



ISSN: 2230-9926

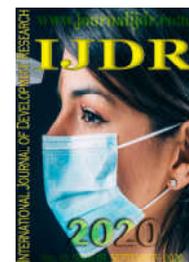
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# IJDR

International Journal of Development Research

Vol. 10, Issue, 09, pp. 40916-40920, September, 2020

<https://doi.org/10.37118/ijdr.20090.09.2020>



RESEARCH ARTICLE

OPEN ACCESS

## PREDICTION OF HIGH-BIOMASS SORGHUM QUALITY USING NEAR INFRARED SPECTROSCOPY TO MONITORING CALORIFIC VALUE, MOISTURE, AND ASH CONTENT

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### ARTICLE INFO

#### Article History:

Received 20<sup>th</sup> June 2020

Received in revised form

06<sup>th</sup> July 2020

Accepted 14<sup>th</sup> August 2020

Published online 30<sup>th</sup> September 2020

#### Key Words:

Biofuel, Multivariate calibration, Bioenergy crop, Partial least squares regression.

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### ABSTRACT

High-biomass sorghum is a crop that has great potential as a source of biomass for energy generation, due to its high productivity, drought tolerance and for being mechanizable. Thus, culture is an alternative to vegetable biomass to be used in electric energy cogeneration processes. The objective of the work was to develop multivariate calibration models, using the near infrared spectroscopy, for analysis of gross calorific value, moisture, and ash content in high-sorghum biomass. At samples were analyzed by reference methods and the results associated with the near infrared spectrum of each sample. Then they were developed for each parameter, multivariate calibration models using the partial least square (PLS) algorithm. A high correlation was obtained between the values predicted by the model and the values obtained by reference method for all properties evaluated. Ratio of prediction to deviation (RPD) and range error ratio (RER) values, respectively, above 3 and 10, for all the models constructed, thus being considered adequate for carrying out quantitative analyzes of chemical composition in the qualification of the sorghum biomass as a source of raw material for energy cogeneration and optimization of biomass conversion technologies.

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Citation: Maria Lúcia F. Simeone, Rafael A. C. Parrella, Cynthia M. B. Damasceno and Robert E. Schaffert. "Prediction of high-biomass sorghum quality using near infrared spectroscopy to monitoring calorific value, moisture, and ash content.", *International Journal of Development Research*, 10, (09), 40916-40920.

### INTRODUCTION

The biomass represents one of the most abundant and promising alternatives for raw materials based on clean and renewable energy sources. Direct combustion is the main technology applied for the production of heat and mechanical energy from biomass, with socioeconomic and environmental advantages, such as a reduction in the balance of emissions of products harmful to the environment (Burin *et al.*, 2015). With high production of biomass, sorghum [*Sorghum bicolor* (L) Moench] has a great potential for burning in boilers of large or thermoelectric plants (May *et al.*, 2014). The genotypes characterized as high-sorghum biomass have a good adaptability, reaching a height of 6.0 m in height and a fresh mass production of 120 t.ha<sup>-1</sup> to 150 t.ha<sup>-1</sup>, with a growth cycle of 180 days. With the prospect of a 50% increase in the demand for electricity in Brazil until 2030 (Brazil, 2015), an increase in the demand for biomass is also expected to meet this growth. Currently, cogeneration plants in the sugar and alcohol sector are supplied with sugarcane bagasse during the

harvest, which extends from April to December in the Center-South region of Brazil. For the rest of the year, most plants are out of operation and there is no electricity production (Burin *et al.*, 2015). In this scenario, the cultivation of biomass sorghum is an opportunity to minimize the effect of seasonality inherent to the cultivation of sugarcane, extending the operation of the plants during the off-season. In the identification of sorghum biomass genotypes with characteristics suitable for this energy conversion technology, there is a need to characterize the chemical composition of the biomass as described by Karampinis *et al.* 2012. Inherent properties of biomass are influenced by many factors including plant genetics, growing environment, harvesting method, storage, climatic conditions, and seasonal variations (Everard *et al.*, 2012). Gross calorific value (GCV) of biofuel is one of the critical properties of interest of industry and it is directly influenced by its chemical composition. The gross calorific value of a fuel can be defined as the amount of fuel energy released in the form of heat during the complete combustion of a unit of mass of the analyzed fuel.

