Fast analysis of high heating value and elemental compositions of sorghum biomass using near-infrared spectroscopy

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Abstract

Near-infrared spectroscopy (NIR) is an efficient, low-cost sensing technology that has potential as an accurate biomass characterization method. The objective of this study is to develop NIR models in conjunction with chemometrics to determine high heating value (HHV) and elemental compositions of sorghum biomass. Partial least squares (PLS) regression and principle component regression (PCR) were used to develop calibration models with full and reduced wavelength regions. In general, models from reduced wavelength regions yielded higher calibration and prediction accuracies. Models to predict HHV, carbon, hydrogen, nitrogen, sulfur, and oxygen contents of sorghum biomass were well developed. HHV value, carbon, hydrogen, nitrogen, sulfur, and oxygen contents were predicted with R^2 of 0.96, 0.96, 0.87, 0.86, 0.84, and 0.83 for validation sample sets, respectively. HHV and carbon content models had excellent prediction accuracy, whereas hydrogen, nitrogen, sulfur and oxygen models could provide reliable predictions. Those models provide good insight into the relationship between chemical

27 bonds and HHV and elemental composition of sorghum biomass, allowing a rapid and accurate
28 determination of HHV and elemental composition at low cost (from 200 to 1 USD) and reduced
29 the time (from 100 to 1 min).

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31 **Keywords:** sorghum; heating value; elemental composition analysis; near-infrared; chemometric
32 analysis

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1. Introduction

Renewable energy derived from biomass is an desirable alternative to conventional fossil fuel because biomass is abundant and has no negative impact on the environment [1]. Sorghum is a favorable feedstock for biofuel production because of its high yield efficiency and high tolerance to drought [2]; furthermore, sorghum yield ranks third among cereal crops in the Midwest region of the United States [3]. Efficiency of biomass utilization for biofuel significantly depends on physical and chemical properties, which vary according to plant species, growing environments, harvest times, and storage conditions [4]. Recently, some biomass utilization have been directly related to energy production, such as thermal application for greenhouse heating [5]. Therefore, analysis of biomass properties is essential to understand, predict, and control conversion systems. HHV is an important thermal property to characterize how much energy can be produced by the combustion of a unit of biomass. Elemental composition analysis, including carbon, oxygen, hydrogen, nitrogen, and sulfur, is necessary to calculate biomass heat content, perform mass and heat balances in the bioconversion process, and predict potential pollution complications during biomass thermal processes. The high heating value (HHV) and elemental composition of biomass feedstock determine the theoretical biomass conversion efficiency and have a great impact on bio-industry economics. In order to effectively use different types of biomass and to fast predict biomass energy potential, studies have explored correlations between HHV and elemental composition of biomass [6-9], but determination of HHV and elemental composition is limited by costly, labor-intensive conventional methods. HHV has been determined by absorbed heat from completed combustion in an oxygen bomb calorimeter, a process that usually requires at least 25 min per measurement, plus sample pelleting and replication. Elemental composition

analysis is also time-consuming and expensive; therefore, rapid, inexpensive methods of analyzing biomass properties must be developed.

NIR is a rapid measurement technique based on spectrum absorption by a specific function group within a biomass. NIR has shown advantages in harsh industrial biomass utilization, such as high-throughput, online, and non-destructive prediction [10, 11]. Some studies have used NIR to predict carbon, hydrogen, and nitrogen in coal [12, 13]. Alciaturi et al. [14] built an NIR model of the heating value of coal with good correlations ($R > 0.9$). NIR has also been used to model carbon and nitrogen of soil and plant materials [15] and analyze HHV and elemental composition of biomass. Lestander and Rhen [16] effectively modeled HHV of Norway spruce with a coefficient of variation of 0.6%. Allison et al. [17] studied the feasibility of using NIR to predict nitrogen content of two dried grass species in different growing conditions with $R^2 = 0.94$. HHV and carbon content of miscanthus and short-rotation coppice willow have been modeled by NIR with $R^2 = 0.99$ and 0.88, respectively [18]. Follow-up research determined the accuracy of visible and NIR models by PLS regression method with a root mean square error of cross-validation (RMSECV) of 0.30 MJ Kg⁻¹ and coefficient of determination (R^2) of 0.97 [19]. Lestander et al. [20] reported NIR provided excellent prediction of HHV of bio-coal from torrefaction and pyrolysis of biomass, and the optimal model can explain 96% variance of HHV. Via et al. [21] modeled HHV changes of sweetgum, loblolly, pine, and switchgrass after torrefaction with NIR and Fourier Transform infrared spectroscopy (FTIR). The NIR model developed by Gillespie et al. had high accuracy for prediction of HHV of wood, miscanthus and herbaceous energy grasses with $R^2 = 0.94$ [22]. Recently, the performance of HHV and elemental composition prediction of 222 straw samples using NIR has been studied [6, 23]. Xiao et al. [24] reviewed NIR coupled with multivariate data analysis as a high-throughput technique

