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Original Article

Determination of fumonisin content in maize using near-infrared hyperspectral imaging (NIR-HSI) technology and chemometric methods

Determinação do teor de fumonisina em milho usando tecnologia de imagem hiperespectral no infravermelho próximo (NIR-HSI) e métodos quimiométricos

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Abstract

Maize (*Zea mays* L.) is of socioeconomic importance as an essential food for human and animal nutrition. However, cereals are susceptible to attack by mycotoxin-producing fungi, which can damage health. The methods most commonly used to detect and quantify mycotoxins are expensive and time-consuming. Therefore, alternative non-destructive methods are required urgently. The present study aimed to use near-infrared spectroscopy with hyperspectral imaging (NIR-HSI) and multivariate image analysis to develop a rapid and accurate method for quantifying fumonisins in whole grains of six naturally contaminated maize cultivars. Fifty-eight samples, each containing 40 grains, were subjected to NIR-HSI. These were subsequently divided into calibration (38 samples) and prediction sets (20 samples) based on the multispectral data obtained. The averaged spectra were subjected to various pre-processing techniques (standard normal variate (SNV), first derivative, or second derivative). The most effective pre-treatment performed on the spectra was SNV. Partial least squares (PLS) models were developed to quantify the fumonisin content. The final model presented a correlation coefficient (R^2) of 0.98 and root mean square error of calibration (RMSEC) of 508 $\mu g.kg^{-1}$ for the calibration set, an R^2 of 0.95 and root mean square error of prediction (RMSEP) of 508 $\mu g.kg^{-1}$ for the test validation set and a ratio of performance to deviation of 4.7. It was concluded that NIR-HSI with partial least square regression is a rapid, effective, and non-destructive method to determine the fumonisin content in whole maize grains.

Keywords: *Zea mays* L., mycotoxins, fumonisins, non-destructive analysis, hyperspectral image near infrared, partial least squares (PLS).

Resumo

O milho (*Zea mays* L.) possui importância socioeconômica por constituir um dos alimentos básicos na nutrição humana e animal. Porém, o cereal é suscetível ao ataque de fungos produtores de micotoxinas que podem causar danos à saúde. Os métodos mais utilizados para detectar e quantificar micotoxinas são caros e demorados e métodos alternativos para a detecção das micotoxinas são uma necessidade. O presente trabalho tem como objetivo utilizar espectroscopia no infravermelho próximo com imagem hiperespectral (NIR-HSI) e análise multivariada de imagens para desenvolver um método rápido e preciso para quantificação de fumonisinas em grãos inteiros de seis cultivares de milho naturalmente contaminadas. Cinquenta e oito amostras, cada uma contendo 40 grãos, foram submetidas ao NIR-HSI e posteriormente divididas em um conjunto de calibração (38 amostras) e um conjunto de predição (20 amostras) com base nos dados multiespectrais obtidos. Os espectros médios foram submetidos a diversas técnicas de pré-processamento (variação normal padrão – SNV, primeira derivada ou segunda derivada). O melhor pré-processamento dos espectros foi SNV e um modelo de mínimos quadrados parciais (PLS) foi desenvolvido para quantificar o teor de fumonisinas. O modelo final apresentou coeficiente de correlação (R^2) de 0,98 e raiz quadrada do erro médio quadrático de calibração (RMSEC) de 508 $\mu g.kg^{-1}$ para o conjunto de calibração, R^2 de 0,95 e raiz quadrada do erro médio quadrático de predição (RMSEP) de 508 $\mu g.kg^{-1}$ para o conjunto de validação do teste e relação desempenho/desvio de 4,7. Conclui-se que o NIR-HSI com regressão parcial de mínimos quadrados pode ser um método rápido, eficaz e não destrutivo para determinar o teor de fumonisinas em grãos integrais de milho.

Palavras-chave: *Zea mays* L., micotoxinas, fumonisinas, análise não-destrutiva, imagem hiperespectral no infravermelho próximo, mínimos quadrados parciais (PLS).

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1. Introduction

Maize (*Zea mays* L.) is rich in nutrients, vitamins, and minerals and is an essential food for human and animal nutrition. It confers significant health advantages to the organism. Although cereal maize is cultivated extensively and consumed worldwide, the highest grain production and consumption levels occur in the United States, China, and Brazil (Erenstein et al., 2022). However, cereals are highly susceptible to attack, both pre-harvest and during storage, by toxigenic fungi of the genera *Fusarium* and *Aspergillus*. The fungi produce a range of secondary metabolites known collectively as mycotoxins. These reduce the quality and nutritional value of food and represent a severe problem in food and feed security (Parrag et al., 2020). For example, the *Fusarium* species commonly observed in maize can produce various mycotoxins such as fumonisins and deoxynivalenol (DON). A few of these are known to cause fatal diseases in livestock and primary liver cancer risk in humans (Parrag et al., 2020; Claeys et al., 2020).

