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Rapid Detection of Kernel Rots and Mycotoxins in Maize by Near-Infrared Reflectance Spectroscopy

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Near-infrared (NIR) spectroscopy is a practical spectroscopic procedure for the detection of organic compounds in matter. It is particularly useful because of its nondestructiveness, accuracy, rapid response, and easy operation. This work assesses the applicability of NIR for the rapid identification of micotoxigenic fungi and their toxic metabolites produced in naturally and artificially contaminated products. Two hundred and eighty maize samples were collected both from naturally contaminated maize crops grown in 16 areas in north-central Italy and from ears artificially inoculated with *Fusarium verticillioides*. All samples were analyzed for fungi infection, ergosterol, and fumonisin B_1 content. The results obtained indicated that NIR could accurately predict the incidence of kernels infected by fungi, and by *F. verticillioides* in particular, as well as the quantity of ergosterol and fumonisin B_1 in the meal. The statistics of the calibration and of the cross-validation for mold infection and for ergosterol and fumonisin B1 contents were significant. The best predictive ability for the percentage of global fungal infection and *F. verticillioides* was obtained using a calibration model utilizing maize kernels ($r^2 = 0.75$ and SECV = 7.43) and maize meals ($r^2 = 0.79$ and SECV = 10.95), respectively. This predictive performance was confirmed by the scatter plot of measured *F. verticillioides* infection versus NIR-predicted values in maize kernel samples ($r^2 = 0.80$). The NIR methodology can be applied for monitoring mold contamination in postharvest maize, in particular *F. verticilliodes* and fumonisin presence, to distinguish contaminated lots from clean ones, and to avoid cross-contamination with other material during storage and may become a powerful tool for monitoring the safety of the food supply.

KEYWORDS: NIR; ergosterol; fumonisin B_1; *Fusarium verticillioides*; *Zea mays*

INTRODUCTION

A chronic problem with maize grown in Europe is the infection of kernels by fungi. *Fusarium* species are among the most common mycota associated with maize plants, causing diseases of seedlings, roots, stalks, and ears. Ear infection can reduce yield and quality and results in mycotoxin accumulation in kernels (*1*) *Fusarium verticillioides* is the most frequent fungal species isolated from maize; together with *Fusarium proliferatum*, both belonging to the Liseola section, (*2*) it is able to produce fumonisins, the most important being fumonisin B_1 (FB1). Both the fungi and the toxin can be found in symptomless maize kernels (*3*). Fumonisins have been recognized as a potentially serious problem in maize-fed livestock. They are linked to several animal toxicoses and are also suspected carcinogens. Fumonisins in *in vitro* mammalian cell studies have been shown to inhibit sphingolipid biosynthesis. It is likely that inhibition of this pathway accounts for at least some aspects of their toxicity. The etiology of *Fusarium* ear rot is poorly understood, although physical damage to the ear and certain environmental conditions can contribute to its occurrence (*1*). *Fusarium* species can be isolated from most field-grown maize. The relationship between seedling infection and stalk and ear diseases caused by *Fusarium* species is not clear. Genetic resistance to visible kernel mold has been identified, but the relationship between visible mold and fumonisin production has still to be elucidated (*4*). A survey on fumonisin production by *Fusarium* species isolated from maize, sorghum, wheat, barley, and mixed feed in Europe showed that the highest production of fumonisins was obtained with strains isolated from maize (average = 1.3 mg/kg). Fumonisin levels detected in field-grown

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maize fluctuate widely, depending on location and growing season, but both preharvest and postharvest surveys indicate that the potential for high levels of fumonisins exists (*5*−*7*). High incidence and levels of fumonisin contamination resulted in Italy with levels up to 51.7 mg/kg (*6*). Data originated from surveys carried out in Europe clearly indicate a widespread occurrence of fumonisins in maize products and a potential for human and animal exposure to fumonisins throughout Europe.