to evaluate HHV and other important properties. In our previous study, both structural polysaccharides and soluble sugars were predicted using NIR with PLS method at reliable predication with R^2 of 0.87 and 0.90 for structural polysaccharides and soluble sugars, respectively [25]. In addition, this study is part of a sorghum biomass genomics and phenomics project to investigate the relationship between genotype and selectively phenotype in 100 sorghum accessions with maximum of genotyping diversity, allowing efficient and cost-effective genome sequencing and breeding. NIR model development is high-throughput and cost-effective method to link the observed phenotypic variation for biofuel conversion and genes and germplasm variation.

Although NIR has been used extensively in the food, pharmaceutical, and agricultural fields [26-28], to our knowledge, no published information exists on HHV and elemental composition modeling of sorghum biomass using NIR. Unlike biomass yield, which can be easily measured by a biomass harvester, measurement of HHV and elemental composition of biomass needs advanced instruments and requires trained professional with high cost and labor intensive. With NIR technology, chemical composition and physical properties of biomass can be rapidly and accurately measured. The main advantages of NIR spectroscopy technology are that sample preparation is simple, analysis is fast and precise, and many constituents can be analyzed at the same time. Therefore, NIR technology can enable us to develop high-throughput phenotyping methods and to allow us to obtain a broad view of genetic diversity in biomass germplasm. The objective of this research was to develop standardized NIR models in conjunction with chemometrics to high-throughput and cost-effective determine HHV and elemental composition of sorghum biomass. This is the first report to model HHV and elemental composition of sorghum biomass via NIR coupled with multivariate data analysis. The NIR method significantly

reduced the time (from 100 to 1 min) and cost (from 200 to 1 USD) of the determination of biomass HHV and elemental composition. Last but not least, this study leverage a high-throughput, cost-effective phenotype of HHV and elemental in sorghum biomass to facilitate plant breeding and genetics studies. Future work of the NIR model should focus on developing a model system to achieve rapid analysis of genomics at low cost to assist plant feedstock improvement.

2. Materials and Methods

2.1 Materials

Samples of the most representative 100 accessions of sorghum used in this study were harvested in Lubbock, TX in October 2012. Samples were provided by the United States Department of Agriculture National Plant Germplasm System (USDA-NPGS), in which 44,774 accessions of sorghum originated from 116 countries. After harvest, biomass samples were air-dried at 60 °C and ground to particle size of 0.5 mm (UDY cyclone mill, Fort Collins, CO) for HHV and elemental analysis. After grinding, all samples were stored in sealed plastic bags. All measurements were carried out in a short time at a lab with stable environmental conditions of 22 °C and 40% humidity. All chemicals were purchased from Sigma (St. Louis, MO).

2.2 Reference methods

HHV was determined using a calorimeter (IKA-Calorimeter C 200, IKA-Werke GmbH and Co. KG, Staufen, Germany) with a benzoic acid standard. Approximately 1.00 g of each pelleted sample was put into an adiabatic bomb calorimeter and burned to ash. Powder samples were compacted into pellets for measurement in order to reduce error caused by incomplete combustion resulting from dry, loose samples blown away during sudden release of volatiles.