In this context, mycologists and phytopathologists are particularly concerned regarding the increase in the contamination of foodstuffs by the *Fusarium* species. Moreover, industrial food processors are seeking rapid, inexpensive methods to detect and quantify mycotoxins in primary source materials (Femenias et al., 2020). The maximum content of fumonisins $B_1 + B_2$ in maize is 5,000 $\mu g.kg^{-1}$ according to Brazilian legislation (Brasil, 2011) and 4,000 $\mu g.kg^{-1}$ according to the European Commission (Khodaei et al., 2021).

Several techniques can be applied for the qualitative and quantitative analysis of mycotoxins. These include high-performance liquid chromatography (HPLC) coupled with fluorescence detection and/or mass spectrometry (MS), and enzyme-linked immunosorbent assay (ELISA). HPLC is the most commonly employed method because it provides remarkable sensitivity, selectivity, and precision. It requires extensive sample preparation, which is destructive, time-consuming, expensive, and results in a large amount of chemical waste (Khodaei et al., 2021; Femenias et al., 2020).

Near-infrared hyperspectral imaging (NIR-HSI) is a rapid, effective, non-invasive, and non-destructive method for analyzing a wide range of biological materials. This procedure generates a set of NIR spectral images representing a narrow wavelength band at all pixel points within a two-dimensional image plane.

Wang et al. (2014) verified the use of NIR-HSI to detect aflatoxin B_1 (AFB_1) contaminants in maize kernels. In this study, AFB_1 solutions with concentrations of 10, 20, 100, and 500 ppb were applied to the surfaces of grains. The spectral data were subjected to principal component analysis (PCA) followed by stepwise discriminant factor analysis. The classification model achieved an accuracy of 88%. This demonstrated the efficiency of the method for detecting levels of AFB_1 even at low concentrations.

In a recent study, Parrag et al. (2020) evaluated the use of NIR-HSI to detect cornmeal samples that had been naturally or artificially contaminated with *F. graminearum*, *F. verticillioides*, or *F. culmorum*. The sample spectra were subjected to Savitzky-Golay smoothing and standard normal variate (SNV) transformation to reduce the effects of noise on the signal. Partial least squares discriminant analysis (PLS-DA) was used to predict the level of contamination on the samples. A partial least squares regression (PLSR) method was applied to predict the amounts of fumonisins and DON. Based on the promising results of this study, the authors contend that HSI has the potential to be used as a preliminary test method for determining the mycotoxin content in feed materials.

NIR-HSI has also been employed to characterize parameters related to the seed quality of other cereals, legumes, and oilseeds (Feng et al., 2019; Jia et al., 2020; Freitag et al., 2022).

For example, Femenias et al., 2020 evaluated the detection of DON in wheat samples using an NIR-HSI approach. Herein, all the spectra were pre-treated and processed by PLSR and LDA. They obtained a root mean square error of prediction (RMSEP) of 501 $\mu g.kg^{-1}$ for the analyte with an accuracy of 85.4% for the validation set.

In a recent study, Kim et al. (2023) proposed a method for classifying single contaminated and co-contaminated aflatoxin and fumonisin in ground maize samples by applying hyperspectral imaging techniques, including reflectance in the visible and near-infrared (VNIR) and short-wave infrared (SWIR) regions combined with machine learning algorithms. SWIR imaging with the support vector machine model resulted in higher classification accuracies compared to VNIR models. In addition, the results revealed that this technique can be used in routine analysis models.

Thus, NIR-HSI can assist in the early identification of fungal contamination. This factor may also contribute to the timely control of plant diseases and represents a viable technique for detecting and quantifying mycotoxins. Recent studies underline that such infrared spectroscopic platforms have great potential for the rapid analysis of mycotoxins (Freitag et al., 2022). Moreover, NIR-HSI can assist food and feed industries by providing a rapid and inexpensive strategy to distinguish between batches of raw materials that are free from fungi and those contaminated with mycotoxins. This would prevent the consumption of contaminated food (Femenias et al., 2020). This study aimed to develop a rapid and accurate method for quantifying fumonisins in whole maize kernels using NIR-HSI and chemometric techniques.

