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## Research Article

## Prediction of chemical compositions of crushed maize used in meat animal ration using near infrared spectroscopy and multivariate analysis

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## Abstract

The aim of this study was to test the ability of near infrared (NIR) reflectance spectroscopy to predict the proximate components in crushed maize which is used in meat animal ration. In total 100 samples were prepared among these 75 samples were in calibration set and 25 samples were in validation set. For detection of chemical composition of crushed maize, spectra were collected using DLPNIR scan Nano Software. Partial least square regression (PLSR) model for calibration and validation were developed through The Unscrambler X software. Accuracies of the calibration models were evaluated using the root mean square error of calibration (RMSEc), root mean square error of validation (RMSEv), coefficient of calibration ( $R^2c$ ) and coefficient of validation ( $R^2v$ ). Generally, the accuracy (i.e. the closeness between actual and the predicted values) of regression model is considered as excellent when the  $R^2 \geq 0.90$ . Predictive ability of the PLSR model is assessed by co-efficient of determination of validation ( $R^2v$ ) and root mean square error of cross-validation (RMSEV). The best model for each trait is selected on the basis of the highest coefficient of determination of validation ( $R^2v$ ) and the lowest root mean square error of validation (RMSEV).  $R^2v$  0.972, 0.971, 0.971, 0.957, 0.960, 0.968 for DM, moisture, CP, EE, CF and ash respectively; and RMSEV values are 0.26, 0.26, 0.24, 0.15, 0.12, 0.19, for DM, moisture, CP, EE, CF and ash, respectively. From the findings it can be concluded that crushed maize proximate components can be predicted through PLS model from reference values using The Unscrambler X software.

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## Introduction

Maize ranks first among all other annual crops in terms of worldwide production, and has a great variety of end uses. Maize is generally valued as a grain crop; however, it has substantial importance as a forage crop and concentrates animal feed in many maize growing regions. The intensive production system depends on balanced compound diets where feed cost represents 65–70% of the total production cost of livestock farming. However, proper attention should be given to evaluate the nutritional quality of feed ingredients in order to supply the adequate amount of balanced diet for animals to maximize the effective production cost (Panda et al., 2014; Kuai et al., 2019).

Wet chemistry is traditionally used to characterize chemical composition of animal feed but these techniques are time-consuming, laborious, expensive, destructive and non-adapted for a real-time feed analysis. This necessitates alternative methods that can eliminate above mentioned disadvantages (Orman and Schuman, 1991). NIR spectroscopy is a method that fulfills such expectations. The application of this technique to assess quality in forage crops was first described by Norris et al. (1976). In maize (*Zea mays* L.), NIRS has been proposed as a tool for the prediction the nutritive value of the forage in breeding programmes (Montes et al. 2006; Matthäus et al. 2001). In this respect, many studies have been carried out to investigate the usability of NIR instruments in the analyses of maize grain quality traits e.g. dry matter (Tallada et al. 2009), protein, starch, fatty acid composition (Baye et al., 2006), and carotenoid composition (Berardo et al. 2004). The scientific literature on NIR spectroscopy roots back to 1930's. Number of the articles published in this area between 1930 and 1940 was only 3, while this number were more than 1000 between 1980-1990 (Egesel and Kahriman, 2012), and climbed over 15000 in 1998 (Pasquini, 2003). In Bangladesh, NIR spectroscopy studies related to animal feed are very scanty (Khaleduzzaman and Salim, 2020), and no information is available on the historical progress. As evident by the google search, domestic studies in this area are rather negligible; and no report has been found on the grain quality of maize. Currently, NIRS of whole grains at the grain elevator is used widely in the USA, Canada, Australia and Europe for evaluation of protein and moisture content of grains (Kays et al., 2005). However, little is known about the potential of NIRS for the nutritional evaluation of locally available ingredients in Bangladesh as well as other parts of Southeast Asia. In addition, quick prediction of nutritional quality of feed ingredients in the animal feed industry is necessary for achieving sustainable animal production. Moreover, 15% to 70% maize is used to develop total mixed rations (TMR) for cattle in Bangladesh context.

The objective of the present research was the evaluation of NIRS as an easy and fast method for the assessment of the chemical composition of crushed maize through multivariate statistical models using partial least square (PLS) regression.

