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Research Article

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Fumonisins are major mycotoxins found worldwide in maize and maize products. Because of their toxicity for both human and animals, European Union regulations were created to fix the maximal fumonisin B1 and B2 content allowed in foods and feeds. Unfortunately, directly measuring these mycotoxins by current analytical techniques is tedious and expensive and most measurement methods do not lend themselves to online control. Alternative approaches to chemical analysis have been developed and involve models that allow the mycotoxin contamination to be predicted based on environmental conditions and analysis by near-infrared (NIR) spectroscopy. In the present work, we use NIR spectroscopy to determine the fumonisin and fungal contents of 117 samples of maize. The determination coefficient between fumonisin and fungal-biomass content was 0.44. We establish herein a threshold for the number of CFUs for fungal biomass beyond which the fumonisin content is likely to exceed the European regulatory level of 4000 μg/kg. In addition, we determine the fungal content by using a NIR-spectroscopy model that allows us to sort samples of maize. Upon calibration, the percentage of well-classified samples was 96%, which compares favorably to the 82% obtained by independent verification.

1. Introduction

Fumonisins are the main mycotoxins produced by the fungi *Fusarium verticillioides* and *F. proliferatum*, which are found worldwide as contaminants of maize and maize byproducts [1].

Fumonisin B1, the most toxic compound of the family, is suspected to be related to the increase in esophageal cancers in some areas (e.g., China and South Africa) [2, 3] and to the rising rate of neural tube defects reported in North and Central America [4, 5]. This compound is also hepatic and nephrotoxic in many animal species [6]. Therefore, the withdrawal of maize batches contaminated by this toxin is a major food-safety concern.

For human and animal health, the hazard associated with exposure to fumonisins led to the creation of European regulations and recommendations that fix the maximal tolerable fumonisin content in foods and feeds, respectively [7, 8].

Currently, the most commonly used methods to determine fumonisin contamination are based on high-performance liquid chromatography (HPLC) coupled with fluorescence detection and/or mass spectrometry and/or enzyme-linked immunosorbent assays [9]. However, these methods depend directly on how representative the sample is of the overall batch of feed or food. In fact, mycotoxin contamination is generally heterogeneous, and characterizing an entire batch of several tons of maize would require preparing many subsamples to form a representative sample

of the sanitary status of the entire lot. The cost and time required for such an analytical method limit its use for rapid screening of raw materials.

Thus, a dire need exists for alternative methods that would enable real-time screening of maize batches vis-à-vis their fumonisin content thereby to determine their subsequent orientation toward human or animal feeding. Toward this end, other specific grain parameters could be used to indirectly predict fumonisin contamination. In fact, because mycotoxins are secondary fungal metabolites, their production is directly linked with fungal development [10]. Therefore, the assessment of fungal contamination may be used to indirectly predict mycotoxin contamination; indeed, previous studies demonstrate that both fungal counts and ergosterol measurements could be used as indirect markers to assess the risk of mycotoxin contamination [11, 12].

Near-infrared spectroscopy (NIR) is now commonly used to verify various quality parameters of cereals [13]. As detailed elsewhere, the NIR absorption spectrum of a substance is obtained from its optical parameters (reflectance, transmission, etc.) in the NIR. The interpretation of these spectra is based on the fact that molecules absorb specific NIR frequencies depending on the characteristics of their structure [14].

To date, two main factors have hindered attempts to use NIRS to directly measure mycotoxin content in the field: (1) mycotoxins are present in quantities too small (in the order of parts per million) for direct detection. Their detection is thus associated with a complex ensemble of information related to the growth of the fungus on the cereal. (2) The associated prediction errors are too high to satisfy the European regulation EC number 1127/2007. For instance, Vignola et al. [15] reported that 68% of the predicted values lie within $\pm\sigma$ (where σ is the standard error of prediction) of the mean predicted value, and 95% lie within $\pm 1.96\sigma$. If we consider, for example, $\sigma = 3610\,\mu$g/kg [16], then NIR of an uncharacterized sample would predict a contamination level of $\pm 1.96 \times 3610\,\mu$g/kg. This would actually correspond to $7000\,\mu$g/kg, whereas the European Union (EU) limit for deoxynivalenol is $1750\,\mu$g/kg (EC number 1881/2006/CE). The details for each study and the associated standard errors of prediction were published by Levasseur-Garcia [17].

However, some recent studies demonstrated that NIR could be used to discriminate between fungal species grown in culture media, and NIR has even been used to differentiate between toxigenic and nontoxigenic isolates [18, 19]. Therefore, the aim of the present study is to evaluate NIR as an indirect method that uses fungal counts as indirect markers to assess the risk of fumonisin contamination in maize. For that, we characterized 117 samples of maize for fungal and fumonisin contamination by using both conventional methods and NIR.