2. Material and Methods

2.1. Maize samples

For this study, fifty-eight corn grain samples with approximately 500 g were obtained from field plots where the grains were naturally contaminated with fumonisin. Therefore, these were representative of real situations observed in the field. The plots were established at Embrapa Maize and Sorghum (Sete Lagoas, MG, Brazil) in 2018. Each sample was homogenized to obtain a subsample of approximately 40 grains. Before the imaging, these grains were placed in a 10 x 20 cm Teflon holder (10 rows x 4 columns) for spectral scanning as described below in the section on NIR-HSI imaging system and acquisition

and multivariate analysis of images. After the maize subsamples were scanned, they were assayed using HPLC for the fumonisin concentration (μg.kg^{-1}) described in the HPLC determination of fumonisin concentration in the maize samples section. The samples were divided into a 2/3 calibration set (38 samples) and 1/3 prediction set (20 samples) according to the Kennard-Stone algorithm (Kennard and Stone, 1969).

2.2. NIR-HSI imaging system

A SisuCHEMA-SWIR chemical imaging system (Specim, Spectral Imaging Ltd., Oulu, Finland) was employed to obtain hyperspectral images of the sample sets in the 1000-2100 nm spectral range with 256 wavelength bands (6.25 nm spectral sampling) and a spatial resolution of 10 nm. The high-performance camera was equipped with a 50 mm lens (50 mm field-of-view) and a frame rate of 60 Hz to acquire images with a pixel size of 150 × 150 μm. A line-scan camera was employed for the signal acquisition. The signal intensity of the pixels (x-y spatial dimension) was recorded using ChemaDAQ software (Specim, Spectral Imaging Ltd.) to generate a sub-image cube comprising 256 × 256 pixels at each wavelength.

2.3. Acquisition and multivariate analysis of images

The system was permitted to stabilize for 30 min before the collection of spectra to prevent interference from the surroundings and ensure consistency in the information derived from the samples. The acquisition of the NIR images of the samples included the digitization of 40 whole maize grains of the linear array by shifting the detector along the x- and y-axes (spatial dimensions) of the image at 256 wavelengths (spectral dimension). The spatial and spectral data from the resulting 3D hypercube were used to obtain the average spectrum for each sample set. The images were saved in the RAW format for further treatment to remove the average background and reveal the cleaned-up spectrum. The hyperspectral images were analyzed using Prediktera Evince v.2.6.0 analysis software (UmBio, Umeå, Sweden).

2.4. HPLC determination of fumonisin concentration in maize samples

2.4.1. Reagents and chemicals

Methanol and acetonitrile (HPLC grade) were supplied by Scharlab (Sentmenat, Spain). The analytical standard mixture of fumonisins B_1 and B_2 (50 μg. mL^{-1} of each in acetonitrile:water) was purchased from Sigma-Aldrich (St Louis, MO, USA; product #34143). Water was obtained from an ELGA® water purification system (Elga LabWater, High Wycombe, UK).

2.4.2. Quantification of fumonisins by HPLC

The maize samples were ground separately in an IKA (Campinas, SP, Brazil) model A11 basic analytical mill. For each sample set, 10 g of powdered material was extracted with 50 mL of water:methanol:acetonitrile (50:25:25 v / v) for 40 min with agitation (at 200 rpm) on an orbital shaker (model 109; Nova Ética, Vargem Grande do Sul, SP, Brazil). The extract was centrifuged at 3000 rpm for 10 min and filtered through a qualitative filter paper to remove the impurities. An aliquot (5 mL) of the filtrate was removed, mixed with 20 mL of phosphate-buffered saline (PBS), and filtered through a glass microfiber filter. A portion (10 mL) of the mixture was transferred to a syringe attached to a Vicam® (Waters, Milford, MA, USA) FumoniTest immunoaffinity column and washed under the action of gravity with 10 mL of PBS solution to remove impurities. The fumonisins were subsequently eluted with 2.5 mL of HPLC-grade methanol injected at one drop per second. The eluent was collected in a glass cuvette held in a water bath at 50-55 °C and subjected to a flow of dry compressed air until the solvent was removed entirely. The dried residue was resuspended in acetonitrile: water (1:1 v / v) and a 50 μL aliquot of the solution mixed with 50 μL of derivatizing reagent containing *O*-phthalaldehyde and 2-mercaptoethanol. The derivatized sample was injected into a Waters Alliance Model 2695 HPLC separation system equipped with a C18 reverse-phase column. The fluorescence detector was set at an excitation and emission of 335 nm and 440 nm, respectively. The retention times of fumonisins B_1 and B_2 were approximately 4 and 9 min, respectively. The fumonisins were quantified using a calibration curve constructed using an analytical standard mixture of fumonisins B_1 and B_2. For each batch of 20 samples, a reference sample from the Trilogy Analytical Laboratory (Washington, MO, USA) with a total fumonisin content of 4.1 ± 0.5 μg.kg^{-1} was analyzed.