Fungal contamination of foods and feeds has been estimated and measured by different methods (*8*). Ergosterol, the predominant sterol that plays an essential role as a component of cell membrane and other cell constituents of molds, absent in higher plants, has been proposed as a fungal contamination marker (*9*). Ergosterol being a primary and not a secondary metabolite, it is directly related to fungal growth. This molecule is easily detectable by high-performance liquid chromatography (HPLC) (*10*) or thin-layer chromatography (TLC) (*11*). It is a very stable molecule, and its transformation into ergocalciferol (vitamin D_2) is very slow or nil in grains and flours preserved with normal techniques. Correlations between ergosterol and different mycotoxins were investigated in winter rye (*12*), in bread-making cereals, and also in transgenic (Bt) and standard maize (*13*). The ergosterol "normal range" for some raw materials corresponds to a "current" or unaltered nutritive quality of products that therefore can be fed to animals without causing toxicity problems. However, it is well-known that fungal mass is not strictly related to mycotoxin content; therefore, a quantification of fumonisin is preferable when a toxicological evaluation of maize grain is requested. Fumonisin analysis is well managed using HPLC, which is an accurate but expensive technique and not suitable for a real-time response.

A nondestructive measurement system that could accurately monitor simultaneously these parameters in real time and with minimum effort and cost is very desirable for quality evaluation of agricultural commodities. In the past few decades, many researchers have focused on the potential use of near-infrared spectroscopy (NIR), a practical spectroscopic procedure for the detection of organic compounds in matters. In the field of agriculture, food, medicine, paper, polymer, etc., intense and aggressive interest has been direct toward NIR spectroscopy because of its nondestructiveness, accuracy, rapid response, and easy operation (*14*). This technique is widely used for milk and dairy products (*14*), to determine proteins, lipids, and moisture in meat products (*15*), and it has been also applied to the quantification of carotenoids in durum wheat and maize flour (*16*), and other minor components in some vegetables (*17*).

Current methods of fungal detection in foods and feeds are time-consuming and do not make use of recent technological developments in analytical methods (*18*). Some infrared spectroscopic techniques have been developed to identify samples infected by fungi. Photoacoustic infrared spectroscopy (IR-PAS) and diffusive reflectance spectroscopy (DRS) have been used for single-kernel analysis to detect fungal infections and toxic metabolites (*19*, *20*). However, the investigation of single kernels makes it difficult to draw conclusions for the sample as a whole. Furthermore, these techniques require sensitive instruments, which are not suitable for use in the field. Mid-infrared spectroscopy with attenuated total reflection (ATR) and the NIR spectroscopy are good methods for the investigation of naturally contaminated corn samples (*18*). NIR has been applied to the detection of scab-damaged kernels and the estimation of deoxynivalenol (DON) and ergosterol in wheat (*21*). A recent work by Peterson and Åberg (*22*), on scab-damaged wheat prepared by dilution series, has also indicated the possibility of measuring DON level by whole-grain NIR transmittance, whereas Delwiche and Hareland (*23*) tried to identify and characterize the more specific NIR wavelengths that could form the basis of a commercial sorting device or instrument.

The objective of this research was to assess the applicability of NIR for the rapid quantification of mycotoxigenic fungi and their metabolites ergosterol and FB1 produced in naturally and artificially contaminated maize kernels and flours by combining NIR spectroscopy with multivariate statistical methods.

MATERIALS AND METHODS

Maize Samples. Two hundred and eighty maize samples were analyzed. Samples were collected during the 3-year period from 2001 to 2003 from naturally contaminated maize crops; maize hybrids were of different maturity classes, grown in 16 areas in northern and central Italy and from artificially inoculated ears [F_3 inbreds from a cross between susceptible (Lo1153*opaque*2) and tolerant (Mp313E) parents]. Each sample consisted of 3 kg of seeds, dried at 40 °C and accurately mixed before subsampling to ensure homogeneity. Two subsamples of 0.5 kg were drawn from each sample, one for fungal analysis and the other, after grinding with a laboratory mill to pass a 1.0 mm sieve, for metabolite analysis.

The set of samples was split into two subsets. The larger set (220 samples) was used to calibrate the NIR analysis and to cross-validate the equation derived. The second set (60 samples) was used to test the goodness of fit of the developed equations. All samples were analyzed in duplicate.