Moisture, and ash content are also important thermal properties to qualify the biomass. Llorente and Garcia cited by Everard *et al.*, 2012 considering that gross calorific value and net calorific value mainly of various biomasses depend on ash and moisture content, (i.e. calorific values are inversely proportional to the ash and moisture content). Biomass is often valued on their moisture (M) content due to its influence on calorific value, combustion optimization, storage management and harvesting properties, (Wu *et al* 2011), and high M also increase transportation costs, drying costs as well. Ash content influences combustion efficiency increases disposal costs due to slagging, fouling, corrosion, agglomeration and may cause cleaning problems to burner and designing and operating systems (Everard *et al.*, 2012, Sirisomboon *et al.*, 2020). To know the quality of the biomass it's necessary to realize the phyco-chemical characterization. Usually the characterization of the biomass is realized by conventional wet chemical analysis methods. These methods are accurate and credible but are also time-consuming, expensive, labor-intensive, and usually destructive (Pasquini, 2018). Thus, the prediction of thermal properties in a rapid, non-destructive manner would allow breeding program biomass to qualify and improve the high-sorghum biomass. In trading, gross calorific value, moisture, and ash content are important traits in the specification of biomass to set a price.

To cover this demand, a faster and efficient method was developed alternative to reference methods, which uses infrared spectroscopy (NIR) associated with the development of multivariate calibration models. This approach has been widely used to determine its potential to predict qualitative and quantitative attributes in the food (Pasquini, 2018), the feedstock's composition (Fagan *et al.*, 2011) and suitable for real-time monitoring of important parameters in alcoholic fermentations (Nascimento *et al.*, 2017) saving time and money with no loss precision or accuracy relative to the calibration methods (Fagan *et al.*, 2011).

The use of an NIR spectroscopy model for classifying biomass quality was recently investigated by Fagan *et al.* (2011), who developed models based on near infrared (NIR) spectroscopy to predict GCV, moisture, ash, and carbon content. The results of moisture and HHV value models were suitable for use in any application, the carbon and ash prediction models were fair and could be used in a screening application. Zhang *et al.* (2017) built an NIR model to determine high heating value (HHV) and elemental components of sorghum biomass using the 75 samples in the calibration set. HHV and carbon content models had excellent prediction accuracy, whereas hydrogen, nitrogen, sulfur, and oxygen models could provide reliable predictions. In our previous studies, NIR spectroscopy based on PLS method has been used to predict cellulose, hemicellulose, lignin and theoretical ethanol yield in sorghum biomass (Guimaraes *et al.*, 2014), sucrose, glucose and fructose in sweet sorghum juice (Simeone *et al.*, 2017) and allow us to a cost-effective high-throughput phenotyping of genetic diversity in sorghum germplasm growth in Brazil. As NIR spectroscopy is applied to the study of grasses in biofuels research, it should be explored as a rapid method to determine calorific value, moisture, and ash. The objective of this work was to develop a multivariate calibration model using near-infrared spectroscopy to determine the gross calorific value (GCV), moisture (M), and ash (A) content in high biomass sorghum to collaborate with breeders and genetic studies to improve the thermal properties and optimization of biomass

conversion technologies of sorghum biomass as bioenergetic culture.

## MATERIALS AND METHODS

**Preparation of samples:** The experiment was conducted in the field experimental area of Embrapa Maize and Sorghum, in Sete Lagoas (19°28' 57"S, 44°15'08"W), and Nova Porteirinha (15°47' 00"S, 43°18'00" W) MG, Brazil, using cultivars of Embrapa's sorghum breeding program. The 812 samples of high-sorghum biomass were grown in different soil and climatic conditions and were harvested at different growth stages. The samples were collected at dough stage e maturation stage during 2015, 2016 and 2017. Fertilizer management weed and pest control, and other agricultural practices were performed as recommended for sorghum cultivation following May *et al.* 2014. The samples were dried at 65 ° C in an air circulation oven LS102 / 960 (Solab, Piracicaba, Brazil) and then ground in a Wiley knife mill up to 1 mm granulometry.