2.5. Chemometric methods

The collected NIR-HSI spectra were preprocessed using mean centering. Additionally, the scattering and baseline deviations were corrected by standard normal variate (SNV) transformation, or first or second derivative and 11-point Savitsky-Golay filter (with or without variable selection) using The Unscrambler v. 10.5 software (AspenTech, Bedford, MA, USA). The spectral pre-treatment methods were used individually or in combination. The preprocessing technique was optimized according to the modeling performance.

Quantitative spectral analysis was performed using chemometric approaches based on the partial least squares (PLS) algorithm. PLS models were developed to quantify fumonisins in grains. PLS regression calibrations were evaluated 1) based on the coefficient of regression (R^2), root mean square error of calibration (RMSEC), and RMSEP and 2) using the ratio of performance to deviation (RPD). The RPD is defined as the ratio between the measured standard deviation and prediction error (Haaland and Thomas, 1988).

3. Results and Discussion

3.1. Spectral analysis

Figure 1 shows the NIR raw spectra of the maize samples. The vibration bands associated with the O-H, N-H, and

C-H groups of the nutrients in the grains are broad and overlapped. The key absorption peaks of the maize samples at approximately 1200 and 1465 are attributed to the combination and overtones of the O–H and N–H stretching vibrations of the chemical information regarding maize composition (Caporaso et al., 2018). The absorption bands associated with the H-bonds of the O–H stretching from water and the O–H combination from polysaccharides are located at 1920 nm and 2090 nm, respectively (Figure 1). Less intense bands appeared at 1170/1270 nm (associated with polysaccharide components) in the C–H stretch second overtone and O–H stretch of the combination bands (Workman, 2021).

3.2. Fumonisins analysis in maize samples

For 58 ground maize samples, fumonisins were analyzed by the concentration range from 217 to 12,411 μg.kg^{-1} contaminated maize. The concentration ranges of fumonisins of the naturally contaminated samples are used in this study. The minimum, maximum, and mean fumonisin levels determined by the HPLC analysis of the calibration and prediction sample sets of maize grains are shown in Table 1. The calibration set addressed the widest range of fumonisins B1 + B2 concentrations.

3.3. PLS model results

Based on the NIR-HSI data, the PLS algorithm was employed to develop models for predicting fumonisin levels in maize grains. The acquired spectra were subjected to three pre-processing procedures, and the performances of the quantitative determination models were compared (Table 2). The best pre-treatment of the spectra was selected based on the regression models that provided the lowest RMSEP and highest RER values. Consequently, the best models exhibited high correlation coefficients (R^2) and low RMSEC, RMSEP, and bias (Pasquini, 2018). In addition, the RPD, which relates the squared error of the predictions to the variance and range in the original reference data, should preferably be at least 3.0 (Darren et al., 2022).

The present study obtained the best results for the calibration set containing the average spectra of 38 maize samples subjected to SNV pre-processing. To evaluate the prediction performance of the PLS models, the predicted fumonisins of the maize grains were plotted against the reference values for all the datasets (Figure 2). The samples are linearly distributed around a diagonal line in the plot of reference versus values predicted by the PLS model.

This model involved 10 latent variables and presented an R^2_c of 0.98 and RMSEC of 508 μg.kg^{-1} for fumonisins B_1 + B_2 in maize grain. For the test set with 20 maize samples, the model presented an R^2_p of 0.95 and RMSEP of 505 μg.kg^{-1}. This measures the prediction's average accuracy (i.e., the difference between the true and estimated values). The model displayed a good prediction capability, as indicated by the high values of R^2 and the marginal difference between RMSEC and RMSEP.

The developed calibration models were also evaluated based on RPD and RER. The RPD of this model was 4.7. This indicates a remarkable predictive capability as verified by

Table 1. Fumonisins content (μg.kg^{-1}) of maize grains in the calibration and prediction sample sets as determined by the HPLC method.

