPPH method, was large compared with the differences between samples. The relatively low fraction of explained variation in sugar release by the NIR calibration is therefore likely to be caused by a large uncertainty in the HTPPH assay compared with the small differences between samples. As analyses of sugar release, NIR spectra and biochemical composition are not performed on the exact same sample, sampling issues could be important.

**Table II.** Calibration models using NIR spectra to predict total sugar release (TS), glucose release (Glu), xylose release (Xyl), chemical components and anatomical fractions. One model uses chemical components and anatomical fractions to predict total sugar release. Models are presented with number of optimal principal components (PC), root mean square error of cross-validation (RMSECV), percentage of explained  $Y$  variance ( $R^2$ ) for spectra recorded on air-dried or oven-dried ( $80 \text{ }^\circ\text{C}$ ) samples and validated with either repeated random cross validation (RRCV) or site segmented cross validation (SSCV). All calibrations were done with 73 samples. In RRCV calibration set consist of 20 random samples repeated 5 times, while SSCV has a calibration set of the samples from first Abed site ( $n = 40$ ) then Sejet site ( $n = 39$ ). Negative percentage of explained variance is a result of unstable calibrations.

X	Y	NIR air-dried						NIR $80 \text{ }^\circ\text{C}$					
		RRCV			SSCV			RRCV			SSCV		
		PC	RMSECV	$R^2$ %	PC	RMSECV	$R^2$ %	PC	RMSECV	$R^2$ %	PC	RMSECV	$R^2$ %
NIR	TS $\text{g g}^{-1}$ dm	3	0.014	56	3	0.016	46	6	0.012	62	3	0.017	40
Chemistry + anatomy	TS $\text{g g}^{-1}$ dm	1	0.018	23	2	0.019	21	1	0.018	25	2	0.019	21
NIR	Glu $\text{g g}^{-1}$ dm	3	0.010	38	1	0.013	-1	5	0.009	44	2	0.011	25
NIR	Xyl $\text{g g}^{-1}$ dm	4	0.005	68	3	0.006	73	5	0.005	69	5	0.005	76
NIR	%cell	1	1.247	18	1	1.332	32	1	1.246	19	1	1.262	38
NIR	%ash	9	0.230	94	2	0.559	71	12	0.171	96	1	1.262	-21
NIR	%lignin	2	0.742	16	1	0.753	27	1	0.776	2	1	0.808	17
NIR	%hemi	3	0.852	24	1	0.898	17	5	0.826	23	1	0.890	19
NIR	%Leaves	4	1.755	61	6	2.747	34	5	1.775	52	3	1.950	65
NIR	%Ears	3	2.811	20	1	4.276	-11	3	2.886	16	1	3.896	-2
NIR	%Stem	4	3.413	40	1	6.399	-2	3	3.602	28	3	3.928	56



**Fig. 1.** Plot of average measured total sugar release ( $n = 3$ , error bars are standard deviations) in grams glucose plus xylose per gram dry matter biomass from 79 wheat samples versus total sugar release predicted (cross-validated) for each sample by NIR calibration.

When performing triplicate measurements of each analysis, however, we do not see alarming data variations to indicate sampling problems within our milled straw samples. Isci et al.<sup>11</sup> reported a standard error of cross-validation of 2.0% theoretical maximum ethanol yield and a standard error of laboratory results of 2.2% theoretical maximum ethanol yield. Calibration thus had lower uncertainty of NIR estimates than the uncertainty of laboratory results, suggesting an over-fitted model. Although prediction capacity for ethanol yield was high (correlation coefficient between measured and predicted values was 0.96), calibration was only performed on 24 samples.<sup>11</sup>

With our data set we also had the opportunity to study if measurements of chemical composition and anatomical fractions together would be able to predict the release of total sugar. A model with chemical and anatomical data as the X matrix and total sugar release as the Y matrix explained up to 25% variance with a RMSECV of 0.018  $\text{g g}^{-1} \text{ dm}$  (Table II). Results were only slightly affected by validation method, indicating good transferability of the calibrations. Chemical and anatomical composition thus seems to be important for sugar release of the samples (predicting 25% variance), but is a poorer predictor of total sugar release than NIR spectra (predicting 56% variance). NIR was a better predictor of the release of xylose than the release of glucose (Table II), and calibrations for xylose were more robust in terms of transferability and oven-drying the samples.

