



Prediction of maize flour adulteration in chickpea flour (*besan*) using near infrared spectroscopy

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Abstract The present study was performed to develop Near-infrared spectroscopy based prediction method for the quantification of the maize flour adulteration in chickpea flour. Adulterated samples of Chickpea flour (*besan*) were prepared by spiking different concentrations of maize flour with pure Chickpea flour in the range of 1–90% (w/w). The spectra of pure Chickpea flour, pure maize flour, and adulterated samples of Chickpea flour with maize flour were acquired as the logarithm of reciprocal of reflectance ($\log 1/R$) in the entire Visible-NIR wavelength range of 400–2498 nm. The acquired spectra were pre-processed by 1st derivative, standard normal variate, and detrending. The calibration models were developed using modified partial least square regression (MPLSR), partial least square regression and principal component regression. The optimal model was selected on the basis of highest values of the coefficient of determination (RSQ), one minus variance ratio (1-VR) and lowest values of standard errors of calibration (SEC), and standard error of cross-validation (SECV). MPLSR model having RSQ and 1-VR value of 0.999 and 0.996 having SEC and SECV value of 1.092 and 2.042 was developed for quantification of maize flour adulteration in chickpea flour. Cross validation and external validation of the developed models resulted in RSQ of 0.999, 0.997 and standard error of prediction of 1.117, and 2.075, respectively.

Keywords Adulteration · *Besan* · Chickpea flour · Maize flour · Modified partial least square regression · Near infrared spectroscopy

Introduction

Food adulteration refers to the addition of superfluous components that are not normally present within the original food substances (Moore et al. 2012). Less expensive non-authentic substances are added by the unscrupulous dealers in a food product without the purchaser's knowledge, for getting more economic gains (Spink 2011). This type of act is known as economically motivated adulteration. It can be the cause of public health food risks when the added ingredient is having some toxic effects. Nowadays, the authenticity of foodstuffs and related food fraud is becoming a big challenge for governments, industry, and organizations involved in setting of food standards.

According to the Food and Agriculture Organization (FAO) of the United Nations, "Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life." Access to adequate food must be reliable (Boyac-Gündüz, et al. 2021). In the era of the COVID-19 pandemic crisis, the consumers are looking to protect themselves and their immune system by adopting healthier diets (Galanakis 2020), therefore, they must get safe food. If the food they are looking for, is adulterated and is not authentic and pure, it may pose harmful effects. Further, when the food item is to be used by the food industry, the genuineness of base material is an important and indispensable part of their quality control system (Jaakola et al. 2010). In order to enforce strict measures for quality

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control, there is a growing requirement for the development of rapid, low-cost and effective analytical approaches to test adulteration.

Chickpea flour or *besan*, refers to powdered sample which is processed by grinding dehulled Bengal gram (*Cicer arietinum* L.). As per Indian standards, it shall not contain any added coloring matter or any other foreign ingredient (PFA 1955). Chickpea flour is rich source of proteins. The protein content in the range of 24.08–28.62% has been reported for different chickpea flour fractions (Bala et al. 2020). It finds applications in various Indian traditional foods. It has been used in the preparation of *pakoras*, *boondi*, *Indian curry*, *ladoo*, at household and at industrial level. Its demand further increases during festive seasons. Because of its commercial importance, it is prone to adulteration especially in the unsupervised sector. The adulterators are usually adulterating *besan* with low priced produce such as maize flour (Verma 2018; Times of India 2019; Presswire 2021) and other cheap legume flours namely pea and *khesari* (FAC 2009a, 2009b; Dattatreya et al. 2011). Since maize flour is a cereal flour, it does not contain any harmful component and is commonly consumed in India for food purposes. Therefore, adulteration of *besan* with maize flour does not cause any harmful effects to the human body. But it is considered as a type of food fraud as the consumer pays more money and does not get the desired nutritional value from the product. *Besan* is rich in proteins while maize is a cereal crop with about 8–9 percent protein content. Therefore, on buying and using adulterated *besan* one may not get the desired taste and texture from the processed products and has to pay higher amount for nutritionally inferior product.