The elemental composition of sorghum biomass samples was measured with CHNS/O Elemental Analyzer (PerkinElmer 2400 Series II, PerkinElmer Inc., Waltham, MA). Two to three milligrams (accurate to 0.001 mg) of the ground sample with fine uniform particle size was weighted into tin capsules using a PerkinElmer AD-6 Autobalance (PerkinElmer Inc., Waltham, MA). The ground sample was packed with foil, introduced into the combustion chamber through a funnel, and burned under a pure oxygen atmosphere. Gases from combustion (CO_2 , N_2 , SO_2 , and H_2O) were separated in a quartz column containing copper wires detected by a thermoconductometer detector. Elemental compositions were reported as a percentage of initial dry weight (w/w, db).

2.3 Multiple linear regression analysis

Multiple linear regression (MLR) analysis was carried out to estimate the relationship between HHV and elemental compositions. Pearson correlations between two parameters and multiple stepwise regression of all parameters were analyzed using SAS (SAS Institute, Inc., Cary, NC). All significance analysis was at significance level 0.05.

2.4 NIR measurement

NIR spectra were measured with an Antaris II FT-NIR analyzer (Thermo Scientific Inc., Madison, WI, USA) in reflectance mode. A sample cup spinner (Thermo Scientific, WI, USA) with an Integrating Sphere module was used to quickly and reliably obtain bulk information from powder biomass. Spectra were collected by rotating the sample cup through the NIR beam. Each spectrum was averaged with 32 scans at a resolution of 4 cm^{-1} in the wavelength range of 4,000–10,000 cm^{-1} .

2.5 Spectra pretreatment and chemometric analysis

Spectra pretreatment and chemometric analysis was conducted by TQ Analyst 8.6.12 (Thermo Scientific Inc., Madison, WI). Pathlength was calibrated using standard normal variate (SNV) and multiplicative signal correction (MSC). The Savitzky-Golay filter was used to reduce random noise. First and second derivatives were used as a pretreatment method to resolve spectra peak overlap and eliminate linear baseline drift. The first derivative was the rate of change of absorbance with respect to wavelength, whereas the second derivative corresponded to the curvature or concavity of the graph. First and second spectra formats were compared.

To avoid bias in the subset and achieve a calibration set and validation set with a ratio of 3, the entire set of 100 sorghum biomass samples was sorted ascendingly by measured value. One in every four samples was randomly assigned to the prediction set (25), with remaining samples as calibration samples (75). A full spectra range from 4,000 to 10,000 cm^{-1} was used unless otherwise specified. PLS and PCR methods described in the literature [11] were used for model development. Model performance was evaluated in terms of the coefficient of determination (R^2), root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP), and ratio of standard deviation of calculated set (SD_y) to RMSEP (RPD), calculated with the equations:

$$RMSEC = \sqrt{\frac{\sum_{i=1}^{n_c} (\hat{y}_i - y_i)^2}{n_c}} \quad (1)$$

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n_p} (\hat{y}_i - y_i)^2}{n_p}} \quad (2)$$

$$RPD = \frac{SD_y}{RMSEP} \quad (3)$$

where n_c and n_p are the number of samples in calibration set and prediction set, respectively, $\hat{y}_i$ and y_i are the predicted value and measured value of the i^{th} sample, respectively, and SD_y is standard deviation of calculated set.

The Chauvenet test was used to eliminate outliers defined as the points at distances in the principle component space greater than 3.0. Predicted residual error sum of squares (PRESS) diagnostic function was used to determine the number of factors necessary for calibration.

3. Results and Discussion

3.1 Samples statistics

Table 1 summarizes the range, mean, and standard deviation of HHV and elemental composition of sorghum biomass samples using reference methods. The representative 100 accessions of sorghum biomass samples showed good range and distribution: 15.67 to 16.99 MJ/kg with standard deviations of 0.26 MJ/kg for HHV, 39.65 to 42.37% with standard deviation 0.57% for carbon content, 4.26 to 6.4% with standard deviation 0.43% for hydrogen content, 0.47 to 1.65% with standard deviation 0.22% for nitrogen content, 0.24 to 1.5% with standard deviation 0.17% for sulfur content, and 50.13 to 53.24% with standard deviation 0.59% for oxygen content. In addition, the range, mean, and standard deviation of the calibration set covered the validation set, and both subsets showed consistent distribution for robust model construction. The range of HHV and elemental composition were similar to those reported by previous research [29, 30]. In generally, biomass has similar HHV ranged from 12 to 18 MJ/kg, whereas coal has higher HHV of 26-30 MJ/kg [31].