### 3.2. Calibrations and Predictions of Chemical Components

Complex models (9 and 12 PCs) could predict 94–96% of the variance in ash content (Table II). Curiously, for the ash predictions we observed a large difference in the calibration performances between the two different validation methods (Table II). RMSECV is two to seven times

higher for site specific validation than for random validation, indicating that there are problems with the transfer of the RRCV based calibration models from site to site and therefore also outside the calibration set. When using spectra from air-dried samples the prediction performance after SSCV is lowered to 71%, whereas the spectra from oven-dried samples have no prediction value for ash at all. The fact that the RRCV model had a much better  $R^2$  value and used more components than the SSCV signified that the good performance of the RRCV was only valid within the sites used for calibration and that the model is not transferable to other sites. Oven-drying samples have apparently introduced a site variation in NIR spectra, rendering spectra unable to predict ash content in new samples from another site. Problems with transferability of models predicting ash content of wheat straw was also found by Bruun et al.<sup>18</sup> Our results indicate that these problems are accentuated when the samples are dried.

The percentage of explained variance for predictions of cellulose, lignin, and hemicellulose evaluated by RRCV and based on the spectra of the air-dried samples was 18%, 16%, and 24% with RMSECV of 1.2, 0.74, and 0.85 using one, two, and three components. Similar results were obtained for the calibrations based on the oven-dried samples except for the calibrations of the lignin content which did not seem to work on dried samples. Comparing RMSECV with the SDL for each component in the acid-hydrolysis established that the uncertainty of the NIR estimates were 1.5, 1.1, and 0.42 times that of the uncertainty of the reference method. Predicting a greater percentage of variance in cellulose, lignin or hemicellulose therefore requires better reference methods for samples like ours with a relatively narrow span in chemical composition. As achieving much more accurate results from the compositional analyses used as reference method is unlikely, it also appears impossible to achieve a higher fraction of explained variance when datasets with such a narrow span in composition is used. The average values of lignin, ash and carbohydrates measured with the NREL method sum to approximately 88% of the dry weight of samples (Table I) and attempting to close the mass balance further by including extraction would have changed the composition of preprocessed biomass which we aim to predict.

Lomborg et al.<sup>12</sup> developed calibrations of glucan, xylan, arabinan and lignin of wheat straw samples and obtained squared correlation coefficients ( $r^2$ ) between measured and predicted values of  $r^2 = 0.83, 0.82, 0.77$  and  $0.72$  and root mean square error of prediction, RMSEP, of 0.60, 0.43, 0.12 and 0.38. It would seem that Lomborg et al.<sup>12</sup> were more successful in developing calibrations than in the present study. The calibrations of Lomborg et al.<sup>12</sup> are however performed on a small dataset (44 samples with up to 18% outliers) and validated in a full-cross validation, which is likely to result in more optimistic

results than the cross-validations employed in the present study. We have not been able to calculate the SDL of the reference method for chemical compositions in Lomborg et al.<sup>12</sup>