Conventional method for the detection of maize flour in the adulterated sample of chickpea flour is based on the microscopic detection of maize starch (Anonymous 2018) and indirectly can be tested by estimating the protein content. For microscopic identification of starch high end equipment i.e. microscopes and trained personnel are required. While for estimation of protein content the method is cumbersome, more time consuming and destructive in nature. Moreover, no chemical methods are available to distinguish between the two flours by addition of some chemical reagent and also, the visual inspection for detection of maize flour from *besan* is very difficult as maize flour and *besan* have nearly same color index. Considering non-availability of direct methods for determining the presence of maize flour in *besan*, there is dire need to develop a rapid, sensitive, reliable and robust method for detecting adulteration of maize flour in *besan*.

Recently, vibrational spectroscopy such as near infrared spectroscopy (NIRS) with chemometrics has become a powerful tool for detection of food fraud. NIRS is gaining widespread acceptance in many fields of analytical

chemistry because of certain advantages linked to it. It requires a little or no sample preparation, is easy to operate, nondestructive and has potential for on line-applications. The technique has some disadvantages also viz. the cost of equipment is quite high and for every commodity and every parameter new models have to be developed. Further, calibration pool has to be updated and strengthened by adding new samples. In spite of all these disadvantages, use of NIRS for nondestructive detection of adulteration in different commodities has been reported by many workers. NIRS has been employed for detection of adulteration of turmeric with starch (Kar et al. 2019). Aykas and Menevseoglu (2021) implemented this technology for detection of green pea and peanut adulteration in pistachio while Genis et al. 2021 studied green pea and spinach adulteration in pistachio. Amirvaresi et al. 2021 compared the potential of near-infrared (NIR) and mid-infrared (MIR) spectroscopy coupled with chemometrics for detection of adulteration of saffron style, calendula, safflower, and rubia in Iranian saffron samples. Near-infrared (NIR) spectra have also been utilized to quantify the amount of adulteration in durum wheat flour with common bread wheat flour (Cocchi et al. 2006). Further, in case of chickpea flour, use of NIR technology has been related to estimate the moisture, fat, protein, and carbohydrate (Kamboj et al. 2017), neutral detergent fibre and acid detergent fiber content (Font et al. 2021) while Rathore et al. 2021 evaluated nutritional quality of Chickpeas in terms of protein and amino acids. No studies based on NIRS are available for quantification of maize flour adulteration in chickpea flour. In this context, we hereby propose NIRS based method for the detection of maize flour adulteration in chickpea flour (*besan*). In the present work, MPLSR, PLSR and PCR were applied to develop calibration models and the models obtained were validated using cross-validation as well as external validation.

Materials and methods

Materials

Twelve numbers of Chickpea dal and maize samples were purchased from varied locations in the local market of Ludhiana, Punjab and processed under laboratory conditions to get Chickpea and maize flour. A flour mill with a 0.5 mm pore sized sieve (Natraj, Scorpio Entreprises, Ahmedabad, India) was used. BSS sieve (85No.) was used to prepare samples with uniform particle size. Samples were kept in closed polypropylene containers till further use.

Preparation of adulterated samples

For preparing Chickpea flour samples maize flour as adulterant, twelve different chickpea flour (*besan*) samples were mixed with different concentrations (1, 2, 3, 6, 8, 10, 16, 20, 30, 40, 50, 60, 70, 80, 90) of maize flour in the range of 1–90% (w/w). This resulted in generation of 180 adulterated sample which along with 24 samples of pure maize and pure besan yielded 204 samples. Samples (10 g each) were prepared as per method of Lohumi et al. 2017 with modifications. Samples were weighed and mixed manually; each sample was sieved thrice for uniform mixing and transferred in closed vials and further mixed in lab vortex mixer at high speed. To maintain uniform moisture content, samples were heated at 55 °C (2 h), and mixed again before analysis.