Fungus Isolation from Kernels. Fifty kernels were randomly sampled from each maize kernel sample; they were surface-disinfected in a 1% sodium hypochlorite solution for 2 min and then in a 90% ethanol solution for 2 min and rinsed in sterile water. The samples were dried on paper, under a sterile hood, plated on potato PCNB agar, and incubated at 25 °C under an 8 h light photoperiod for 7 days. The colonies grown from each kernel were subcultured for identification on potato dextrose agar (PDA) according to the method of Burges et al. (*24*).

Ergosterol Analysis. Ergosterol analysis was performed essentially by using the Schwadorf and Müller (*10*) method. The whole analysis was performed in subdued light. Briefly, 150 mL of ethanol, 50 mL of methanol, and 10 g of potassium hydroxide were added to 25 g of sample. The mixture was refluxed for 30 min at 80 °C, cooled to 20 °C, and filtered through a folded filter paper; 20 mL of the filtrate was transferred to an Extrelut column (Merck), and after 15 min, ergosterol was slowly eluted with 90 mL of *n*-hexane in a round-bottom flask. The eluate was evaporated to dryness at 35 °C by rotary evaporation, quantitatively transferred to a 10 mL volumetric flask, and brought to volume with *n*-hexane/isoamyl alcohol (98:2, v/v). A 125 × 4 mm i.d., 4 μm, Superspher Si-60 column (Merck) was used at ambient temperature, with a mobile phase of *n*-hexane/isoamyl alcohol (98:2) at 1.0 mL/min. The UV detector was set at 280 nm. Ergosterol standards between 20 and 200 ng were injected; the retention time was ∼7.1 min.

Fumonisin B_1 Analysis. *HPLC Technique.* FB1 was determined according to the method of Shephard et al. (*25*). A 25-g sample was extracted with 100 mL of methanol/water (3:1, v/v) by shaking for 120 min. After filtration through folded filter paper, an aliquot of 10 mL of the filtrate was applied to a SAX Isolute strong cation exchange cartridge (IST, Hengoed, U.K.), previously conditioned by successive passage of 5 mL of methanol and 5 mL of methanol/water (75:25, v/v).

The cartridge was washed with 8 mL of methanol/water (75:25) and with 3 mL of methanol, and finally FB1 was eluted with 10 mL of 1% acetic acid in methanol. The eluate was evaporated to dryness under a stream of nitrogen and the residue redissolved in 1 mL of methanol. FB1 contained in a 50 mL aliquot of the extract as derivatized with 200 mL of o-phthaldialdehyde (OPA) solution. This solution was prepared by adding 5 mL of 0.1 M sodium tetraborate and 50 mL of 2-mercaptoethanol to 1 mL of methanol containing 40 mg of OPA. The OPA−mercaptoethanol derivative of FB1 was quantitated by reversed-phase HPLC with fluorescence detection (λ_{ex} = 335 nm, λ_{em} = 440 nm). The column was a 125 × 4 mm i.d., 5 μm, Select-B RP-8

with a mobile phase of acetonitrile-acidified (2% acetic acid) water (43:57). The flow rate was 1.2 mL/min.

ELISA Technique. Briefly, to obtain the sample solution, 5 g of ground sample was put into a suitable container and 25 mL of methanol/water (70:30, v/v) added; the mixture was then shaken vigorously for 3 min with a shaker. The extract was filtered through a Whatman no. 1 filter. The filtered sample extract was dilued 1:14 with distilled or deionized water. Fifty microliters of the diluted filtrate was used per well in the Ridascreen fumonisin **(**R-Biopharm, Darmstadt, Germany) test.