The Pearson correlation coefficient was employed to analyze relationships between variables. As shown in Table 2, a strong correlation was observed between HHV and carbon ($R = 0.841$, p

< 0.01), HHV and oxygen ($R = -0.692$, $p < 0.01$), and carbon and oxygen ($R = -0.822$, $p < 0.01$). This result suggests that sorghum biomass with higher carbon content and lower oxygen content could release additional heat after combustion. Nitrogen element in sample was converted to simple gas N_2 in combustion process and showed a negative correlation between it and HHV, possibly as a result of relationships between elements such as carbon and nitrogen ($R = -0.527$).

A MLR model was developed; regression coefficient results of the model are listed in Table 3. Although only one model was obtained from MLR, this model showed good performance, with a high $R^2 = 0.84$ and low p-value < 0.001 with F value of 237.18, as shown below:

$$\text{High heating value} = 1.325 + 0.841 \times \text{Carbon} \quad (4)$$

In this equation, hydrogen, nitrogen, sulfur, and oxygen were not significantly independent, even though HHV showed significant correlations with nitrogen and oxygen. A possible reason is that there were strong collinearity between carbon, nitrogen and oxygen. MLR with stepwise exclusively selected optimal variables rather than all significant variables in Pearson correlation. As carbon content of sorghum biomass sample increased by 1%, HHV of the sample increased 0.841 KJ, significantly differing from a majority of previous results [6, 8, 32]. This result could have been caused by strong multicollinearity between independent variables [33]; however, Tillman [34] proposed a classic equation in which only carbon content was correlated with HHV. From MLR regression results, prediction of HHV from elemental composition is a fairly reliable method ($R^2 = 0.84$), a less expensive, more accurate method to measure HHV and elemental composition must be found.

3.2 Samples spectra

The near-infrared spectrum of 100 sorghum biomass samples in the 4000-10000 cm^{-1} range is presented in Fig. 1. In general, all NIR spectra exhibited three main peaks. The first peak was relatively wide, between 6550 and 7100 cm^{-1} , in relation to cellulose (6711 cm^{-1}) and O-H stretch in the first overtone of H_2O (6897 cm^{-1}) and the C-H combination of aromatic structures from lignin (6915 cm^{-1}) [35]. The second peak at 5180 cm^{-1} was associated with O-H stretch/HOH deformation from a combination of cellulose [35]. The third peak could correspond [36] to the asymmetrical C-O-O stretch in the third overtone of cellulose (4745 cm^{-1}) [35].

3.3 NIR model development for HHV

Calibration models for predicting HHV were developed using PCR and PLS methods. Table 4 shows that PLS always had better performance than PCR based on larger R^2 and RPD, thereby indicating that PLS is a more powerful quantitative analysis for HHV of sorghum biomass. For PLS analysis methods, all relevant spectral information in the analysis region or regions of the calibration spectra were condensed into a set of principal components. Each principal component (PC) represented an independent source of spectral variation in the data. PC analysis plots were helpful in examining sample groups on the basis of similar characteristics and in identifying outliers. Fig. 2A shows plots of PC analysis scores of HHV. PC1 described a majority of variation in the calibration spectra and accounted for 86.78% of variation explanation; PC2 represented 8.99% of variation among the samples. Distribution of sample dots was wide and symmetrical, suggesting that the samples were representative and there were no obvious outliers.

Spectra processes such as derivatives were applied to improve model prediction. The performance of models after first and second derivatives treatment is summarized in Table 4. Neither first nor second derivative treatment significantly increased goodness-of-fit of calibration