#### 4. CONCLUSION

NIR could predict 56% of the variance in total sugar release (glucose and xylose) with a RMSECV of 0.014 g g<sup>-1</sup> dm. In terms of monomeric sugar release, NIR was a better predictor of xylose release (max  $R^2 = 76\%$ ) than of glucose release (max  $R^2 = 44\%$ ). Percentage of explained variance for predictions of cellulose, lignin, hemicellulose, and ash was 18%, 16%, 24%, and 94%. The relatively low percentage of explained variance in total sugar release was mainly due to the uniformity between samples, all consisting of wheat straw, which rendered uncertainty of replicates in HTPPH assay to be large compared with variance between samples. NIR spectroscopy is therefore concluded to have potential as a method for assessing sugar release of wheat straw. Predictions of sugar release made from NIR calibrations was significantly better than predictions made from measurements of chemical and anatomical composition, which further accentuates the usefulness of NIR spectroscopy. Predictions of chemical components made from NIR calibrations were, however, limited by the reference method on our uniform sample-set. Despite successful prediction of ash content, site-specific cross-validation indicated that there might be problems with model transferability from site to site.

**Acknowledgments:** The current project was funded by the Danish strategic research council through the OPUS project (case file 2117-05-0064). The extensive work of measuring the chemical composition of all wheat materials was done by the lab technicians at Department of Forest and Landscape, University of Copenhagen. Support by the

Office of Biological and Environmental Research in the DOE Office of Science for the BioEnergy Science Center (BESC) was essential to the development of the HTPPH system applied in this research.

#### References

1. C. E. Wyman, *Trend. Biotechnol.* 25, 153 (2007).
2. S. R. Decker, R. Brunecky, M. P. Tucker, M. E. Himmel, and M. J. Selig, *Bioenergy Res.* 2, 179 (2009).
3. M. H. Studer, J. D. DeMartini, S. Brethauer, H. L. McKenzie, and C. E. Wyman, *Biotechnol. Bioeng.* 105, 231 (2010).
4. M. A. Sanderson, F. Agblevor, M. Collins, and D. K. Johnson, *Biomass and Bioenergy* 11, 365 (1996).
5. M. Blanco, A. C. Peinado, and J. Mas, *Biotechnol. Bioeng.* 88, 536 (2004).
6. C. J. Shih and E. A. Smith, *Anal. Chim. Acta* 653, 200 (2009).
7. D. Templeton, A. Sluiter, T. Hayward, B. Hames, and S. Thomas, *Cellulose* 16, 621 (2009).
8. E. J. Wolfrum, A. J. Lorenz, and N. de Leon, *Cellulose* 16, 577 (2009).
9. B. R. Hames, S. R. Thomas, A. D. Sluiter, C. J. Roth, and D. W. Templeton, *Appl. Biochem. Biotechnol.* 105, 5 (2003).
10. A. J. Lorenz, J. G. Coors, N. de Leon, E. J. Wolfrum, B. R. Hames, A. D. Sluiter, and P. J. Weimer, *Crop Sci.* 49, 85 (2009).
11. A. Isci, P. T. Murphy, R. P. Anex, and K. J. Moore, *Bioenergy Res.* 1, 163 (2008).
12. C. J. Lomborg, M. H. Thomsen, E. S. Jensen, and K. H. Esbensen, *Bioresour. Technol.* 101, 1199 (2010).
13. A. Sluiter, Determination of structural carbohydrates and lignin in biomass, NREL laboratory analytical procedures, National Renewable Energy Laboratory, Golden, CO, USA (2004).
14. R. P. Overend and E. Chornet, *Philosophical Transactions of the Royal Society of London Series A-Mathematical Physical and Engineering Sciences* 321, 523 (1987).
15. H. Martens, J. P. Nielsen, and S. B. Engelsen, *Anal. Chem.* 75, 394 (2003).
16. R. J. Barnes, M. S. Dhanoa, and S. J. Lister, *Appl. Spectrosc.* 43, 772 (1989).
17. A. Savitzky and M. J. E. Golay, *Anal. Chem.* 36, 1627 (1964).
18. S. Bruun, J. W. Jensen, J. Magid, J. Lindedam, and S. B. Engelsen, *Industr. Crops Prod.* 31, 321 (2010).

Received: 19 July 2010. Revised/Accepted: 27 September 2010.