NIR Data and Analysis. Dispersive near-infrared reflectance (NIR−vis) data (including the visual region) were collected using a model 6500 spectrophotometer from Foss NIRSystems, Inc., Silver Spring, MD. The spectrophotometer uses a split detector system with a silicon (Si) detector between 400 and 1100 nm. The spectrophotometer used a tungsten halogen lamp and had an internal ceramic standard. The light fell on the sample at an angle of 90°, and the NIR−vis spectra were measured in reflectance mode at an angle of 45°. The NIR−vis reflection spectra were recorded using a transport sample cup with a quartz window. All spectroscopic data were recorded in duplicate as log R^{-1}, where R is the reflectance, in the wavelength range of 400−2500 nm every 2 nm, to give a total of 1050 data points per sample; these data were stored in a PC. Approximately 4 g of ground grain or 20 g of whole kernels was sampled for each entry. The software for scanning, mathematical processing, and statistical analysis was supplied with the spectrophotometer by Infrasoft International (ISI, Port Matilda, PA).

Data Processing. Equations for NIRS prediction were developed using the algorithms "Symmetry and Calibrate" (version 4.2, Infrasoft International, NIR Systems Inc.) with the modified partial least-squares regression (MPLS) option. The mathematical transformations (1, 4, 4), (1, 5, 5), (2, 6, 8), (2, 8, 6), and (3, 10, 10) (first, second, and third derivative, gap over which derivative was calculated, number of data points used in first smoothing, and no second smoothing) were used for prediction of regression equation models. The best equation for each parameter was selected using the lowest standard error of cross-validation (SECV); this was obtained by dividing the data into sets of four and predicting each fourth value from calibrations developed from the other three values. Samples with large residuals were omitted, and cross-validation was performed again. Validation of MPLS models includes testing the prediction ability of the model and determining the optimum number of principal components. All models were validated by full cross-validation, also known as leave-one-out validation (*26*). The root-mean-square error of cross-validation (RMSECV) was used as a measure of the model's ability to predict unknown samples; RMSECV contains information on both the precision and accuracy of the model, and it can be calculated by the formula

$$\mathrm{RMSECV} = \sqrt{\frac{\sum_{i=1}^{N}(y_{\mathrm{cv}} - y_{\mathrm{ref}})^2}{n}}$$

where n is the number of samples, y_{cv} is the value estimated by the cross-validated model, and y_{ref} is the reference value. RMSECV is computed in original units. Prior to calibration developments, multiplicative scatter correction (MSC) (*27*) was applied. MSC is a method used to remove additive and/or multiplicative effects in spectroscopic data to prevent them from dominating the information (signal) in the data. The MSC was made as a full MSC for both spectroscopic methods and included the correction for both offset (additive effects) and amplification (multiplicative effects).

RESULTS AND DISCUSSION

NIR spectroscopy measures overtones and combination bands of the fundamental molecular vibrations found in the infrared region. NIR spectroscopy is commonly used as a rapid noninvasive spectroscopic method in food and feed industry sectors (*28*). Previous studies made use of the combination of NIR spectroscopy and chemometrics in an attempt to determine the content of constituents such as humidity, proteins, and lipids in soft and durum wheat, barley, oats, maize, soy, soft and durum wheat flours, and oleaginous seeds are widely accepted in the trade of these products (*29*). Furthermore, the Canadian Grain Commission (*30*) promoted this kind of instrumental analysis in wheat grading and classification, as the data they produce possess a precision comparable with that of the respective official methods of analysis and can be obtained at much lower unit cost.

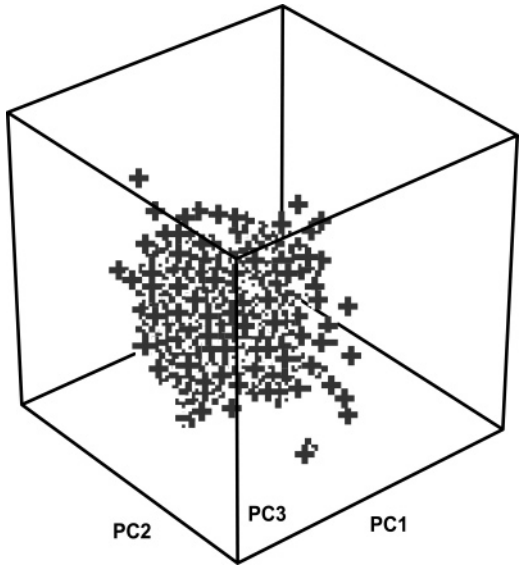


Figure 1. Symmetry distribution of kernel maize population using the main three selected principal component scores PC1, PC2, and PC3 representing 34.5, 23.6, and 14.0% of the variance of the model, respectively.