## Materials and Methods

### Required equipment and preparation of instruments

The equipment required were Zipper Bag, Refrigerator, Crucible, Microwave oven, Burette, Conical flask, Pipette, Jar, Water bath, Petri dishes, knife, water and tissue paper. All necessary instruments were cleaned with hot water and detergent powder and then autoclaved and dried properly before starting the experimental activities.

### Sample collection

Crushed maize samples were collected from 10 different feeds shops and sample size was 100 from different bags. All samples were collected from local commercial market known as Vailly Bridge Bazar at Shibchar in Madaripur district. NIR method of analysis can greatly varies depending on the particle size of feed samples. The crushed maize samples were immediately transferred to the Animal Science Laboratory, Bangladesh Agricultural University, Mymensingh. The information on particle size was maintained by grinding in same sieve after collection from different seller of crushed maize at Animal Science laboratory.

### Sample preparation

Collected crushed maize samples were numbered from 1 to 100. Each zipper bag contained 100 g maize sample. At first spectra of each sample was taken for NIR experiments and then it was used to evaluate the chemical characteristics.

### NIR spectra measurement

NIR reflectance spectra of maize samples were recorded in the laboratory using a spectrophotometer (DLP NIRscan Nano EVM, Texas Instruments Inc., Texas, USA) at room temperature (20° C). The NIR spectrophotometer used for this study works in the wavelength range of 900-1700 nm with an optical resolution of 10 nm intervals between contiguous bands with a total of 228 bands. For NIR analysis, the sample window of spectrophotometer was placed over the sample surface and the spectra were recorded at 3 different locations. The NIR spectrum for each sample was recorded as log 1/R (reflectance) for each wavelength in the NIRS region.

### Partial least squares (PLS) regression

Partial least squares regression method was followed to develop prediction equation. The dataset used in this study was split into two sets: a calibration set (75 samples) and a test set (25 samples). The test-set samples were selectively chosen by The Unscrambler X software, in which the samples were equally distributed over the spectra variation to cover the entire variation range in both datasets. The PLSR method was used to develop prediction equations for maize chemical composition using NIR spectra. The calibration set was evaluated by applying the following metrics: the coefficient of determination for calibration ( $R^2_c$ ) and the root mean square error for calibration (RMSEC). The prediction equations developed from the calibration-set were also evaluated on the independent test-set samples (test-set validation). The validation errors from the test-set are combined in the coefficient of determination for prediction ( $R^2_p$ ) and the root mean square error for calibration (RMSEP).

### Proximate composition (Reference or measured value)

Proximate composition such as Dry Matter (DM), Moisture, Ether Extract (EE), Crude Protein (CP), Crude Fiber (CF) and Ash were carried out according to the methods (AOAC, 2005).

Dry matter derived using the fundamental reference technique (AOAC, 2005). Ten grams (10) of sample was taken into a Petridis after weighing it with a lid. The petri dish containing the sample was placed inside the oven set at 105°C for 24 hours with the lid open. Following that, the petri dish was placed in a desiccator to cool, and the weight of the dish with the sample was then noted. The dry matter percentage was then estimated by using equation:

$$\% \text{ DM} = \frac{\text{Wt. of the petridish with sample} - \text{Wt. of the petridish}}{\text{Initial sample wt.}} \times 100$$

Moisture content of the sample was determined by deducting the dry matter % with 100. The formulais mentioned below:

$$\% \text{ Moisture} = 100 - \text{Dry matter \%}$$

Crude protein of the sample's was ascertained using a method outlined by AOAC (2005). Wet digestion of the material, distillation, and titration are the steps taken in this method. A boiling tube was filled with 3 g of the sample, 25 ml of concentrated sulfuric acid, and a cube of a catalyst tablet made up of 0.15 g of  $\text{TiO}_2$ , 5 g of  $\text{K}_2\text{SO}_4$ , and 0.15 g of  $\text{CuSO}_4$ . To enable digestion, the tube was heated at a very low temperature (Hashem et al., 2023; Mia et al., 2023). The digested sample was then diluted with 100 ml of distilled water, 5 ml of  $\text{Na}_2\text{S}_2\text{O}_3$ , and 10 ml of 40% NaOH. After include an anti-growth agent, the sample was once more diluted with 10 CC of boric acid. Using a 25 ml burette and titrating with HCl of 0.1 N standards, the amount of  $\text{NH}_4$  in the distillate was calculated. Without the sample, a bare was condition existed. The amount of crude protein was calculated by multiplying the valued protein that was extracted by a conversion factor. Thus:

$$\% \text{ crude protein} = \frac{\text{ATV} - \text{TOTB} \times 0.1 \text{NHCl} \times 0.014 \times \text{CF}}{\text{Weight of the sample}} \times 100$$

Where; ATV= Actual Titre Value, TOTB= Titre of the blank, CF= Conversion Factor, HCl= Hydrogen chloride (ml).