2. Materials and Methods

2.1. General Procedure. Figure 1 shows the general procedure for classifying maize according to fumonisin content.

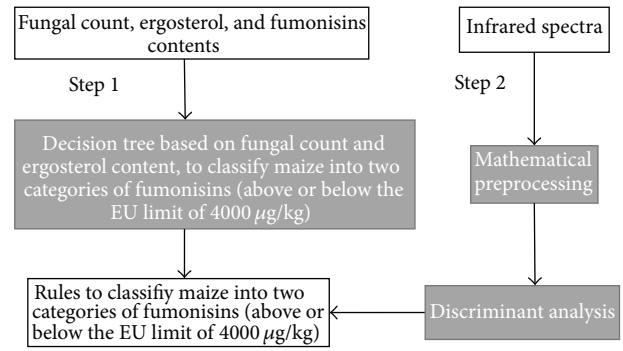

Figure 1: Procedure for classifying maize according to fumonisin content.

This procedure involves two steps. The first step considers only the benchmark values obtained in the laboratory: the ergosterol content, the number of colony-forming units (CFUs), and the fumonisin concentration. In this first step, a sequence of rules is embedded in a decision tree to determine whether the fumonisin content in a batch of maize is above or below the European regulatory threshold for human consumption ($4000\,\mu$g/kg) [8]. A decision tree is a method of classification that uses a tree-like model. It is a flowchart-like structure consisting of internal nodes, leaf nodes, and branches. Each internal node represents a test, each outgoing branch corresponds to a possible outcome of the test, and each leaf node represents a class [20].

The second step is based on NIR. The spectra are first processed by mathematical algorithms and then are used as input into a discriminant analysis to automate the implementation of the decision tree from Step 1.

Our objective is to evaluate the fungal count and the ergosterol content as indirect indicators to efficiently predict whether the fumonisin content of a batch of maize is greater than or less than the EU limit. If this is possible, then rules must be created that allow users in the field to classify maize using fungal count and ergosterol content. In addition, these rules must be automated according to NIR spectra from maize samples.

Thus, the sequence of these two steps should constitute a model that, based on the NIR spectra, allows maize samples to be classified according to whether their fumonisin content is above or below the EU regulatory limit.

2.2. Chemicals. All solvents (methanol, ethanol, toluene, and acetonitrile) and reagents (O-phthalaldehyde, pyrogallol, and potassium hydroxide) used for mycotoxin and ergosterol measurements were analytic grade and were purchased from Prolabo (Paris, France).

2.3. Maize Samples. A total of 117 samples of maize were used in this study. Samples were harvested in 2007 in Italy, Denmark, France, Hungary, The Netherlands, and Poland. Each sample consisted of 1.5 kg of grains dried at 40°C. Two 0.750 kg subsamples were drawn from each sample. One subsample was used to quantify ergosterol, count CFUs, and

identify the fungal species. The other subsample (0.150 kg) was used for mycotoxin analysis and to collect the infrared spectra.

2.4. Determination of Fumonisin B1 Content. Fumonisin B1 (FB1) was quantified by HPLC as described previously [21]. Briefly, FB1 was extracted and purified as described by Le Bars et al. [22]; namely, by mixing with 100 mL of methanol-water solution (3 : 1) and grinding for 3 min in a Warring blender. After filtration by a fluted number 3 filter (Fioroni, VWR, Fontenay Sous Bois, France), 10 mL of the extract was applied to Bond-Elut SAX cartridges (500 mg, 2.8 mL; VWR, Fontenay Sous Bois, France) and eluted with 14 mL of acidified methanol (0.5% acetic acid). These extracts were evaporated to dryness under a gentle stream of nitrogen and then dissolved in methanol.

For HPLC quantification, 25 μL of extract or 25 μL of standard was derivatized with a mixture of 25 μL of borate buffer (pH 8.3), 25 μL of water, and 25 μL of O-phthaldialdehyde (15 mM) and separated by HPLC with an M2200 pump (ICS, Toulouse, France), a Prontosil C18, 5 μm, 250 × 4 mm column equipped with a precolumn (ICS, Toulouse, France), and an 8450 fluorescence HPLC monitor (Shimadzu, Kyoto, Japan). The HPLC used liquid-phase NaH_2PO_4 (0.1 M, pH 3.3)/methanol (25/75 v/v), a 1 mL/min flow rate, a 10 μL injection volume, and fluorescence detection at the excitation (emission) wavelength 335 nm (440 nm). The spectra were quantified by comparing the peak area measured by a Pic3 data system from ICS (Toulouse, France) with a standard calibration curve. The mean retention time was 7.5 and 17.5 min for FB1 and FB2, respectively. The quantification limits were 0.1 and 0.2 μg/g for FB1 and FB2, respectively [23].