NIRS spectra collection

Near-infrared spectra were collected in reflectance mode using spectrophotometer (6500 Model, Foss NIR Systems Inc., Laurel, MD, USA). For this purpose each flour sample was filled in a ring cup, provided with the equipment. The spectrum of each sample was recorded as log (1/R) in the Vis–NIR range of 400 to 2498 nm with an interval of 2 nm. Each spectrum represents the average of 32 scans. ISI Scan Software (Windows version 3.0, Foss and Infrasoft International LLC, USA) supplied with the equipment was used for acquiring the spectral data.

Preprocessing of spectral data

Spectral data were imported to WinISI 4.0 software version (Infrasoft International, Port Matilda, PA, USA) for further analyzing the data. Outliers were removed using software with the help of Mahalanobis distance (MH) or Global H (GH). MH describes the distance of an individual sample from the center of sample population. The score algorithm selects spectra which are different from other spectra and remove them as outliers. An outlier is data value far away from the center with respect to the underlying covariance structure. Samples with $GH > 3$ were regarded as outliers as per control limit set with the software and were then removed from the dataset used for calibration and validation. Data set were randomly split by using software into calibration set and validation set. A total of 204 samples were used for acquiring spectral data, out of these 13 were removed as outliers, 145 were used as calibration and 46 spectral data sets for validation. Preprocessing of spectral datasets was performed prior to model development. Standard normal variate + detrending (SNV + DT) and derivative pretreatments were employed. SNV pretreatment requires that the mean and standard deviation of

spectral values to be the calculated. 1st derivative (1:4:4:1), second derivative (2:4:4:1) where different digits refers to—derivative order: segment of the derivative, smoothing segment, and second smoothing segment, respectively were employed. A second derivative provides spectral patterns showing peaks and valleys which correspond to the point of inflection on either side of the log (1/R) peak while the first derivative calculations result in a spectral pattern of peaks pointing downward only (Shenk et al. 1992).

Chemometrics analysis

The variability among spectra can be observed as patterns or loadings. These patterns also known as factors or scores were produced using Principal component Analysis (PCA). For quantitative analysis of maize adulteration in chickpea flour, calibration and prediction models were developed using Principal Component Regression (PCR), Partial Least Square Regression (PLSR), and Modified Partial least square regression (MPLSR). These regression models utilized the spectra in the spectral data matrix (X) to predict amount of adulterant in chickpea flour in column vector (Y). For a model to exhibit good prediction, the value of coefficient of determination (RSQ) and an estimate of RSQ (1-VR) should be high while the classification errors viz., standard errors of calibration (SEC), and standard error of cross-validation (SECV) should be less. As per Williams and Norris (2001) R^2 should be near 1, but a R^2 of over 0.90 shows excellent performance and less than 0.82 means poor performance. The number of terms or latent variables was chosen according to the criterion of the lowest prediction error in cross-validation and the evaluation of the explained variance in the X and Y blocks. The performance of the developed calibration equation was evaluated from cross-validation and external validation and various statistics such as, bias and standard error of performance (SEP) were calculated. Based on the lowest value of SEP, the best possible calibration model was selected.

Analytical characteristics test

In order to assess the limit of detection (LOD) and limit of quantification (LOQ), method of Liu et al. (2013) was applied. For this purpose, low levels of adulteration were added to the calibration (1–9%) set one after other and the lowest possible concentration which could be distinguished from pure chickpea flour after PCA was considered as LOD. LOQ was considered as the lowest concentration added to the calibration set when the developed model showed R^2 of > 0.90 for quantitative analysis. To measure the precision of the MPLS model, ratio of standard error of performance to standard deviation (RPD) was considered. RPD is given as $\sigma/\text{Root mean square of the error of}$