Fat content of the sample was measured following the automated method (Soxtec system HT) recommended by AOAC (2005). During the procedure, an analytical weighing balance was used to weigh almost 10 g of the sample product that was wrapped in sifting paper. The material was then placed in an extraction vessel. The vessel was thoroughly cleaned, oven dried, and refrigerated in a desiccator before weighing. The crude fat content was then removed from a flask after adding 25 ml of petroleum ether as a solvent. Following successful extraction, the solvent was removed by oven drying, and the flask and the substance it contained were then chilled in a desiccator and reweighed. The crude fat content percentage was then estimated by using the equation:

$$\% \text{ Fat} = \frac{\text{Weight of the fat extracted}}{\text{Weight of the sample}} \times 100$$

Crude ash of the sample was determined after heating it at 550–600°C for 2 hours in a container. The method outlined by AOAC (2005) was used to ascertain the sample's crude ash concentration. In the procedure, 5 g of each sample was measured in triplicate into a container, which was then heated nearly 550°C in a soundproof furnace until light grey ash was visible and an exact weight was realized. After cooling in a desiccator to minimize moisture absorption, the sample product was reweighed to determine the ash content. The crude ash percentage was then estimated by using the equation:

$$\% \text{ Ash} = \frac{\text{Wt.of ash content}}{\text{Weight of the sample}} \times 100$$

### Statistical analysis

Descriptive statistical analysis of measured values of quality attributes was performed using the statistical software, Statgraphics Centurion XV.I.

## Results and Discussion

### Descriptive statistics of proximate components of crushed maize of crushed maize

Compositional values of crushed maize samples are given in Table 1, expressed on a fresh basis, that is, in the same form as samples were scanned. To provide an overview of the structure of the samples used in the investigation, the mean, range, standard deviation (SD), coefficient of variance (CV) for dry matter (DM%), moisture (%), crude protein (CP%), ether extract (EE%), crude fiber (CF%) and ash (%) for all crushed maize samples were determined by laboratory methods as reference values are summarized in Table 1.

**Table 1.** Descriptive statistics of proximate components of crushed maize

Descriptive statistics	Proximate components					
	DM	Moisture	CP	EE	CF	Ash
Mean	89.9546	10.0829	8.4007	3.086	1.8878	11.5873
Minimum	79.63	7.88	5.35	2.1	1.1	9.24
Maximum	92.12	20.37	12	7.95	5.2	15.37
STD	1.607165	1.568172	1.427198	0.777795	0.624101	1.090291
CV	1.77	15.55	16.99	25.20	33.06	9.41
BY NIRS	97.9%	97.8%	97.7%	96.5%	96.7%	97.5%
Measured value by NIRS	88.07	9.86	8.21	2.98	1.83	11.30

In Table 1 shows descriptive statistics of proximate components of maize. The crushed maize sample was quite normal. In case of DM the mean was 89.95, CV% is 1.77, minimum value is 79.63 and maximum value is 92.12. For Moisture mean is 10.0829, CV% is 15.55, minimum value 7.88, maximum value is 20.37. Then the mean, minimum, maximum value of CP is 8.4007, 5.35 and 12 respectively which is quite normal and similar to Egesel and Kahrman (2012). The mean, minimum, maximum value of EE is 3.086, 2.1 and 7.95, respectively which is quite normal and similar to Xue et al., (2015). CF and ash content is quite normal and their minimum, maximum and CV% was normal. All measured value of proximate compositions by NIRS almost similar to reference values (Table 1).

### NIRS calibration and prediction statistics for different parameters

In Table 2, NIRS calibration and prediction statistics for different parameters is shown.