Table 1. Summary of Traits of the Calibration and Cross-Validation Set in Maize Grain and Flour, Showing Number of Samples (*N*), Range and Mean of Values, Standard Deviation (SD), Standard Error (SE), and Outliers

Trait	*N*	min	max	mean	SD	SE	outlier
			Grain				
Fungi infection (%)	220	28	100	86.0	22.3	1.50	0
F. verticillioides (%)	217	2	100	59.0	24.7	1.68	3
			Flour				
Ergosterol (mg/kg)	160	0.78	41.52	7.7	7.1	0.56	20
Fumonisin B_1 (mg/kg)	180	0.01	19.6	5.0	3.9	0.30	20
Fumonisin ELISA (mg/kg)	180	0.03	11.9	5.9	3.2	0.24	20

The mean values, standard deviations (SD), and standard errors for total infected kernel, *F. verticillioides* infected kernels, ergosterol, and FB1 content in the samples used as calibration set are shown in **Table 1**. Total kernel infection ranged from 28 to 100%, with a mean of 86% in naturally contaminated samples, whereas the mean incidence of *F. verticillioides* was 59%.

The mean ergosterol content in maize flour samples was 7.7 mg/kg, ranging from 0.78 to 41.52 mg/kg. The mean FB1 level detected by HPLC was 5.0 mg/kg, ranging from 0.01 to 19.6 mg/kg, whereas the FB1 detected by ELISA test (FE) ranged from 0.03 to 11.9 mg/kg with a mean value of 5.9 mg/kg.

The distribution symmetry of the population used in this study, based on three main selected principal components (PC_s) that describe the systematic information present in the data, is represented in **Figure 1**. The percentages of variance explained for PC1, PC2, and PC3 were 34.5, 23.6, and 14.0, respectively. The data are approximated by linear combinations of the orthogonal PC_s. PC_s decompose the original data matrix in a