**Physico-chemical analysis:** Moisture content was measured according to the standard ASTM E1756-08 method. The high-biomass sorghum samples were dried using a hot-air oven 102-250 model (American Lab, Piracicaba, Brazil) at 105 °C until a constant weight was observed. Gross calorific value was obtained in a calorimeter C2000 (Werke GmbH and Co. KG, Staufen, Germany). Approximately 1.00 g of each pelleted sample was put into an adiabatic bomb calorimeter and burned to ash. Ash content was measured at 550 °C by burning samples to a constant weight in a muffle furnace GP-2000G-M (GP Científica, Belo Horizonte, Brazil). The analyzes were performed in duplicates.

**Near infrared spectra data calibration and validation:** The spectra of the high-biomass sorghum samples were obtained in FT-NIR spectrometry equipment, model NIRFlex 500 (Buchi Labortechnik, Flawil, Switzerland) in triplicate, in the region of 4,000 to 10,000  $\text{cm}^{-1}$ , with a resolution of 4  $\text{cm}^{-1}$  and 32 steps per spectrum. To correct the scattering effects of light and baseline shifts, spectra were pre-processed using the standard normal variation - SNV and Savitzky-Golay first derivative. Standard normal variate is a pre-treatment used quite often in near infrared to remove the scatter. It is applied to every spectrum individually. The average and standard deviation of all the data points for that spectrum is calculated. Every data point of the spectra is subtracted from the mean and divided by the standard deviation (Mikola *et al.*, 2020; Bi *et al.*, 2016). The Savitzky-Golay first derivative (1st) is a polynomial derivative filter. The method used a smoothing of the spectra prior to calculating the derivative to decrease the detrimental effect on the signal-to-noise ratio than conventional finite-difference derivatives would have (Lee, Liong, & Jemain, 2018). The data were also centered on average. The Kennard-Stone algorithm (Kennard; Stone, 1969) for the selection of the sample set to be used in the development of the calibration model (2/3 of samples) and validation (1/3 of samples).

The gross calorific value, moisture, and ash were obtained by reference methods and were associated with the mean of NIR spectra of each sample. Then, they were developed for each constituent of the biomass the multivariate calibration models using the partial least square (PLS) algorithm. All PLS models were constructed with cross-validation by the full method. In this method one sample was removed systematically from the

data set, then a PLS model was constructed with the remaining samples to predict the value of the Y-variable for the removed sample. This process continued until each sample had been excluded from the data set and used for validation. External validation of the PLS model for the prediction of gross calorific energy value was carried out with another sample set of sorghum biomass not included in model development. For all model development, The Unscrambler® software version 10.5.1, (CAMO Software Inc., Oslo, Norway) was used. Statistical indicators (ASTM International, 2012) used to assess performance and validation of the models were: square root of the mean calibration error (RMSEC), square root of the mean cross-validation error (RMSECV), square root of the mean error of prediction (RMSEP) and R<sup>2</sup> (coefficient of determination) for the calibration and validation set. Spectral outliers were determined by the analysis of the F-residuals versus leverage statistics represent three different kinds of outliers. The residual statistics on the ordinate axis describe the sample distance to model, whereas the leverage describes how well the sample is described by the model. The high residual variance may be due to non-important regions of a spectrum, for instance. Samples with high leverages have a stronger influence on the model than other samples; they may or may not be outliers, but they are influential.