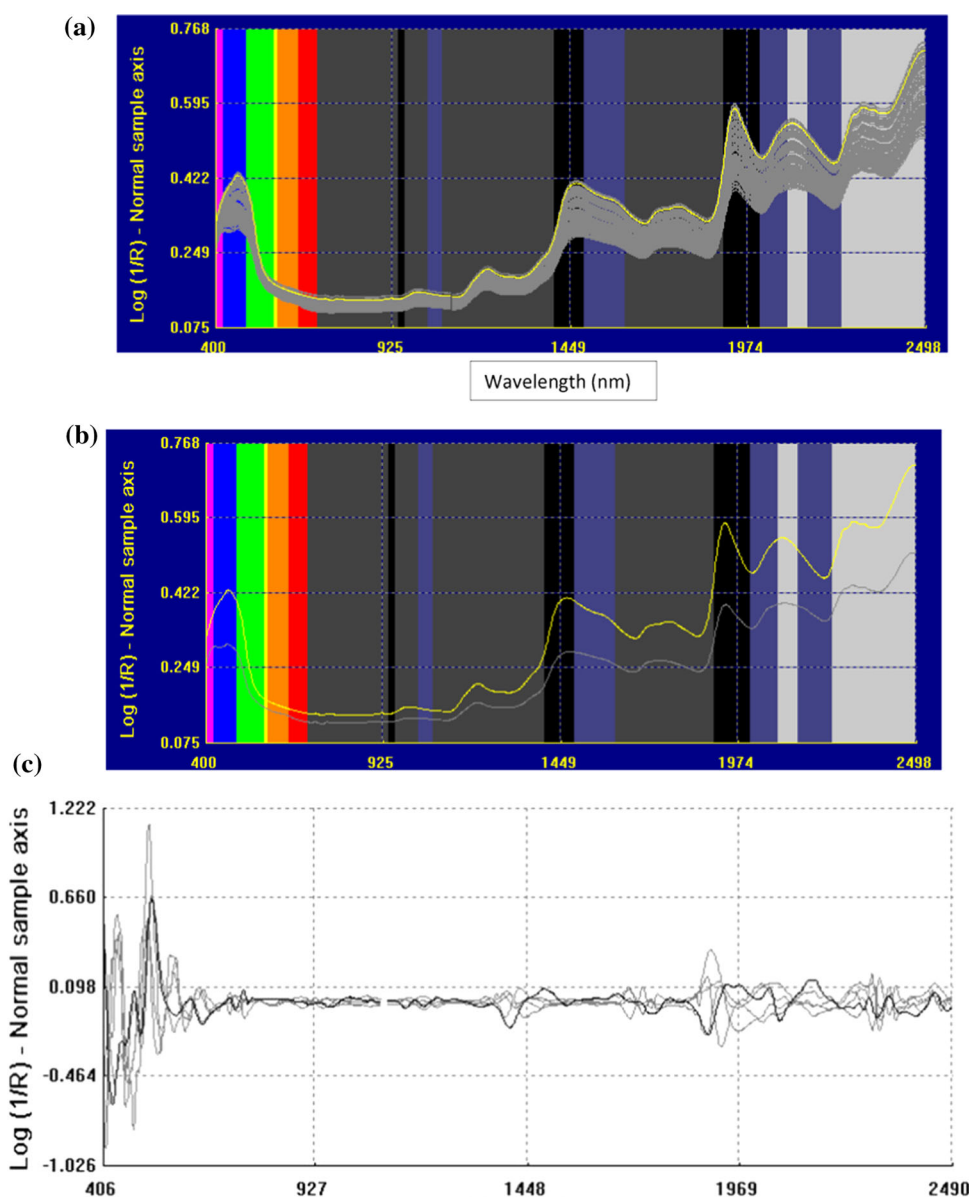
performance (RMSEP).RMSEP can be calculated from SEP and bias ($RMSEP^2 = SEP^2 + bias^2$). RPD value above 8 is considered excellent and calibration can be used for any purpose and value below 2.3 indicates a poor performance of calibration model (Agelet and Hurburgh 2010). Accuracy of model is determined from the values of SEC, SECV and SEP. For repeatability studies, sample with known adulteration concentration (10%) was scanned 5 times under similar conditions and mean SD of prediction value was presented. For reproducibility same sample with known adulteration concentration (10%) was prepared by three different Lab personnel and prediction was checked.