**Table 2.** NIRS calibration and prediction statistics for different parameters

Parameters	Calibration					Test-set Validation				
	n	R <sup>2</sup> <sub>c</sub>	SD	Range	RMSE <sub>c</sub>	n	R <sup>2</sup> <sub>v</sub>	RMSE <sub>v</sub>	RPD	RER
DM	75	0.979	1.607165	12.49	0.23	25	0.979	0.26	4.10	48.03
Moisture	75	0.978	1.568172	12.49	0.23	25	0.978	0.26	6.03	48.03
CP	75	0.977	1.427198	6.65	0.21	25	0.977	0.24	5.94	27.70
EE	75	0.965	0.777795	5.85	0.14	25	0.965	0.15	5.18	39.00
CF	75	0.967	0.624101	4.1	0.11	25	0.967	0.12	5.20	34.16
Ash	75	0.975	1.090291	6.13	0.17	25	0.975	0.19	5.73	32.16

(R<sup>2</sup><sub>c</sub> = calibration coefficient; R<sup>2</sup><sub>v</sub> = validation coefficient; SD = standard deviation; RMSE<sub>c</sub> = standard error of calibration; RMSE<sub>v</sub> = standard error of validation; RPD = residual standard deviation; RER = range error ratio).

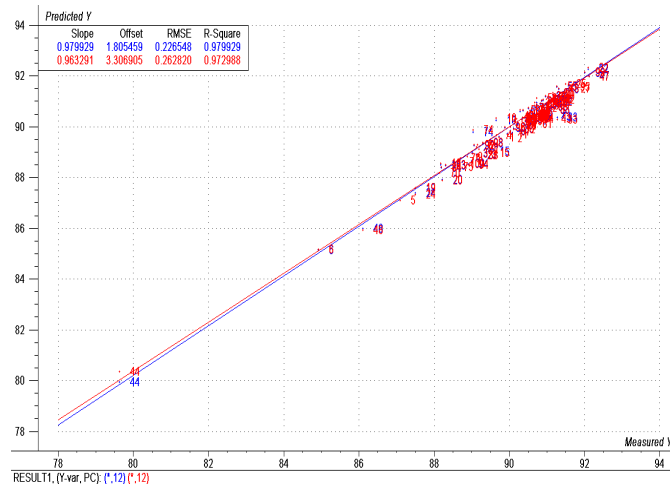
A wide and even distribution in composition, along with precise reference analysis techniques are recognized as important characteristics of the calibration set of samples, in order to obtain a successful equation (Hashem et al., 2022a, 2022b and 2022c; Cen and He, 2007). Calibration co-efficient were 0.979, 0.978, 0.986, 0.977, 0.965, 0.967, 0.975 for DM, Moisture, CP, EE, CF and ash, respectively, where Egesel and Kahrman, (2012) showed that CP, EE, ash for maize grain were 0.990, 0.823, 0.926 respectively. Generally, the accuracy of multivariate calibration model is considered as excellent when the R<sup>2</sup> is 0.90 or higher (Hashem et al., 2021; Kamruzzaman, 2016). However, it is always expected to obtain R<sup>2</sup> as close as 1. In our study R<sup>2</sup><sub>c</sub> for moisture and DM were 0.979 and 0.98 whereas Tøgersen et al., (1999) found for moisture 0.71–0.86 and Alomar et al., 2003 stated for DM it was 0.77.

Predictive ability of the PLS model is assessed by co-efficient of determination of validation (R<sup>2</sup><sub>v</sub>) and root mean square error of Leverage Correction (RMSE<sub>v</sub>). The best model for each trait is selected on the basis of the highest co-efficient of determination of validation (R<sup>2</sup><sub>v</sub>) and the lowest root mean square error of validation (RMSE<sub>v</sub>). R<sup>2</sup><sub>v</sub> 0.972, 0.971, 0.971, 0.957, 0.960, 0.968 for DM, moisture, CP, EE, CF and ash respectively; and RMSE<sub>v</sub> values are 0.26, 0.26, 0.24, 0.15, 0.12, 0.19, for DM, moisture, CP, EE, CF and ash respectively. So, results above values indicate that PLS model has prediction ability.