Accuracy of the generated PLS models was attested by trueness and precision studies. The model's predictive ability was assessed with the dimensionless parameters ratio of prediction to deviation (RPD) defined in Eq. (1) and range error ratio (RER) defined in Eq. (2). RPD values above 2.5 indicate that Model discriminates between minors and the highest values of the responses and RER values above 10 are indications of good prediction accuracy. (Williams and Norris, 2001; Calegari *et al.*, 2020).

$$RPD = SD_{val}/RMSEP \quad \text{equation 1}$$

$$RER = (Y_{max} - Y_{min})/RMSEP \quad \text{equation 2}$$

## RESULTS AND DISCUSSION

The characteristics of the calibration and validation sets for the GCV, moisture, and ash content in high-sorghum biomass are shown in Table 1. Analyzing the values of the mean and standard deviation, made possible to detect the wide variability among high-sorghum biomass samples. The raw spectra of the 812 samples are presented at figure 1. The spectral regions for which dominant peaks were found between 7200 and 6600, 6000 and 5500, 5400 and 4600 and 4600 and 4000 cm<sup>-1</sup> can be attributed to O–H stretch first overtone, C–H stretch first overtone, O–H combination bands and C–H combination band regions, respectively (Workman Jr. and Weyer, 2008). The peaks of the raw spectra were like sweet sorghum (Guimarães *et al.*, 2014). Among the pretreatments applied before calibration, a procedure that presented a good performance in the elimination of the multiplicative light scattering effect was the second derivative Savitzky-Golay (SG-2), with 11 points on the right and on the left for the obtain the best model for the gross calorific value. For moisture and ash content the standard normal variate (SNV) followed by first derivative Savitzky-Golay (SG-1), with 9 points on the right and on the left, were applied to the spectra to obtain the best calibration model. Latent variables (LVs) can be used to reduce the dimensionality of data, and the optimal number of latent variables (LVs) was determined by the lowest value of

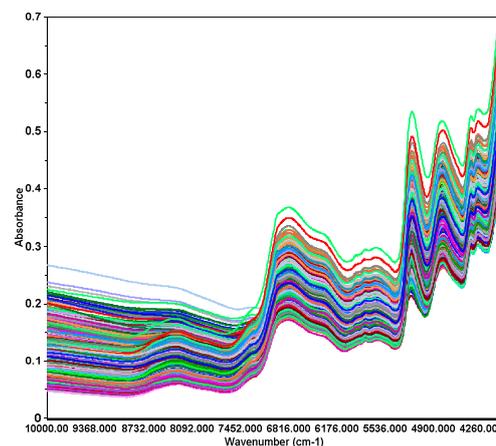


Figure 1. Raw spectra of the high-sorghum biomass samples obtained in the range of 10,000 to 4,000 cm<sup>-1</sup>.

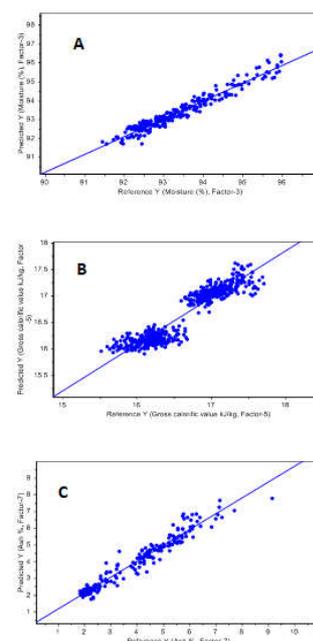


Figure 2. Scatter plot of predicted versus measured values for calibration set and validation set of high-sorghum biomass: a) gross calorific value (kJ.kg<sup>-1</sup>), b) moisture content (%), c) ash content (%)

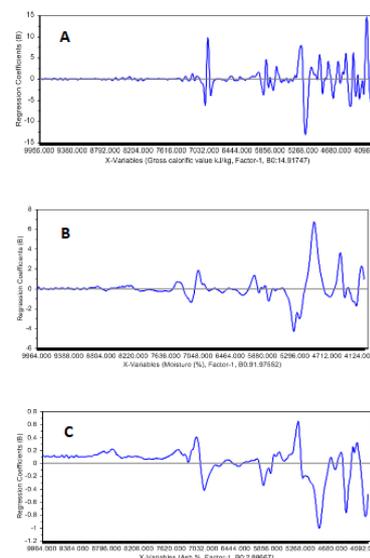


Figure 3. Regression coefficients plots of PLS models using high-sorghum biomass for a) gross calorific value, b) moisture content, M (%), c) ash content, A (%)