Results and discussion

NIR spectra

All the 204 flour samples were scanned to get their spectra in the wavelength range of 400-2498 nm. The acquired spectra of chickpea flour, maize flour and adulterated samples of chickpea flour are represented as raw spectra in Fig. 1A and pure chickpea flour and pure maize flour are shown in Fig. 1B. It is noticeable from Fig. 1A that spectra of different samples along X- axis appear similar but the differences in log 1/R values can be observed along Y-Axis. Spectral differences were also obvious with respect to the different adulteration levels (1–90%). In raw spectra peak were observed corresponding to 1208, 1496,

Fig. 1 **A** Spectra of pure *besan*, pure maize and adulterated samples of besan with maize in the spectral range of 400–2498 nm. **B** Spectra of pure *besan*, pure maize samples in the spectral range of 400–2498 nm. **C** 1st derivative Plot of pure *besan*, pure maize and adulterated samples of chickpea flour or *besan* with maize flour (0–90%)



1724, 1936, 2308 nm. The spectral peaks located at 1412 nm, 1450 nm, 1465 nm, between 1400 and 1440, 1936 nm, and 1940 nm and between 1900 and 1950 have been assigned to water (Martinez Valdivieso et al. 2014; Lu et al 2000; Osborne et al. 1993). The absorption band at 1200 nm due to 2nd overtone of C–H stretch; methyl and methylene, is assigned to starches, lipids and/or proteins, respectively (Lu et al. 2000).

Modeling in whole spectral range of 400–2498

Spectral files (.cal file) and laboratory values were imported to the software (WinISI 4.0) for multivariate data analysis. Generally the spectral data is affected by scattering effects, random noise and overlapping peaks. To overcome these problems, data were preprocessed using SNV and DT followed by 1st derivative prior to model development. The derivative spectra are corrected spectra for overlapping peaks and baseline corrections (Fig. 1C). It is considered that pretreated spectra provide enhanced spectral features related to specific compositions to the NIRS (Yi et al. 2017).

Principal component analysis yielded histogram (Supplementary Fig. 1) which showed pure *besan* and adulterated samples at levels 1 and 2% were clubbed in one group while adulterated sample of *besan* with 3% maize flour was separated in other group. Therefore, LOD was achieved as 3%.

Most of the variability among spectra exists as loadings or patterns and can be observed with compositional changes. Principal component analysis (PCA) loadings spectra generated spectral loading peaks which were observed at 1402, 1698, 1730, 1890, 1900, 1926, 2068, 2296, 2314, 2338, 2356 nm. These observed bands correspond to absorption by stretching-bending. In literature attributions of the NIR bands in the regions of 1400–1600 nm and 2000–2350 have been assigned to different agricultural and food products. 1420–1700 nm corresponds to N–H overtone and relates to differences in protein content (Yi et al. 2017). From 1900 to 2500 nm absorption information is reported to be associated with primary combination bands of one or more overtone bands. The spectral region at higher wavelengths is characterized by the absorptions of constituents like starch, cellulose and protein (Cocchi et al. 2006).

The spectra of all studied samples were ranked according to their H distance (Mahalanobis distance) from the mean spectrum of all the spectra. $H > 3$ norm was employed to remove the outliers. The calibration models were developed using remaining datasets. The calibration method development was performed using Principle component (PC), Partial Least Square (PLS), and Modified Partial Least Square (MPLS) regression methods on

pretreated spectral data. The statistical data pertaining to the developed calibration models of PCR, PLSR and MPLSR is presented in Table 1. The developed models were evaluated using cross validation using the same samples used for building calibration. Coefficient of determination (RSQ) along with standard errors of calibration (SEC), standard error of cross-validation (SECV) was employed to select the best model among the developed models. The results showed that various statistical descriptors of cross validation were better for the MPLSR model developed for quantification of maize flour adulteration in *besan*. Further, number of latent factors required for getting good statistical values was 9, 6, and 5 for PCR, PLS and MPLS. MPLSR model showed R^2 and 1-VR of 0.999 and 0.996 while, SEC and SECV values were 1.092 and 2.047, respectively.