2.5. Fungal Contamination

2.5.1. Ergosterol Measurement. Ergosterol was quantified as per Bailly et al. [24]. Fifteen grams of crushed grains were mixed with 45 mL of methanol, 15 mL of ethanol, 60 μL of pyrogallol, and 6 g of potassium hydroxide in a 250 mL round-bottom flask. The mixture was refluxed with agitation at 80°C for precisely 30 min and then quickly cooled in a water bath to 20°C. The extract was filtered through a GF/A 1.6 μm Whatman paper and the ergosterol was extracted from 15 mL of filtrate by petrol ether (2 × 30 mL with 2 min of strong mechanical agitation) and washed with acid water (pH = 2.5, 2 × 30 mL with 2 min of strong mechanical agitation). The petrol-ether extract was evaporated until no visible solvent was present in the tube and then redissolved in 100 μL of a 98/2 v/v toluene/acetonitrile solution. Three μL of the solution was placed on a silica support plate and separated by migration in an 85/15 v/v toluene/acetonitrile solution. After drying, the support plate was heated to 130°C and held at this temperature for 30 min to make the ergosterol fluorescent. A fluorodensitometer at 365 nm (Shimadzu CS930) was used to quantify the ergosterol concentration. Each extract was analyzed twice and was quantified by comparing with a standard calibration curve obtained from the same plate that was used for the samples.

2.5.2. Fungal Count and Identification. The fungal count of the maize samples was done according to the AFNOR norm [25]. Briefly, 20 g of crushed sample was mixed with 180 mL of tween 80 (0.05%) by mechanical agitation for 30 min. Decimal dilutions (10^{-1} to 10^{-5}) were prepared in tween 80 (0.05%). One hundred μL of each dilution was inoculated in Petri dishes containing a malt medium (2% agar, 2% malt, and 50 ppm chloramphenicol) and a salted malt medium (malt medium + 6% NaCl). Colony forming units (CFU) were counted after 3 and 5 days of incubation at 25°C, and the results were expressed as number of CFU per gram of sample.

To further identify the fungal species, mycelia were planted out on potato dextrose agar (PDA) and grown at 25°C. The identification was done by macro- and microscopic examination of the isolates according to Raper and Fennel for *Aspergillus* [26] and according to Nelson et al. for *Fusarium* [27].

2.6. Near-Infrared-Spectroscopy Procedure. A near-infrared spectrometer (NIR Systems 6500, FOSS Tecator, Sweden) was used in reflectance mode to collect reflectance spectra of whole-maize samples over the spectral range 400–2498 nm. Each spectrum was obtained by averaging 32 scans, and the spectral resolution was 2 nm.

The spectra were collected at room temperature. Measurements were repeated three times and averaged for each sample. The spectra were used for multivariate analysis. The 117 samples were divided randomly into two sets: one set was for calibration to develop the model (100 samples) and one set (17 samples) was to test the model.

2.7. Statistical Analysis

Step 1.

(i) Pearson's Correlation. Pearson-correlation coefficients were used to find any significant relationships between the quantitative variables. The level of dissimilarities was fixed at ±0.5. Statistical analyses were done with XLSTAT version 2014.5.02 (Addinsoft, New York, USA).

(ii) Decision Tree. A chi-squared automatic interaction detector (CHAID) decision tree was used to construct a statistical model from a given training dataset. In the CHAID technique, the first step is to create categories from the ergosterol content and fungal count. Next, a chi-squared test is used to determine the best split with which we predict whether, considering ergosterol content and fungal count, the fumonisin content is greater than or less than the EU limit. In the present work, we use the fumonisin content for human consumption (i.e., 4000 μg/kg) [8].

The model was evaluated by using confusion matrices [28]. Table 1 shows the confusion matrix for a two-class classifier.

The prediction accuracy and classification error can be calculated from the number of samples belonging to

Table 1: Two-way confusion matrix.

	Predicted by model	
	Negative (fumonisin content <4000 μg/kg)	Positive (fumonisin content >4000 μg/kg)
Actual content		
Negative (fumonisin content <4000 μg/kg)	a	b
Positive (fumonisin content >4000 μg/kg)	c	d

a: number of correct negative predictions (maize samples with a mycotoxin content below the limit and properly classified); b: number of incorrect positive predictions (maize samples with a mycotoxin content below the limit and improperly classified); c: number of incorrect negative predictions (maize samples with a mycotoxin content above the limit and improperly classified), d: number of correct positive predictions (maize samples with a mycotoxin content above the limit and properly classified).

the different categories indicated in Table 1 [20]. The prediction accuracy is obtained as follows:

$$\text{Accuracy} = \frac{(a+d)}{(a+b+c+d)}. \quad (1)$$

The classification error is obtained from the confusion matrix as follows:

$$\text{Error} = \frac{(b+c)}{(a+b+c+d)}. \quad (2)$$

The best model is defined as the model with the highest accuracy and the lowest error. Samples in groups a and d are properly classified, whereas samples in groups b and c are not. Group b contains maize incorrectly predicted as positive. These samples have mycotoxin content below the limit but are improperly classified as being above the limit. Samples in group c have mycotoxin content above the limit and are improperly classified as safe.