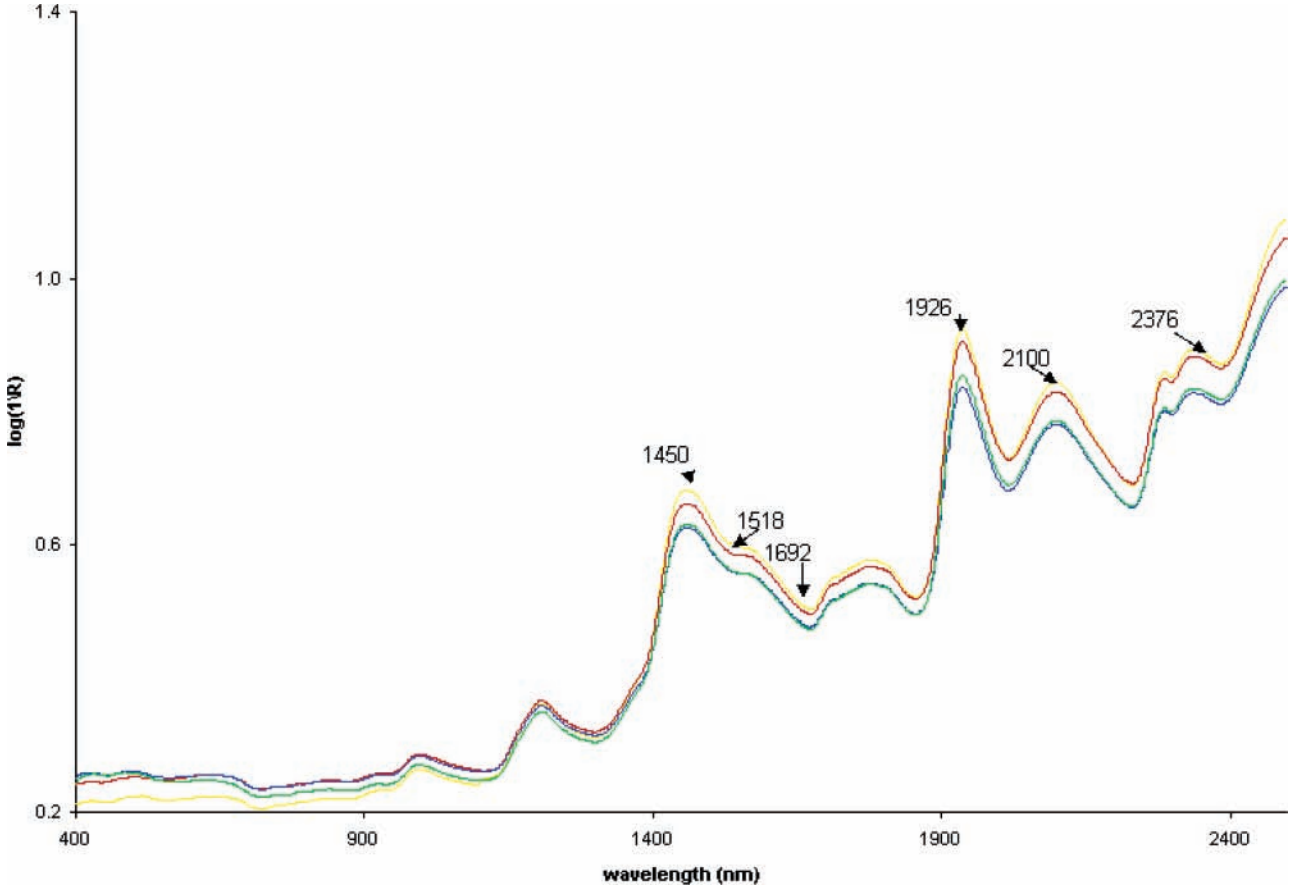


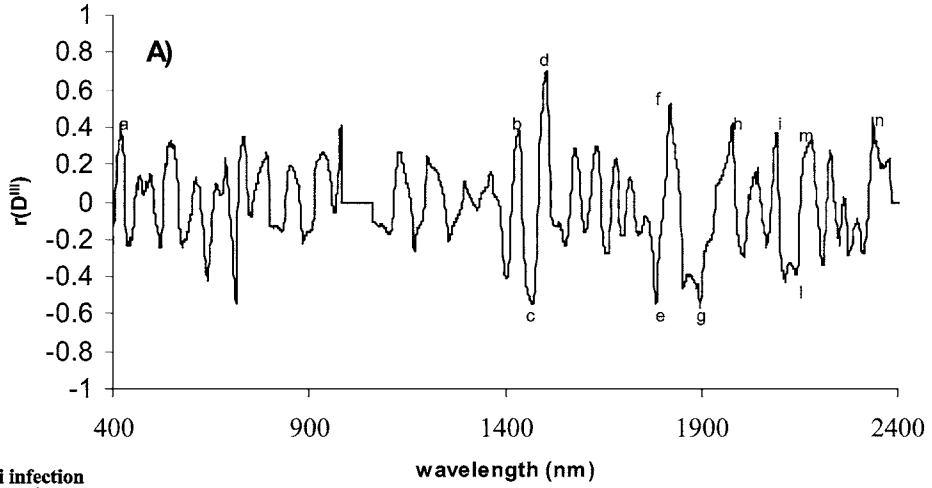
Figure 2. NIR–vis spectra for ergosterol (red), PDA (blue), and PDA–*F. verticillioides* (green), mixed at 25 mg kg^{-1} with dry G-25 Sephadex superfine (yellow).

score matrix and a loading matrix and collect the residual in another matrix. The loadings contain information about spectra, scores contain information about the samples, and the number of PC_s constitutes the traits rank of the matrix.