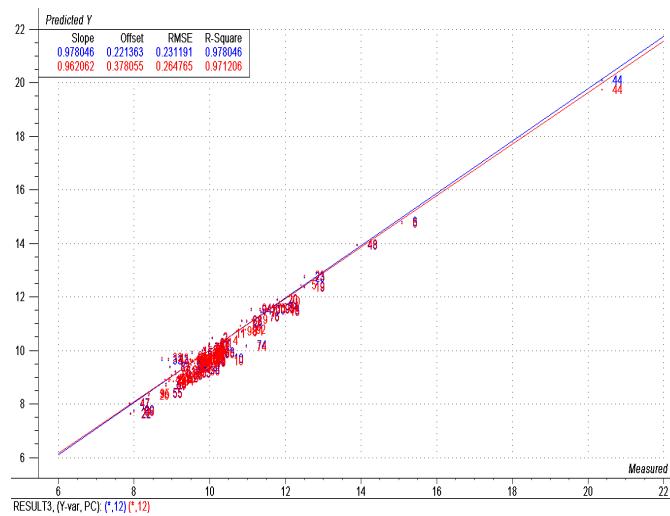
To assess the practical utility of the prediction models the ratio performance deviation (RPD) and the range error ratio (RER) were calculated. RPD were 4.10, 6.03, 5.94, 5.18, 5.20 and 5.73 for DM, moisture, CP, EE, CF and ash respectively. It indicates that all values are adequate for analytical purposes as values above 2.5 are adequate for analytical purposes (Sinnaeve et al., 1994). Range error ratio was 48.03, 48.03, 27.70, 39.00, 34.00 and 32.00 for DM, moisture, CP, EE and ash respectively. The range of the reference data, RER value between 27-48 classify the model as poor to fair and indicate it could be used for screening purposes and RER values between 21 and 30 indicate a good classification suggesting the model could be suitable for application in quality control (Williams, 2007). So, for the above RER values the model is fair to good.

### Development of calibration model based on NIR spectra

Although Nicolai et al., (2007) reported that the NIR region covers the wavelength range from 780 to 2,500 nm, we used the range of 900 to 1,700 nm as done in other studies evaluating meat quality (Hashem et al., 2020; Cecchinato et al. 2011; Rodbotten et al. 2000). Spectral data at full wavelength range (900-1700 nm) with 228 variables were calibrated using PLSR model through Leverage correction. To visualize graphically the performance of the PLS calibration models, the measured value and its predicted values resulting from the optimal PLS models are plotted and displayed in Figure 1, 2, 3, 4, 5 and 6.



**Figure 1.** Prediction of DM content through PLSR model using Leverage correction.



**Figure 2.** Prediction of moisture content through PLSR model using Leverage correction.

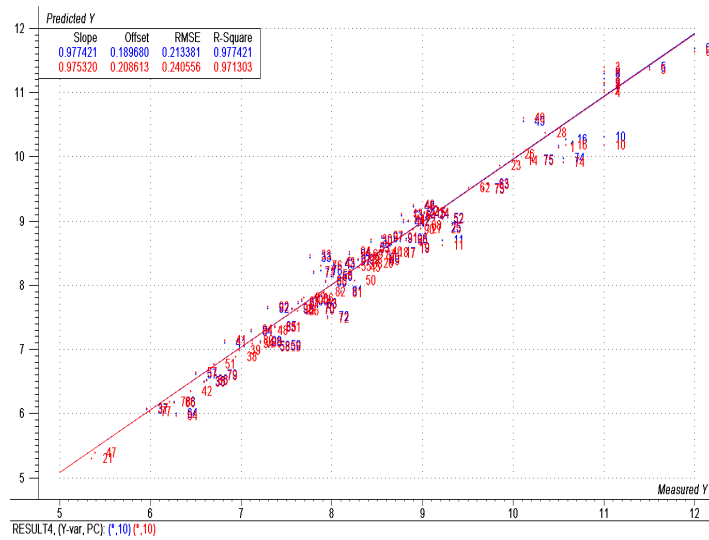


Figure 3. Prediction of CP content through PLSR model using Leverage correction.

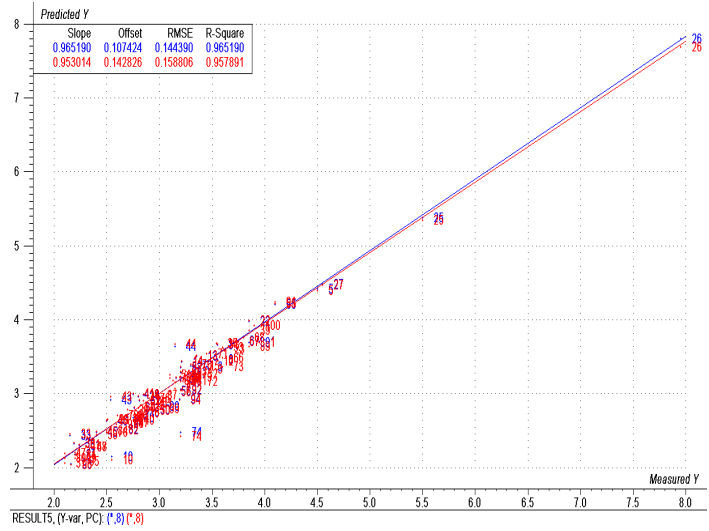


Figure 4. Prediction of EE content through PLSR model using Leverage correction.