Step 2.

(i) Multiplicative Scatter Correction. The spectra were preprocessed to remove the effects of light scattering and to compensate for baseline offsets and bias [29]. Treating a spectrum by multiplicative scatter correction (MSC) involves the following calculations:

$$x_{\text{org}} = b_0 + b_{\text{ref},1} x_{\text{ref}} + e,$$
$$x_{\text{corr}} = \frac{x_{\text{org}} - b_0}{b_{\text{ref},1}}, \quad (3)$$

where b_i are the correction coefficients, e is the unmodeled part, x_{org} is the original spectrum, x_{ref} is the reference spectrum, and x_{corr} is the corrected spectrum.

Preprocessing the MSC was done by using the Unscrambler Multivariate Data Analysis (v. X; CAMO A/S, Oslo, Norway).

(ii) Principal Component Analysis. After preprocessing, a principal component analysis (PCA) was done to highlight sample clustering and to compress the spectral data. A PCA is an orthogonal transformation that converts the set of 1050 possibly correlated infrared variables (400–2498 nm, every 2 nm) into a set of linearly uncorrelated variables called principal components (PCs). PCA is defined so that the first PC accounts for the maximum possible variability in the infrared spectra, with the subsequent principle components accounting for less and less variability [30].

PCA was done by using the Unscrambler Multivariate Data Analysis (v. X; CAMO A/S, Oslo, Norway).

(iii) Quadratic Discriminant Analysis. The quadratic discriminant analysis is a nonlinear model that constructs a nonlinear boundary between principal components (PCs) and fumonisin class. For the decision tree, the model was evaluated by using confusion matrices [20].

Table 2: Fungal contamination of 117 maize samples.

Fungal species	Mycoflora content (log CFU/g)			
	Min.	Max.	Mean	Occurrence (%)
Fusarium (total)	ND*	6.3	5.37	97
F. verticillioides	ND	6.3	5.32	90
F. proliferatum	ND	5	3.69	38
F. subglutinans	ND	5.6	3.23	59
F. oxysporum	ND	2.95	0.95	2
F. poae	ND	2.6	0.48	1
F. equisiti	ND	3	1.15	3
F. graminearum	ND	2	0.48	3
Aspergillus	ND	4.33	2.61	56
Mucor	ND	3.78	2.66	56
Penicillium	ND	4.78	3.54	71
Yeast	ND	4.3	3.08	50
Acremonium	ND	5.48	4.25	69
Cladosporium	ND	4.3	3.06	90

*ND means "not detected".

3. Results and Discussion

3.1. Fungal and Fumonisin Contamination of Maize Samples. One hundred and seventeen maize samples were characterized in terms of their fungal and fumonisin contamination. The fungal content was simultaneously determined by counting CFUs, identifying fungal species and quantifying ergosterol. The results are presented in Tables 2 and 3.

The maize samples were mainly contaminated with fungal species belonging to the *Fusarium* genus, which were found in 97% of the maize samples. *F. verticillioides* was the most important species in terms of both occurrence and

Table 3: Ergosterol and fumonisins content of 117 maize samples.

	Min.	Max.	Mean	Standard deviation	Samples above limit*
Ergosterol content (mg/kg)	2.1	7.5	4.6	1.1	85%
Fumonisins content (μg/kg)	60	9850	2509	2418	26%

*ICMFS or European regulation.

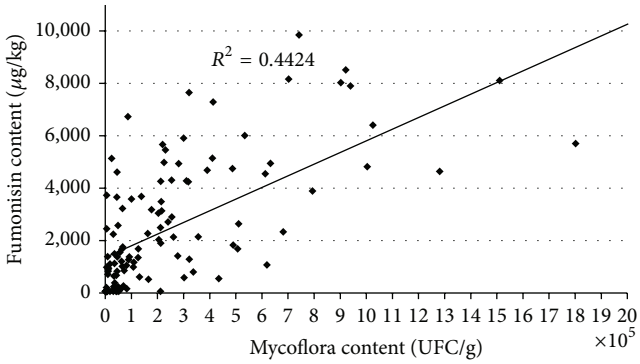

Figure 2: Relationship between fungal count and fumonisins contamination based on 117 samples of maize.