The raw and derivatized NIR spectrum of total fungi, *F. verticillioides* infection, and ergosterol content (**Figures 2** and **3**) were emphasized by the major absorption bands associated with the samples of fungi and ergosterol, mixed with an excipient. The interpretation of the complex NIR spectra of fungi is difficult because the diverse array of chemical compounds present can result in band overlapping, and a specific band in the spectra could be a composite of many bands containing information on more than one type of molecular vibration.

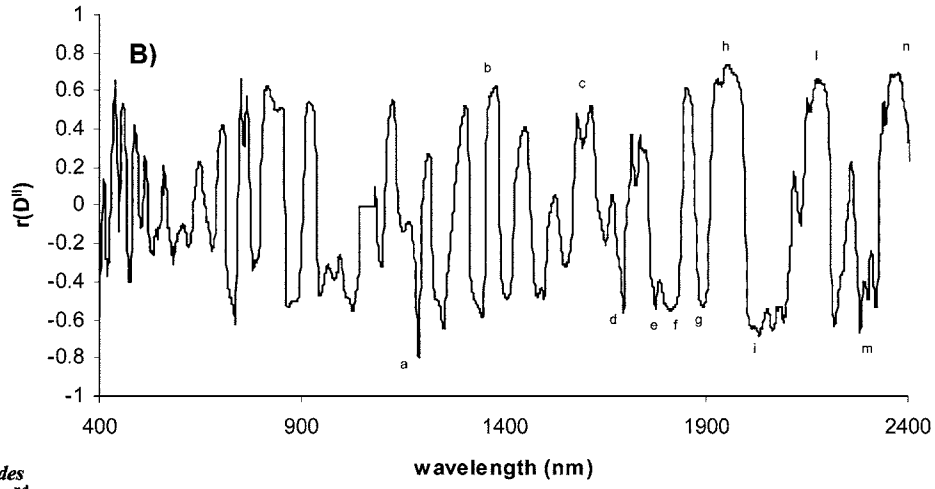
The NIR absorbances for various generic functional groups (*28*) may be correlated to the major fungal constituents. The NIR−vis spectra show several distinct peaks where a functional group absorbs in the NIR region (**Figure 2**). Many differences can be revealed in **Figure 3**, which reports the spectroscopic/chemical relationship of derivatized NIR−vis spectra of these products. NIR−vis bands that have been assigned to chemical functional groups, present in all fungi and macromolecules, can be used to interpret the specific absorption bands generated by the fungal cellular compounds such as chitin and β-glucan, the two main macromolecules that contribute to build the hyphal walls. The spectra of total fungi infection, *F. verticillioides* infection, and ergosterol, were measured and analyzed to demonstrate correlation to some of the bands observed in infected samples (**Figure 3**). The bands at 1430, 1470, 1820, 2140, and 2180 nm related to total fungal infection could be assigned to the first overtone of the OH stretching modes of glucose, NH in most amino acids, and CH combination bands in cis unsaturation. The higher correlation (0.69) for total fungal infection is at 1510 nm, corresponding to the third overtone of OH deformation hydroxyl group and CO stretching of primary amides, which could be ascribed to cutin and β-glucan. The main absorbed bands for *F. verticillioides* infection with higher correlation (−0.79 and 0.70) were observed at 1190 nm and from 1954 to 2378 nm, corresponding to the second overtone of CH stretching mode of carbonyl compounds, the third overtone of NH stretching mode of trans-secondary amides, the first overtone of OH stretching and deformation−combination modes of hydroxyl group, the second overtone of NH bending mode of primary amides, and CO symmetrical vibrations mode; all of these groups could be ascribed to cutin and β-glucan, respectively. The bands with higher relationship (−0.77 and 0.70) of pure ergosterol were observed at 1692 and 1910 nm corresponding, respectively, to the first overtone attributed to CH stretching mode corresponding at the CH_2 group and the third overtone rings deformation mode attributed to $-CH{=}CH_3$ groups of benzene and the first overtone of O−H stretching/O−H deformation−combination modes corresponding to hydroxyl−CH=CH−, $-CH{=}CH_2$, and the third overtone deformation mode of CH.