No doubt values were also good for PLSR and PCR models. PCR is considered as direct application of PCA method and is the basic regression method used for calibration purposes. PLS is improved alternative to PCR and is preferred because the algorithm is fast and developed models show high precision (Agelet and Hurburgh 2010). PLS has several methods such as modified PLS, hybrid PLS and robust PLS, these methods help to improve accuracy of PLS in datasets having noisy data and gives better results as compared to PCR and PLS. This could be the reason that in our results, MPLS model yielded better values of statistical predictors.

Validation of developed equation

Internal as well as external or test set validation was carried out on the developed model. Table 1 represents statistical descriptors of cross and test validation. Cross-validation provides information about the predictive ability of the calibration equation, whereas test validation is performed to check the robustness of the developed calibration equation. Cross validation statistics showed RSQ value of 0.999 and SEP of 1.117 (Table 1). High RSQ obtained for the MPLSR model developed for quantification of the maize flour adulteration in *besan* indicated a good relationship between the laboratory and predicted values (Table 1). Our values emphasized the good prediction ability of the developed calibration model. Prediction plots for adulteration of maize flour in *besan* using cross validation are shown in Fig. 2A.

The test validation was carried out with the set of samples not included in calibration data set. For determining the precision of the calibration model, different statistical parameters namely RSQ, SEP, slope, bias and for prediction ability RPD was considered (Table 1). For external validation, R^2 of 0.997, slope of 0.991, standard error of prediction (SEP) of 2.07% and bias of -1.016 was

Table 1 Statistical parameters in NIRS models for the detection of maize flour adulteration with chickpea flour

Method	Pretreatment	Calibration			Cross validation			Test validation				
		Coefficient of determination (RSQ)	Standard error of calibration (SEC)	Standard error of cross validation (SECV)	One minus unknown variance (1-VR)	Coefficient of determination (RSQ)	Standard error of prediction (SEP)	Bias	Coefficient of determination of calibration (RSQ)	Standard error of prediction (SEP)	Bias	RPD
MPLS	1,4,4,1 and SNV + DT	0.999	1.092	2.047	0.996	0.999	1.117	0.028	0.997	2.075	- 1.016	14.52
PLS	1,4,4,1 and SNV + DT	0.998	1.366	2.267	0.995	0.997	1.821	0.034	0.996	3.012	- 1.561	9.87
PCR	1,4,4,1 and SNV + DT	0.997	1.694	2.282	0.995	0.996	2.452	0.056	0.995	3.451	- 2.310	8.05

Standard deviation (SD) = 33.39

obtained for predicting the adulteration of maize flour in Chickpea flour. RPD of 14.52 was obtained which was more than 8 showing developed model produces accurate estimations for the test validation set. Prediction plots for adulteration of maize flour in *besan* using test validation are shown in Fig. 2B. Supplementary Table 1 represents actual and predicted values of test set. In present study we were able to develop model with R² of 0.999 even upto addition of 1% maize flour adulteration, and according to method described by Liu et al. 2013 LOQ of 1% can be considered. Keeping in mind that maize adulteration ratio will be higher than this value of LOD and LOQ values of developed models since maize flour in *besan* is added to get economic gains, therefore, the proposed model has significance and can be useful for the adulteration detection of maize flour in *besan*. Table 2 describes analytical characteristics of the developed model. Repeatability and reproducibility values of 10.17 ± 0.89 and 10.42 ± 0.98 respectively were observed for 10% adulterated sample. The various other parameters namely linearity given by R² closer to 1 was achieved. While using a wider range of sample 1–2% error has been obtained. RPD value (14.52) of more than 8 has been achieved which provides information about precision and as per literature describes the model with good prediction ability. With near infrared spectroscopy our results have been achieved in comparison with other workers who have tried to separate and quantify admixtures of different flour. Considering that, strengthening of the model is a continuous effort, for which more and more number of samples will be added to lower SEP.