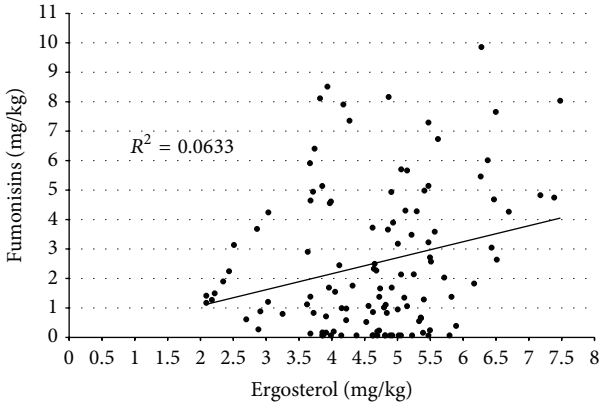

Figure 3: Relationship between ergosterol and fumonisins contamination based on 117 samples of maize.

the number of CFUs, in agreement with reports for European maize [31]. The average fungal count was far higher than the maximum tolerance limits set by the International Commission on Microbiological Specification for Foods (ICMFS), which is 10 000 CFU/g [32, 33]. Only 15% of the samples had fungal counts lower than the ICMSF limit.

The mean ergosterol content of the samples was 4.6 mg/kg with extreme values ranging from 2.1 to 7.5 mg/kg, which are typical values for this type of cereal [34, 35].

Table 3 summarizes the fumonisin content. FB1 was detected in all samples at concentrations ranging from 60 to 9850 μg/kg. Although such contamination is commonly reported for European maize [35, 36], 26% of the samples exceeded European regulations for fumonisin content (4000 μg/kg).

Step 1 (correlation between fumonisin and fungal contamination). To reliably sort maize according to the indirect markers of fumonisin contamination, we investigated the correlation between fumonisin content and markers of fungal contamination (i.e., ergosterol content and fungal count). Figures 2 and 3 show the correlation obtained between fumonisin content and fungal count or ergosterol respectively.

The coefficient of determination between fumonisin content and mycoflora (ergosterol) content is 0.44 with $P > 0.01$ (0.06 with $P = 0.006$). In the two situations, no clear regressions were obtained between the two variables. Thus, the ergosterol content is not proportional to the mycotoxin content. A weak fungal biomass combined with a relatively high level of mycotoxins is possible, as is the opposite. Of several other studies that focused on this subject, some concluded that a correlation exists between these variables [11, 37–41], whereas others either made no conclusion or concluded that no relationship exists, as is the case for the present study [42–47]. This situation may be directly related to the nature of the fungal flora present in the samples. Specifically, the organization of the fungal membranes, the physiology of the species, and especially the sporulation (number and size of spores) may cause the ergosterol concentration to vary between fungal species, thereby leading to a nonlinear relationship between mycotoxin and ergosterol content. In the present study, the dominant species was *Fusarium verticillioides*, which is characterized by numerous small spores with weak ergosterol content. Such differences in ergosterol content between fungal species may explain the absence of a linear relationship between mycotoxin and ergosterol content. Nevertheless, because mycotoxin synthesis is directly related to fungal development, it is probable that a threshold for ergosterol content could be established whereby if the ergosterol content was below the threshold, the mycotoxin concentration would not exceed the regulatory limit.

Decision Tree Based on Fungal Count and Ergosterol Content to Classify Fumonisin Content of Maize as Either above or below EU Limit of 4000 μg/kg. We used the CHAID algorithm to create a classification tree to separate maize samples into two categories: one with a fumonisin content greater than 4000 μg/kg and one with a fumonisin content less than this limit. We used ergosterol content and fungal count as explanatory variables and fumonisin content as a binary dependent variable (above or below the EU limit of 4000 μg/kg).

After calculations, we used the software XLSTAT to check the results of the stages in which the decision tree