Descriptive statistic	Calibration set	Prediction set
Number of samples (*n*)	38	20
Minimum value (μg.kg^{-1})	217	330
Maximum value (μg.kg^{-1})	12,411	9,151
Mean value (μg.kg^{-1})	4,925	2,316
Standard deviation (μg.kg^{-1})	3,917	2,390

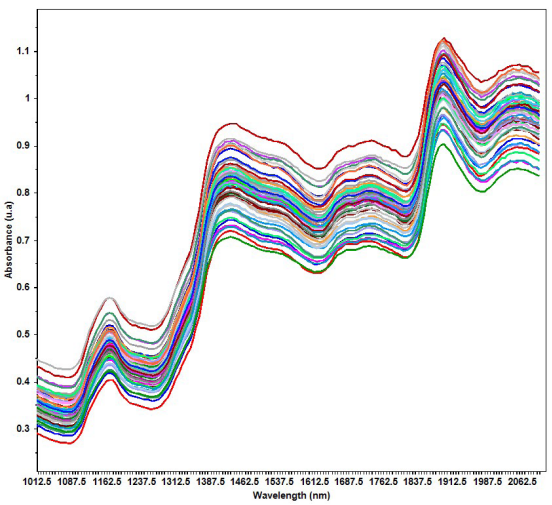

Figure 1. Raw mean absorbance spectra obtained by NIR-HSI from the 58 samples of whole maize grains.

Table 2. Performances of models for the quantitative determination of fumonisin developed using the PLS method from NIR-HSI spectra of sample sets subjected to different spectral pre-treatment procedures.

Pre-processing procedure	LV	R^2_c	RMSEC (μg.kg^{-1})	R^2_p	Bias (μg.kg^{-1})	RMSEP (μg.kg^{-1})	RPD	RER
Standard normal variate	10	0.98	508	0.95	-0.018	505	4.7	17.5
1st derivative	13	0.86	1,447	0.78	-0.023	2,740	0.9	3.2
2nd derivative	12	0.92	1,088	0.72	-0.015	2,486	1.0	3.5

LV: latent variable, R^2_c and R^2_p: determination coefficients of calibration and prediction sets, respectively; RMSEC: root mean square error of calibration, RMSEP: root mean square error of prediction, RPD: ratio to performance deviation, RER: range error ratio.

the predicted versus reference plot (Figure 2) for fumonisins B_1 and B_2 in maize grains. According to Xing et al. (2019), RPD > 2.5 indicates remarkable models and/or predictions. This is because a larger RPD value indicates a higher capability of the model to precisely predict adulteration in new samples. The RER value was 17.5. RER > 10 is considered a good predictability for multivariate calibration (Rambo et al., 2020). AACC Method 39-00.01 (AACC, 1999) provides quality thresholds for model performance based on the RER values: For RER > 4, the calibration is reasonable for sample screening; for RER > 10, the calibration is reasonable for quality control; and for RER > 15, the calibration is suitable for quantification. This model may be used to screen the fumonisin content in maize grains. However, it should be emphasized that the model's accuracy depends on its application and prediction errors (RMSEP).

According to Yang et al. (2022), starches, sugars, and other food matrix components interact with the amino and tricarboxylic acid groups in fumonisin structures. In this context, interpreting the regression coefficients is necessary to avoid possible accidental correlations (Rambo et al., 2020). The NIR spectral region between 1100 and 1300 nm (Figure 3) is influenced by the bending modes of the $-CH_2$ and $-CH_3$ groups in proteins, carboxylic acids, esters, and starches (Caporaso et al., 2018). Meanwhile, multiple bands in the 1200-1540 nm region involve $-CH_2$, C-H, and O-H in-plane deformations (De Géa Neves et al., 2022).

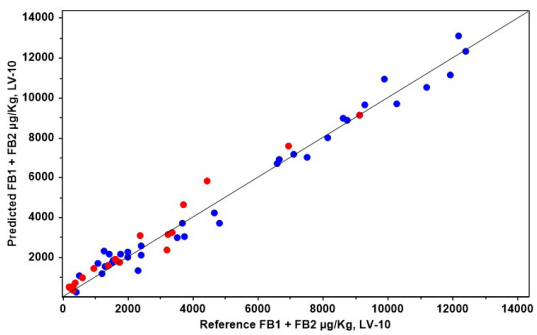

Figure 2. Predicted versus reference plot for the PLS calibration (blue) and validation (red) sets of grain maize samples.