and validation sets, suggesting derivative spectra were not useful for HHV model development because spectra transformation generated false information from mathematical artifacts [37]. To further optimize the HHV model, a reduced spectral range was used to decrease noise. In Fig. 3 A, the mean spectra (red curve) show the average of all spectra. The variance spectra (blue curve), which show variance in all spectra, was produced by calculating the square root of the spectral variance at each HHV value across all spectra. The regression coefficient spectra (black curve) indicate which wavelengths had larger weights in the calibration model. Wavelengths from 6000 to 7000 cm^{-1} were selected as reduced wavelength regions to improve the model because of their high coefficient and high variance, thereby indicating high correlation and larger variation. The reduced-wavelength region had better model performance than the full-range model, with R^2 of 0.96, RMSEP of 0.061KJ/Kg, and RPD of 4.59 for the validation sample set, indicating that this model is able to provide an accurate prediction and could be used in most applications [38]. This result is in good agreement with a previous study in which wavelengths from 6500 to 8500 cm^{-1} were considered the important region in order to predict HHV because this region was assigned to the C-H stretching (second overtone) of aromatic and CH_3 structures [18]. Lestander et al. [10] reported that HHV prediction using peaks of NIR spectra at 6900 and 5200 cm^{-1} , which associated with O-H stretching and bending in water. Moreover, Lestander and Rhen [16] listed covalent bonds contributing to HHV in decreasing order, C=C, S-H, C-H, C-C, C-N, N-H, C=O, C-O, and O-H. All 10 calculated factors were used in calibration models, suggesting that calculated factors were insufficient. Fig. 4A shows the plot of calculated versus actual HHV in the improved model. The model was robust and excellent for predicting HHV of sorghum biomass samples. The optimal model for HHV prediction this study yielded better performance than existing NIR model for coal [14], Norway spruce [16], and short-rotation

coppice willow, whereas slightly worse than HHV prediction model for miscanthus with $R^2 = 0.99$.

3.4 NIR model development for carbon content

Table 5 shows models' performances related to carbon content prediction. Full-range spectra were used in PLS and PCR models to yield similar R^2 of 0.88 in the validation set. However, the PLS model had better fitness in the calibration set, with R^2 of 0.88 and RPD of 3.03, compared to the PCR model, with R^2 of 0.71 and RPD of 2.60 (Table 5). In Fig. 2B, PC1 and PC2 explain 73.52% and 23.81% of variance in the samples' carbon contents. Distribution of sample dots was not as good as in the HHV model, resulting in lower fitness. The first derivative of spectra slightly increased model performance, with R^2 of 0.9 in the calibration set and RPD of 3.39, but the second derivative of spectra did not improve the model. According to the same principle of reduced range as in the HHV model, wavelengths from 4362 to 7000 cm^{-1} in Fig. 3B were optimal for the carbon content prediction model. This range was essential for predicting carbon content because it contained C-H stretching and/or deformation of CH, CH_2 , CH_3 , aromatics, -CHO and cellulose (7000, 5900, 5800, 4550, and 4350 cm^{-1}); O-H stretching and deformation of ROH at 7010 and 4800 cm^{-1} , respectively; C=O stretching (second overtone) of $-\text{CO}_2\text{H}$, CONH, and CONH (5260, 5200, and 4920 cm^{-1}); N-H stretching (first overtone) of CONH_2 (6990 cm^{-1}); and S-H stretching (first overtone) of -SH (5740 cm^{-1}) [18]. Reduced range significantly increased calibration R^2 in raw spectra, first derivative spectra, and second derivative spectra, but reduced range worsened performance in validation R^2 in first derivative spectra and second derivative spectra. The best carbon prediction model using PLS with raw spectra in 4362 to 7000 cm^{-1} had a validation R^2 of 0.96, RMSEP of 0.115%, and RPD of 4.69. For optimal prediction, factor number adjustment was based on the PRESS plot of various calculated factor numbers, in

which PRESS value was inversely proportional to prediction performance. The factor number of 8 in the current model was less than that of calculated factor number (10), suggesting no additional factor numbers were needed for calibration. As shown in Fig. 5, PRESS values in the plot reached the turning point at six factors, gradually decreased, and finally leveled off to a constant value. Although PRESS values eventually decreased below the turning point when more factors were used, model performance was not likely to improve, possibly decreasing if the calibration model became overfit. Fig. 4B compares calculated versus actual carbon content in order to demonstrate excellent prediction of the model for potential use in most applications. This NIR model showed comparable performance as previous researches, such as carbon prediction model for coal [12], heterogeneous plant material [15], as well as switch grass and reed canary grass [17].