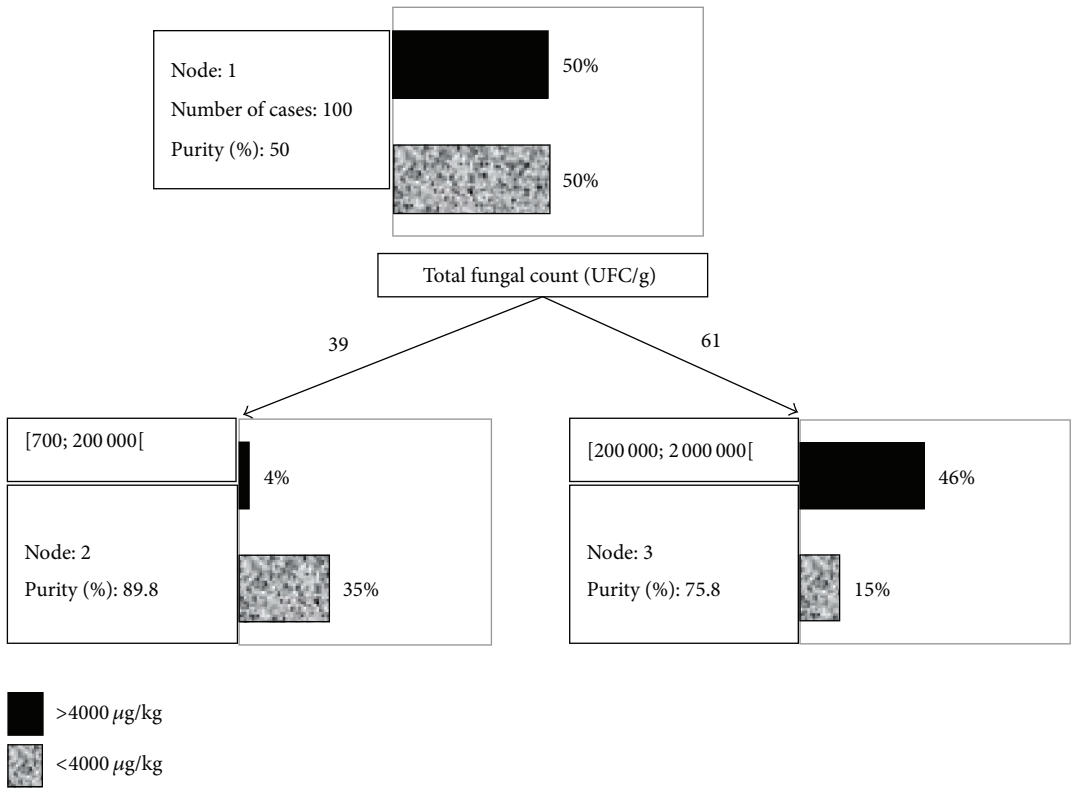

Figure 4: Binary decision tree obtained to predict fumonisin content (greater than or less than 4000 μg/kg) from ergosterol content and fungal count.

Table 4: Confusion matrix for fumonisin contents with fungal count as sorting parameter.

From	To			
	Fumonisin content <4000 μg/kg	Fumonisin content >4000 μg/kg	Total	Well-classified samples
	Calibration			
Fumonisin content <4000 μg/kg	35	15	50	71%
Fumonisin content >4000 μg/kg	4	46	50	92%
Total	**39**	**61**	**100**	**81%**
	Independent verification			
Fumonisin content <4000 μg/kg	10	1	11	91%
Fumonisin content >4000 μg/kg	1	5	6	83%
Total	**11**	**6**	**17**	**88%**

is automatically created and select the most stable tree (i.e., the tree that gives the best purity and called accuracy). The best split is the one that best separates the data into the two groups (i.e., greater than and less than 4000 μg/kg), where a single class predominates in each group. The measure used to evaluate a potential split is called "purity." Thus, the best split is the one that results in the greatest increase in purity of the subsets. In addition, we automatically corrected the class weights.

In Figure 4, intermediate node-division conditions are given next to each node. This tree contains one decisive node (node 1) and two final nodes, called leaves (nodes 2 and 3). For each node, a histogram gives an overview of the distribution of the decision variable (i.e., the number of maize samples with fumonisin content above or below the EU threshold of 4000 μg/kg). The purity is also indicated for each node, and the splitting attribute is the fungal count. Even if ergosterol was used as the explicative variable, it would not be sufficiently explicative compared with fungal count. The attribute used for splitting is 200 000 CFU/g. The resulting decision tree has a simple structure, and only a single question needs to be asked to classify a maize sample; namely, is its fungal count above or below 200 000 μg/kg? The results of applying this model are presented in Table 4.

Applying this detection method to 100 maize samples resulted in 71% of the samples with fumonisin content below

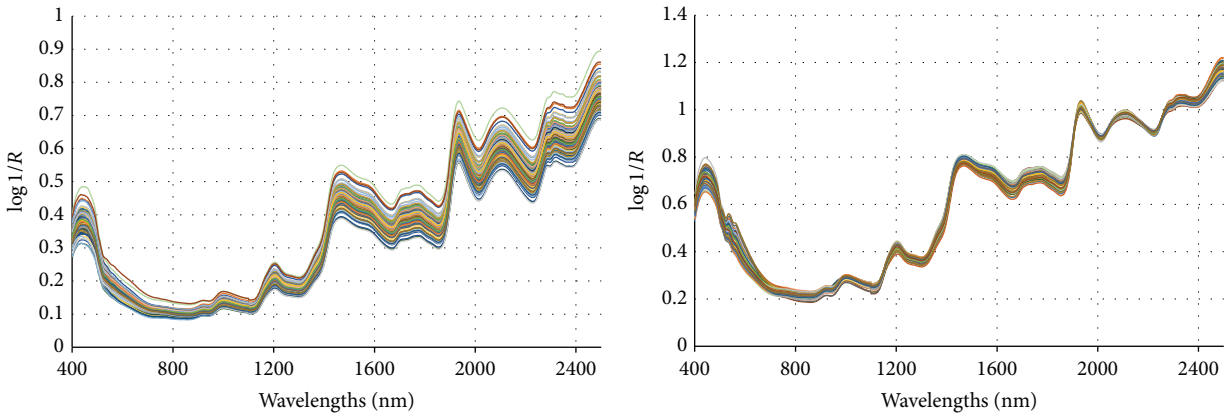

Figure 5: (a) Raw and (b) MSC infrared spectra of 117 maize samples.

the EU limit being properly recognized as such, with this figure climbing to 92% for samples with a fumonisin content above the EU limit. Independent verification by using the 17 control samples resulted in 91% and 83%, respectively.