It has been reported that application of pretreatment and modeling approaches helps to get better precision in the results (Rady and Guyer 2015). Our results are in agreement with those who have applied SNV + DT and derivative pretreatments to improve their results (Yi et al. 2017; Su and Sun 2017; Sen et al. 2018). It was observed that for quantification of maize flour adulteration in chickpea flour was achieved with high accuracy. Differences in the chemical composition of maize and chickpea flours could be one factor (Supplementary Table 2). Maize flour is rich in carbohydrates while *besan* is rich in proteins. Our results were in line with the results of other researchers. Su and Sun (2017) used NIR along with hyper spectral imaging for authenticating the admixtures of wheat flour, cassava flour, corn flour in organic Avatar wheat flour with R² of 0.991–0.986. Ayvaz et al. 2021 studied computer-Based Image Analysis and Near-Infrared Spectroscopy based models to detect adulteration level of bread wheat flour in both einkorn-wheat flour mixtures and the bread made of those mixtures and suggested NIRS use for the flour mixtures.

Development of FT-MIR based PLSR models with R² of 0.9587–0.9898 and 0.99, for detection and quantification of

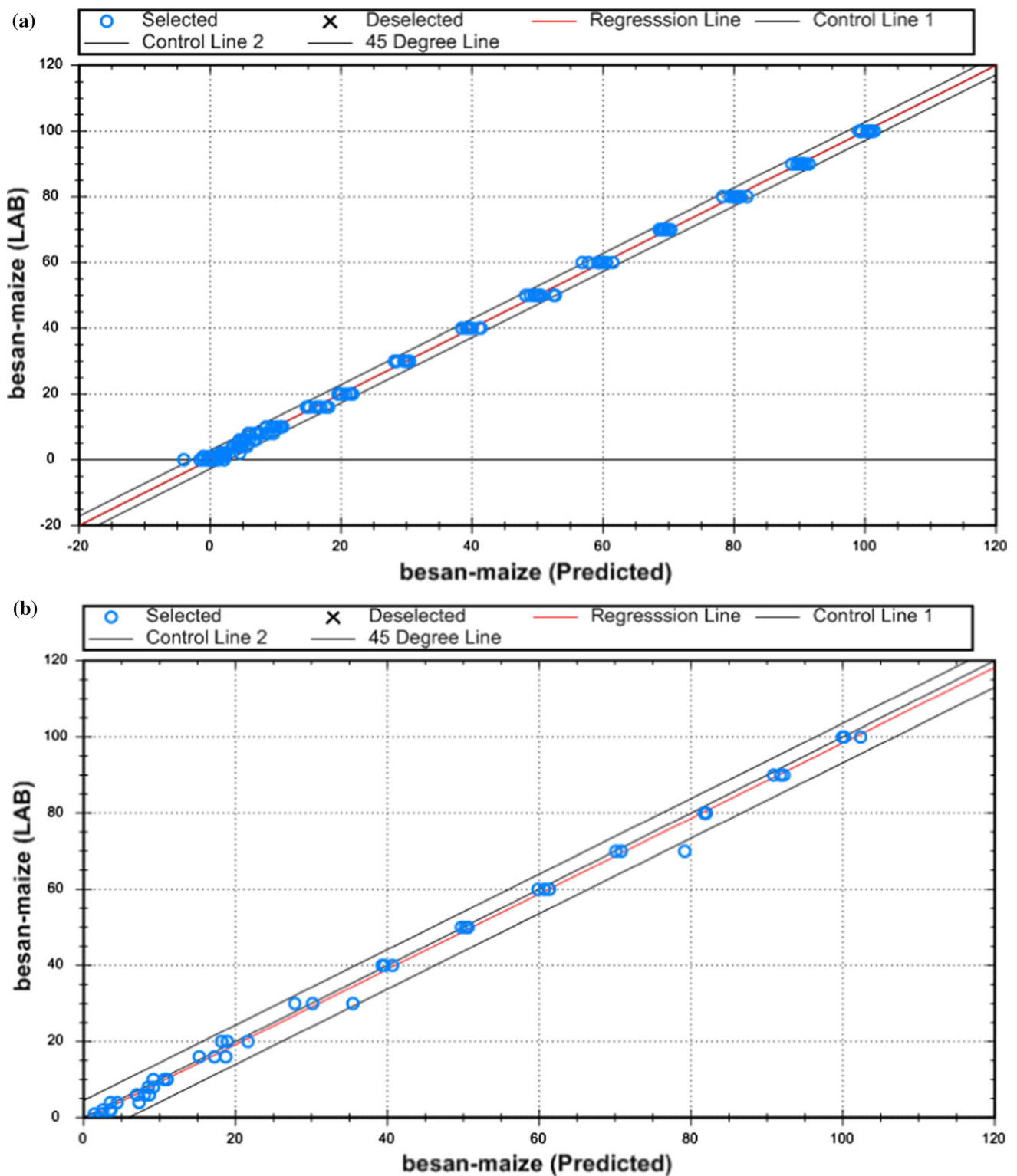


Fig. 2 A. MPLSR-NIRS for predicted and laboratory values of pure and adulterated *besan* sample with maize flour using cross validation. B. MPLSR-NIRS for predicted and laboratory values of pure and adulterated *besan* sample with maize flour using external validation

potato and sweet potato starch adulteration (0–100%, w/w) with lotus root powder (Liu et al. 2013) and starch adulteration (1–35% w/w) with onion powder (Lohumi et al.

2014) has been reported. Ndlovu et al. 2021 developed PLSR model with R^2 of 0.94% to predict gluten adulteration in green banana flour. It has been reported that closer

Table 2 Analytical characteristics of the MPLS-NIR model

Parameters	Statistical parameter	Value
Linearity	R ²	0.999
Accuracy	SEC	1.092
	SECV	2.046
	SEP cross validation	1.117
	SEP test validation	2.075
Repeatability	Mean ± Sd	10.17 ± 0.89
Reproducibility	Mean ± Sd	10.42 ± 0.98
Precision	RPD	14.52
LOD%	–	3