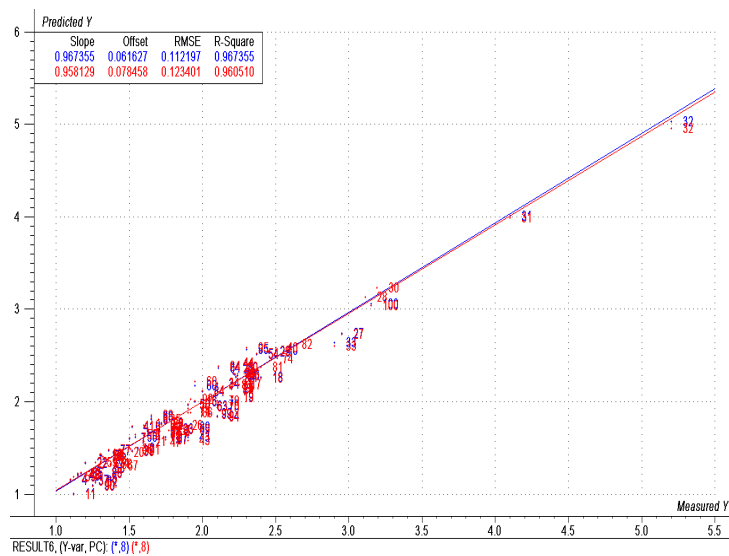
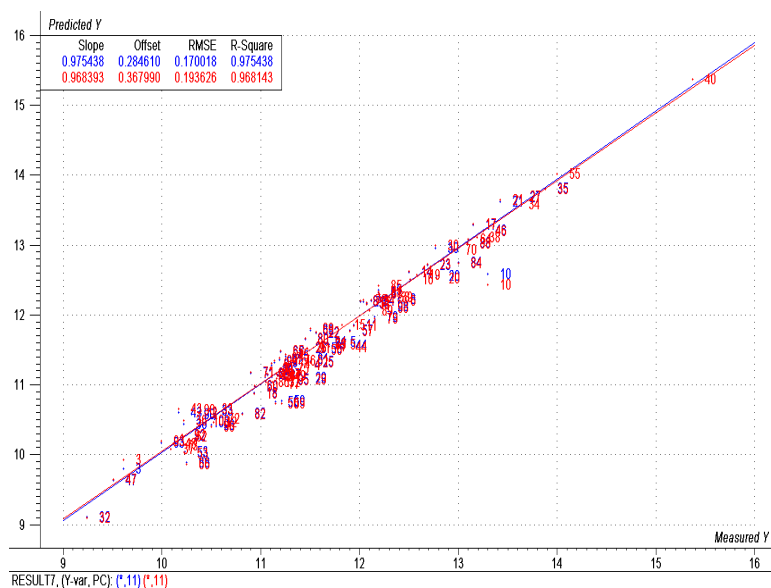


Figure 5. Prediction of CF content through PLSR model using Leverage correction.



**Figure 6.** Prediction of ash content through PLSR model using Leverage correction.

## Conclusions

This study revealed that NIR spectroscopy coupled with PLSR can be successfully utilized as a rapid screening technique to predict the crushed maize quality. From the established PLSR models, we obtained coefficients of determination ( $R^2_v$ ) of 0.98, 0.97, 0.98, 0.97, 0.96, 0.97, 0.97 for DM, Moisture, CP, EE, CF and ash respectively. Partial least squares regression (PLSR) was developed to correlate the NIR spectra of different maize samples and their corresponding reference value. The results demonstrated that the spectral data collected from NIR spectral region combined with appropriate multivariate PLS methods could become an appropriate method to predict simultaneously several maize chemical properties and also an interesting tool to predict the crushed maize quality.

## Conflict of interest

There is no conflict of interest among the authors.

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