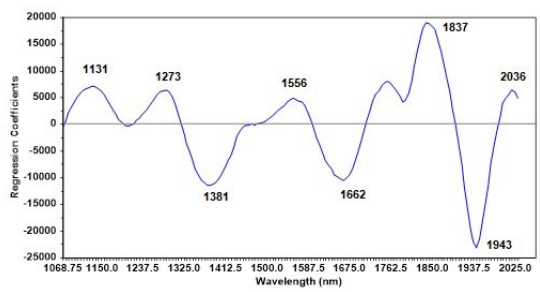

Figure 3. Regression coefficients for fumonisin PLS model of maize samples.

The regression vector displays positive peaks at 1131 nm, 1273, 1556, 1891, and 2036 nm (Figure 3). Laubscher et al. (2023) concluded that FB1 absorbs NIR energy at approximately these wavelengths. The band at 1131 nm corresponds to the absorption of carbohydrates (first harmonic of C-H stretching). Meanwhile, the bands at 1381 and 1556 nm correspond to the second harmonic of C-H stretching, which is related to the symmetrical elongation of C=O and COO in carbohydrates and the deformation angles of the $-CH_2$ and $-CH_3$ groups in fatty acids. These indicate the presence of starch and sucrose (first harmonic of O-H stretch) (López et al., 2017). The second overtone of the C=O bond in the carboxylic acids was identified at approximately 1900 nm. Therefore, it could be responsible for the peak at 1837 nm. Figure 3 shows the negative regression vectors at 1381 nm and 1943 nm. Here, the significant bands are assigned to water, the first harmonic of O-H stretching, and deformations in the O-H group combined with hydroxyl. The band at 1662 nm corresponds to the absorption of proteins, starches, and the second harmonic of N-H stretching (Workman, 2021). The prominent peak profile shown in Figure 3 indicates that the main cause of the variation in contaminated maize grains was the variation in the composition or structure of starch and protein. This, in turn, indicates a decrease in the levels of stored food reserves, as observed earlier by Williams et al. (2012).

Most initial studies involving the application of NIR technology to quantify fumonisins employed Fourier-transform (FT)-NIR spectroscopy. For example, Tyska et al. (2021) quantified the contamination level of total fumonisins, i.e., B1 + B2 and zearalenone, in 200 unknown maize samples, and no significant difference was observed in predicted values using NIR and reference values obtained by Liquid Chromatographic Coupled to Tandem Mass Spectrometry (LC-MS/MS). Kim et al. (2023) developed a short-wave infrared (SWIR) method for screening fumonisin-contaminated milled maize, considering the European legal limit. However, these applications used ground samples.

The application of multispectral imaging employing 10 wavelength bands in the NIR range of 720-940 nm to predict the fumonisin content of milled maize was first described by Firrao et al. (2010). The results showed a significant correlation between the image analysis predictions and the mycotoxin concentrations as determined by a chemical analysis. More recently, hyperspectral imaging employing over 100 wavelength bands in the NIR region has become an essential nondestructive technique for investigating mycotoxin contamination in cereals (Caporaso et al., 2018). Nevertheless, several studies have been conducted in this area by scanning individual grains subjected to artificial contamination. This procedure can modify the final level of contamination and interactions between grains, molds, and mycotoxins. Stasiewicz et al. (2017) described the potential use of a multispectral sorter (470-1550 nm) for identifying and removing aflatoxin- and fumonisin-contaminated grains from bulk mature maize kernels in Kenya. The authors reported that statistically significant ($p < 0.001$) reductions of up to 83% were achieved for grains contaminated with each toxin.

The results obtained in the present study show that NIR-HSI combined with multivariate regression may be a suitable alternative method for determining the fumonisin content in whole maize grains. The technique described circumvents the grinding step that is generally required and is faster and less complex than other machine learning methods. This would facilitate the analysis of many samples in an industrial process.

4. Conclusion

This study demonstrated that a multivariate calibration of the average spectra obtained from whole maize grains using the NIR-HSI technique is an advantageous method for determining fumonisins. It has applications in the identification of contaminated batches and prevention of cross-contamination during maize storage. Nevertheless, because fumonisin levels vary considerably among different cultivars, climatic conditions, and agronomic regions, new samples should be analyzed using this model. Such studies would enable updating of this technology and increase its robustness in quantifying fumonisins. This would, in turn, provide remarkable safety in using maize for human and animal nutrition.

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