**Table 1. Statistics of the high-sorghum biomass physical-chemical properties used in the calibration and validation sets.**

Properties	Calibration					Validation					
	Min	Max	Mean	SD±	No.	Min	Max	Mean	SD±	No.	SE±
GCV (KJ.kg <sup>-1</sup> )	15.52	18.11	16.69	0.51	552	15.83	17.65	16.74	0.51	260	0.17
Moisture (%)	91.80	95.55	93.22	0.83	234	92.19	95.54	93.22	0.77	109	0.40
Ash (%)	1.84	9.16	3.95	1.56	206	1.85	7.63	4.09	1.59	102	0.35

Min — minimum value, Max — maximum value, SD — standard deviation, SE — standard error of analysis, No — number of samples, GCV: gross calorific value.

**Table 2. Results of PLS regression models for the gross calorific value, moisture, and ash content, (%) for the calibration and validation sets of high-biomass sorghum**

Properties	Calibration				Validation					
	N	LV	R <sup>2</sup> <sub>cal</sub>	RMSEC/RMSECV	N	R <sup>2</sup> <sub>val</sub>	RMSEP	RPD	RER	bias
GCV KJ.kg <sup>-1</sup>	552	6	0.88	0.16/0.16	260	0.86	0.19	3.23	11.38	0
Moisture %	234	1	0.93	0.27/0.28	109	0.87	0.27	3.5	15.22	0
Ash %	206	7	0.94	0.36/0.41	102	0.93	0.43	3.44	13.65	0.006

GCV: gross calorific value, N: sample set, LV: latent variable, R<sup>2</sup><sub>cal</sub>: coefficient of determination of calibration set, RMSEC: root mean squared error of calibration, R<sup>2</sup><sub>val</sub>: coefficient of determination of external prediction set, RMSEP: root mean squared error of prediction, and RPD: ratio of prediction to deviation, RER: range error ratio.

predicted residual error sum of squares (PRESS), (Haaland and Thomas, 1988). Consequently, the calibration optimal models were selected to high R<sup>2</sup>, and low RMSEC, RMSECV, RMSEP and bias (ASTM International, 2012). The statistical parameters obtained for the sample sets used for the development of the model's multivariate calibration and external validation for sorghum biomass samples are presented in Table 2. A high correlation (R<sup>2</sup>) was obtained between the values predicted by the model and the values obtained by the reference method for both samples of the calibration set (RMSEC) as well as for the validation set (RMSEP) to gross calorific value (Figure 2a), moisture (Figure 2b), and ash (Figure 2c). Accuracy of the generated PLS models was attested by trueness and precision studies. Trueness of multivariate methods is evaluated by RMSEC, RMSECV and RMSEP. Low calibration and prediction errors of the GCV, moisture, and ash contents (RMSEC 0.16, 0.28 and 0.41%, RMSEP 0.19, 0.27 and 0.43%, respectively). The precision was only estimated at the level of repeatability by estimating relative standard deviations (RSD) for triplicates of three sorghum samples with low, medium, and high GCV, moisture, and ash contents. RSD varied 0.2, 0.1, 0.2% for GCV; 0.2, 0.1, 0.1 % for moisture; 0.3, 0.2, 0.2% for ash, respectively. These values can be compared with the expected values issued from the Horwitz equation (Horwitz, 1982) and acceptable RSD (< 4%) were obtained. The determination coefficient (R<sup>2</sup>) obtained for the set of calibration samples was 0.9, showing a good correlation between the values predicted by the model multivariate developed and the values obtained by the reference methods. RPD and RER ratio relates SEP to variance and range in the original reference data, taking into consideration that RPD should ideally be at least 2.5 and the RER at least 10.0. Williams and Sobering (1996) indicated that the RPD value of 3 or more was recommended. All models in Table 2 presented RPD above 3 and RER above 10 and may be used to screen high-sorghum biomass genotypes.