The content threshold of 200 000 CFU/g seems to be a good benchmark with which we assess fumonisin content in maize. The last step of this work, which we present in the following section, involves using NIRS to rapidly predict the CFU content of maize samples.

Step 2 (using NIRS to screen samples for risk of fumonisin contamination).

(i) Infrared Spectra of 117 Maize Samples. Figure 5(a) shows the raw NIR spectra and Figure 5(b) shows the same spectra preprocessed by MSC.

Figure 5(a) shows that the infrared-reflectance peaks are broad and overlap each other. In Figure 5(b), small differences appear in the infrared preprocessed spectra in three ranges: 400–900, 1500–1800, and 2300–2500 nm. These differences are confirmed by the plot in Figure 6, which shows the mean spectra of samples with fumonisin content above and below the EU threshold of 4000 μg/kg.

Mycotoxins were present in the maize samples in quantities too small (on the order of parts per million) to allow direct detection [17]. Thus, the variations between the spectra shown in Figure 6 may be due to a complex ensemble of information related to the growth of fungus on the cereal, which is related notably with modifications of the protein or carbohydrate level (starch, cellulose, etc.).

(ii) Principal Component Analysis. The entire spectrum (400–2498 nm) was analyzed by PCA. The PCA model was built by using twenty PCs. Figure 7 shows a bidimensional representation of PC 1 and PC 2, which together account for 92% of the variance in the data. The first six PCs account for about 99% of the total variance in the spectra (65%, 27%, 3%, 1%, 1%, and 1%, resp.). All samples were labelled by their fumonisin status (i.e., above or below the EU regulatory threshold).

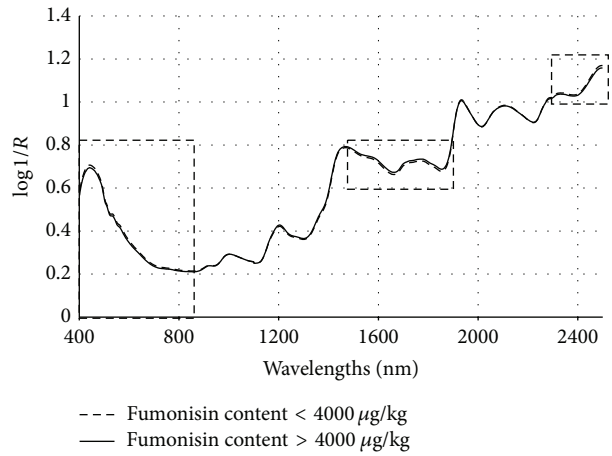

Figure 6: NIR reflectance spectra for maize sample with fumonisin content above (solid line) and below (dashed line) the EU threshold.

Figure 7 shows that the samples do not cluster within the two categories and no obvious outliers appear.

Figure 8 shows the loadings of the first two PCs.

A higher loading means that the corresponding wavelength carries greater weight for explaining the variance in the data. As shown in Figure 8, the wavelength ranges 400–600 and 1900–2500 nm are the most important for PC 1 because of higher loading in these ranges. For PC 2, the most important ranges are 400–600 and 1400–1900 nm.

The first six PCs are used as inputs for a discriminant analysis to discriminate between maize samples having fungal counts greater than and less than 200 000 CFU/g.

(iii) Discriminant Analysis. Starting from the six PCs of the NIR spectra, which together represent 99.9% of the variance, we used a quadratic discriminant analysis to determine if the fungal count of the sample was above or below the threshold of 200 000 CFU/g. Using NIRS to predict the fungal count category of the maize samples gave good results. Table 5

Table 5: Results of discriminant analysis based on NIRS to sort maize samples according to their risk of contamination with fumonisins.

From	To			
	Fumonisin content <4000 μg/kg	Fumonisin content >4000 μg/kg	Total	Well-classified samples
	Calibration			
Fumonisin content <4000 μg/kg	54	1	55	98%
Fumonisin content >4000 μg/kg	3	42	45	93%
Total	**57**	**43**	**100**	**96%**
	Independent verification			
Fumonisin content <4000 μg/kg	10	2	12	83%
Fumonisin content >4000 μg/kg	1	4	5	80%
Total	**11**	**6**	**17**	**82%**