LOQ%	–	1

are the lab and predicted values better is the regression method. Based on similarity of results with others it can be concluded that our MPLSR-NIRS calibration model could be used to rapidly determine maize flour adulteration in *besan*. Therefore, the higher detection accuracy of flour adulteration in our study is rational and reasonably good.

Conclusion

The present study has emphasized the potential of near infra red spectroscopy along with multivariate analyses for the detection of admixtures of *besan* and maize flour. The results revealed that pretreatment of spectral data affects the calibration statistics of developed models. Among PLS, PC and MPLS regression methods, MPLS regression yielded the optimum model (R² = 0.999) which could predict the adulteration of maize flour in *besan* with high accuracy. Standard error of prediction (SEP) value of 1.117 and 2.661 was observed for cross and test validation, respectively. The results revealed that the developed prediction model could be used to quantify the adulteration of maize flour in *besan*.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13197-022-05456-7>.

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Author contributions MB: Conceptualized, designed the experiments, performed the experiments, analyzed the data, authored and reviewed drafts of the paper, and approved the final draft. SS: Performed the experiments, authored and reviewed drafts of the paper, and approved the final draft. SS: Scanned the samples, analyzed the data, authored and reviewed drafts of the paper and approved the final

draft. MD: Analyzed the data, authored and reviewed drafts of the paper and approved the final draft. GK: Analyzed the data, authored and reviewed drafts of the paper, and approved the final draft.

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Declarations

Conflict of interest The author declares that they have no conflict of interest.

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Consent to participate Not applicable.

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Data availability Not applicable.

Code availability Not applicable.

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