The GCV model in this study was compared to those in earlier studies reporting on 100 accessions of sorghum (R<sup>2</sup>= 0.85, RMSEP=0.11 MJ.kg<sup>-1</sup>, and RPD 2.62), (Zhang *et al.*, 2017), and Miscanthus (R<sup>2</sup>= 0.97 and RMSEP 0.33 MJ.kg<sup>-1</sup>, and RPD = 4.54), Everard *et al.*, 2012. The regression coefficient shows the wave number, and absorption would have a strong influence on the model if its regression coefficient was at that high wave number.

Lignocellulose in biomass impacts on its GCV and the regression coefficient of the model is associated with vibrational band of molecules in lignin, hemicellulose, and cellulose. Spectral regions (Figure 3a) between 5800-5400 cm<sup>-1</sup> and 4600-4000 cm<sup>-1</sup> are related, respectively, to the first overtone of C-H stretching, and C-H+C-H and C-H+C-C combination bands. O-H stretching and deformation of ROH at 7052 and 4944 cm<sup>-1</sup>, respectively, attributed to vibrations of the molecules of water and sugars present in the biomass. Two main peaks for the M model (Figure 3b) were 6028 cm<sup>-1</sup> corresponding to the O-H combination of H<sub>2</sub>O, and 5776 cm<sup>-1</sup> and 4476 cm<sup>-1</sup> relating to the first overtone of C-H stretching of CH<sub>2</sub>. O-H stretching and deformation of ROH at 7052 and 4944 cm<sup>-1</sup>, respectively and C=O stretching (second overtone) of -CO<sub>2</sub>H, CONH, and CONH (5260, 5200, and 4920 cm<sup>-1</sup>), (Workman Jr, 2008). For M, the prediction of M in biomass had a good performance. It was based principally on the presence of O-H bonds in structures, for example cellulose, hydroxyl groups and water.

As previously shown by Sirisomboon *et al.*, 2020 the performance of the ash model resulted in good predictions that could be applied toward quality assurance. However, the ash model was a good predictor and be able to predict indirectly, since ash is inorganic, it was not an NIR absorber. Ash and moisture content impact the quality of combustion, showing a negative correlation with GCV like observed by Everard *et al.*, 2012 and Gillespie *et al.*, 2015.

The regression coefficient main peaks for the ash content model (Figure 3c) were at 7120 cm<sup>-1</sup> relating to the second overtone of C-H of aromatic molecules, and 5960 cm<sup>-1</sup> which were related to the first overtone of C-H stretching vibration of CH<sub>2</sub>. An NIR technique for prediction GCV, moisture, and ash content of the high-biomass sorghum could be an alternative method for pricing biomass, thus NIR technique can be applied real time and on-line characterization of the biomass for solid biofuels as pellets, cogeneration or direct burning (Everard *et al.*, 2012). The overall results show that the studies in biomass sorghum breeding, the NIR technique can be quickly and nondestructively and determine whether the samples have higher thermal quality, obtain a distribution map of different varieties of sorghum and the constituents ratio in the samples.

## Conclusion

The use of NIR spectroscopy associated with multivariate calibration methods enabled the development of a fast and nondestructive method for analyzing the levels gross calorific value, moisture, and ash content in high-sorghum biomass. The sample set was broad, representing at least 308 cultivars with different growth stages. It is expected that the use of these models in the analysis routines will make the research into the biomass sorghum breeding program for bioenergy.

## Acknowledgments

The authors are grateful to Brazilian National Bank for Economic and Social Development (BNDES) process number 3013/2014, National Science and Technology Development Council (CNPq) for providing student fellowships, National Institute of Sciences and Technologies Advanced Analytics (INCTAA), proc. n.º: CNPq 465768/2014-8 and Brazilian Agricultural Research Corporation (Embrapa).

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