3.5 NIR model development for hydrogen, nitrogen, sulfur, and oxygen contents

Prediction models for hydrogen, nitrogen, sulfur, and oxygen contents were developed by strategies similar to those outlined above, as summarized in Table 6. PLS analysis was applied in selection of all quantitative methods. All elemental composition had SNV pathlength data processing, with the exception of MSC for hydrogen for which the best model had R^2 of 0.93 for the calibration sample set, R^2 of 0.87 for the validation sample set, and RMSEP of 0.072% and RPD 2.52 with no spectral treatment in the spectral region 4500-9800 cm^{-1} . Prediction nitrogen model was developed with SNV treatment and no spectral treatment in the spectral region 5000-5500 cm^{-1} to achieve good fitness as R^2 of 0.86 for the prediction sample set. Sulfur models had best performance with R^2 of 0.88 for the calibration sample set and R^2 of 0.84 for the prediction sample set in the full wavelength range, which is less robust. One possible reason for the fair performance of nitrogen and sulfur models is the low concentration with relatively higher

variation in samples. Oxygen prediction was fair after treating spectra with the first derivative and reducing the range from 4500-8000 cm^{-1} ; the model had a calibration R^2 of 0.83, a validation R^2 of 0.83, an RMSEP of 0.166%, and an RPD of 3.37. The decreased fitness of the oxygen prediction model was likely caused by the non-independence of oxygen, calculated from the difference between 100% and the sum of carbon, hydrogen, nitrogen, and sulfur. This result also confirmed the strong multicollinearity of MLRs. Although prediction models of hydrogen, nitrogen, sulfur, and oxygen were not as good as HHV and carbon content prediction models, they are still good enough to use with caution in most applications, such as research studies and screenings [38].

4. Conclusion

Models to predict HHV, carbon, hydrogen, nitrogen, sulfur, and oxygen contents of sorghum biomass were well developed. HHV, carbon, hydrogen, nitrogen, sulfur, and oxygen contents were predicted with validation R^2 of 0.96, 0.96, 0.87, 0.86, 0.84, and 0.83, respectively. HHV and carbon content were deemed excellent and suitable for use in any application. The hydrogen, nitrogen, sulfur and oxygen models could be usable with caution in most application such as research study. The NIR method significantly reduced the time (from 100 to 1 min) and cost (from 200 to 1 USD) of the determination of biomass HHV and elemental composition. The future work of the NIR model should focus on developing a model system to achieve rapid analysis of genomics at low cost to assist plant feedstock improvement. This study would be very useful for scientists in research institutions, engineers in bio-industries, and biomass producers, and also filling the gap between plant breeding and various biomass applications.

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321 **Nomenclature**

FTIR	fourier Transform infrared spectroscopy
HHV	high heating value
MLR	multiple linear regression
MSC	multiplicative signal correction
NIR	near-infrared spectroscopy
PCR	principle component regression
PLS	partial least squares
PRESS	Predicted residual error sum of squares
RMSEC	root mean square error of calibration
RMSECV	root mean square error of cross-validation
RMSEP	root mean square error of prediction
RPD	ratio of standard deviation of calculated set
SD _y	standard deviation of calculated set
SNV	standard normal variate

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Fig. 1. Near-infrared spectra of one hundred sorghum biomass samples.

A

B

Fig. 2. Plot of principal component analysis scores of HHV (A) and carbon content (B) in PLS model.

A

B

Fig. 3. Regression coefficient, mean, and variance spectra of HHV (A) and carbon content (B) in PLS model.

A

B

Fig. 4. Linear regression plots of calculated versus actual HHV (A) and carbon content (B).

Fig. 5. Plot of PRESS of calculated factor numbers in carbon content prediction model.