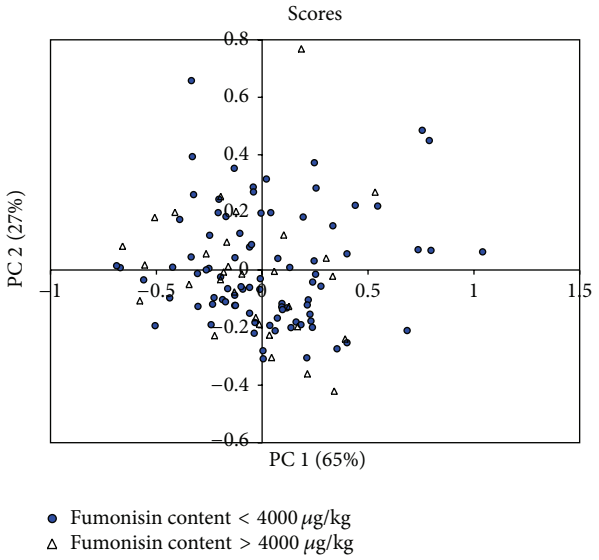

Figure 7: Scatter plot of first two principal components (PC 1 and PC 2) for all spectra. The variance explained by each principal component is indicated in parentheses.

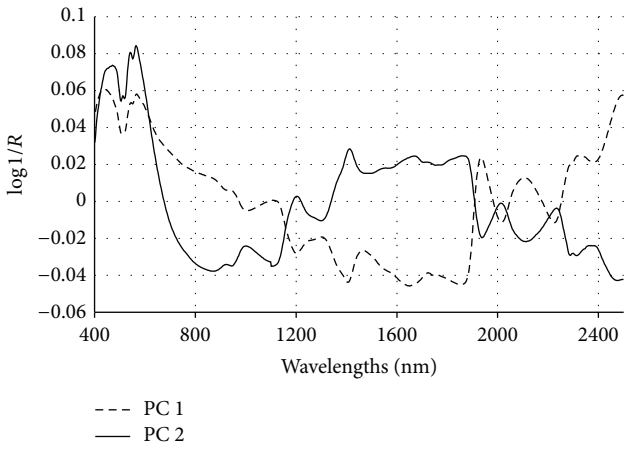

Figure 8: Loading-vector plot of first two principal components corresponding to the dataset constructed from the 117 maize samples.

summarizes the results obtained by using this method to sort maize samples according to their risk of fumonisin contamination.

Upon calibration, we found that 96% of the maize samples were properly classified, which compares favorably with the 82% properly classified by independent verification of the 17 control samples. In both cases, the number of false negatives was low: 3 out of 57 samples (5%) for the proposed calibration method and 1 out of 11 samples (9%) for the independent verification.

Models with which we classify maize contaminated by mycotoxins have been proposed by various authors. Levasseur-Garcia et al. [48] summarize the main results for classifying contaminated samples, with the targets being fungus, mycotoxin, or both. These works deal with the presence of *Aspergillus*, *Fusarium*, or *Penicillium* and the mycotoxins aflatoxin, deoxynivalenol, and fumonisins in samples of wheat, maize, or barley. Overall, the rate of proper classification is good to excellent, which underlines the interest in applying NIRS to the analysis of mycotoxin contamination in grains. However, most works in this area were done on grains that were individually scanned after artificial contamination, which can modify the final level of contamination and/or the interactions between grains, molds, and mycotoxins. The present study was done on relatively large samples (150 g) that were contaminated naturally and are therefore representative of real situations found in the field. In addition, the only study that details false negatives is that of Gordon [49], which reports a 4% rate of false negatives. We could probably decrease the rate of false negatives obtained in our work by increasing the number of samples used to construct our database.

Finally, these models may also allow industry to sort maize according to whether it is destined for human or animal consumption. Batches found to contain fumonisin content greater than the threshold established for human consumption (i.e., 4000 μg/kg) can be oriented toward animal consumption, which has a higher regulatory threshold. Such sorting would improve food safety. The use of a rapid, nondestructive method to inspect grain would also allow grain to be sorted before being stocked in silos.

4. Conclusions

In this work, we searched for correlations in maize samples between mycotoxin content and markers of fungal contamination. We used these correlations to establish indirect markers of mycotoxin contamination, thereby allowing us to predict fumonisin contamination and thus to sort the maize samples accordingly. This study demonstrates that thresholds exist for fungal count that can be used to screen samples according to the risk of fumonisin contamination. In this study, all maize samples that exceeded the EU regulatory limit for fumonisin content (4000 μg/kg) had a fungal count over 200 000 CFU/g. Therefore, we used this threshold in an analysis by near-infrared spectroscopy (NIRS) to screen samples for fumonisin contamination.

This study demonstrates the potential of NIRS as a rapid method for screening maize samples according to their risk of fumonisin contamination. The models developed herein led to proper classification of 96% of the maize samples, versus 82% proper classification obtained by independent verification. Moreover, the number of false negatives (i.e., contaminated samples classified as safe) was low. Therefore, NIRS is a promising alternative to the time-consuming analytical method that is currently used to determine mycotoxin content. With results available within 1 min, the proposed method may prove useful for rapid first screening of maize batches according to their risk of fumonisin contamination. The ultimate goal is to develop this technology into a tool for real-time screening of maize batches.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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