Table 1. Statistics of HHV and elemental composition of sorghum biomass using reference methods

	Full set			Calibration set			Validation set		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
HHV (MJ/kg)	15.67-16.99	16.25	0.26	15.68-16.92	16.24	0.28	15.67-16.99	16.25	0.27
Carbon (%)	39.65-42.37	41.07	0.57	39.65-42.35	41.09	0.59	40.22-42.37	41.02	0.54
Hydrogen (%)	4.26-6.4	5.13	0.43	4.26-6.4	5.12	0.49	4.88-5.49	5.15	0.18
Nitrogen (%)	0.47-1.65	1.01	0.22	0.48-1.48	1.00	0.21	0.47-1.65	1.02	0.25
Sulfur (%)	0.24-1.5	1.19	0.17	0.24-1.50	1.19	0.18	0.8-1.39	1.19	0.15
Oxygen (%)	50.13-53.24	51.61	0.59	50.13-53.24	51.63	0.61	50.15-52.74	51.55	0.56

Table 2. Pearson correlation coefficient between HHV and elemental composition of sorghum biomass

	HHV	Carbon	Hydrogen	Nitrogen	Sulfur	Oxygen
HHV	1	0.841**	0.184	-0.476**	-0.052	-0.692**
Carbon	0.841**	1	0.248*	-0.527**	-0.034	-0.822**
Hydrogen	0.184	0.248*	1	-0.274**	0.001	-0.413**
Nitrogen	-0.476**	-0.527**	-0.274**	1	-0.224*	0.435**
Sulfur	-0.052	-0.034	0.001	-0.224*	1	-0.238*
Oxygen	-0.692**	-0.822**	-0.413**	0.435**	-0.238*	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 3. MLR coefficient results of HHV and elemental composition of sorghum

	Beta	t-values	p-values
Constant	6.175	6.725	0.000
Carbon	0.245	10.959	0.000
Hydrogen	-0.027	-0.478	0.633
Nitrogen	-0.045	-0.705	0.482
Sulfur	-0.024	-0.432	0.667
Oxygen	-0.001	-0.008	0.994

Table 4. Model development for HHV

		Full range (4000-10000 cm ⁻¹)			Reduced range (6000-7000 cm ⁻¹)		
		Spectra	First derivative	Second derivative	Spectra	First derivative	Second derivative
PLS							
	R ² , cal.	0.83	0.82	0.83	0.95	0.99	0.90
	R ² , val.	0.83	0.82	0.58	0.96	0.89	0.52
	RMSEP	0.112	0.118	0.174	0.061	0.089	0.188
	RPD	2.50	2.37	1.61	4.59	3.15	1.49
	Factor used	8	6	6	10	10	5
PCR							
	R ² , cal.	0.71	0.76	0.69	0.80	0.81	0.57
	R ² , val.	0.57	0.71	0.49	0.85	0.78	0.44
	RMSEP	0.177	0.151	0.191	0.107	0.126	0.206
	RPD	1.58	1.85	1.47	2.62	2.22	1.36
	PC used	7	10	10	10	10	10

Table 5. Model development for carbon content

		Full range (4000-10000 cm ⁻¹)			Reduced range (4362-7000 cm ⁻¹)		
		Spectra	First derivative	Second derivative	Spectra	First derivative	Second derivative
PLS							
	R ² , cal.	0.88	0.89	0.68	0.95	0.97	0.99
	R ² , val.	0.88	0.90	0.46	0.96	0.83	0.20
	RMSEP	0.195	0.174	0.428	0.115	0.246	0.516
	RPD	3.03	3.39	1.38	4.69	2.40	1.14
	Factor used	10	7	4	8	8	9
PCR							
	R ² , cal.	0.71	0.73	0.72	0.91	0.81	0.52
	R ² , val.	0.88	0.84	0.76	0.90	0.88	0.07
	RMSEP	0.227	0.241	0.293	0.184	0.199	0.573
	RPD	2.60	2.45	2.01	3.21	2.96	1.03
	PC used	9	10	10	10	10	10

Table 6. Summary of preferred prediction models for hydrogen, nitrogen, sulfur, and oxygen contents

	Hydrogen	Nitrogen	Sulfur	Oxygen
Quantitative method	PLS	PLS	PLS	PLS
Pathlength type	MSC	SNV	SNV	SNV
Spectral region	4500-9800 cm ⁻¹	5000-5500 cm ⁻¹	4000-10000 cm ⁻¹	4500-8000 cm ⁻¹
Spectral treatment	none	none	none	first derivative
R ² , cal.	0.93	0.88	0.87	0.83
R ² , val.	0.87	0.86	0.84	0.83
RMSEP	0.072	0.084	0.063	0.166
RPD	2.52	2.98	2.38	3.37
Factor used	4	8	7	5