The statistics of the calibrations and of the cross-validations for the mold infection and metabolites produced in maize grain kernels and flours were as follows. In mold infection, the standard error of calibration (SEC) and coefficients of determination (R^2) ranged from 4.60 to 8.50 and from 0.74 to 0.90, respectively. Performance on cross-validation of total fungal infection and *F. verticillioides*, expressed as the squared coefficient of correlation (r^2) and SECV, had values that ranged from 0.60 to 0.79 and from 9.40 to 10.53, respectively. The best predictive ability for the percentage of total fungal infection and *F. verticillioides* infection was obtained using a calibration



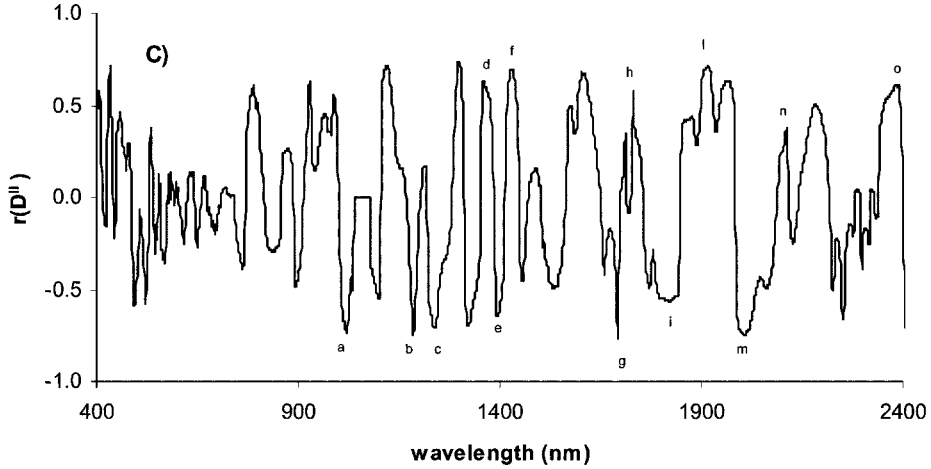
A) Total fungi infection

Wavelength	r(3rd derivative)	chemical bond
a	1430	0.39	O-H stretching 1st overtone sucrose, starch.
b	1470	-0.55	N-H stretching 1st overtone CONHR; C-N stretching 3rd overtone amides O-H stretching 1st overtone internal OH bonds glucose.
c	1510	0.69	O-H deformation 3rd overtone hydroxyl; C = O stretching 3rd overtone primary amines.
d	1784	-0.55	C-N stretching 3rd overtone amides; C-H stretching 3rd overtone aliphatic compounds.
e	1820	0.52	N-H stretching 1st overtone in most aminoacids; C = O vibrations 2nd overtone, open-chain acid anhydrides.
f	1900	-0.54	O-H deformation 3rd overtone primary alcohols.
g	1980	0.42	N-H stretching, 3rd overtone secondary amides; C-N stretching 2nd overtone unsaturated nitrogen compounds; O-H deformation 3rd overtone secondary alcohols.
h	2090	0.37	O-H deformation 2nd overtone, hydroxyl.
i	2140	0.39	C-N stretching 3rd overtone secondary amides, secondary carbon atoms; C-O, O-H stretching 1st overtone combination, primary alcohols; C-H stretching 1st overtone *cis* unsaturation, combination.
l	2180	0.33	N-H bending , 2nd overtone *trans* secondary amides, proteins.
m	2340	0.45	C-N stretching 2nd overtone amides.



B) *F.verticillioides*

Wavelength	r(2nd derivative)	chemical bond
a	1190	-0.79	C-H stretching 2nd overtone, carbonyl compounds.
b	1366	0.62	O-H stretching 1st overtone, secondary alcohols; C-H stretching 2nd overtone, CH_2 and CH_3 groups.
c	1620	0.53	N-H stretching 1st overtone hydrogen bonding, peptide links,protein helices, secondary amides;N-H bending, 3rd overtone, *trans*- secondary amides.
d	1696	-0.56	C-H stretching 3rd overtone, CH_3 groups.
e	1776	-0.55	N-H bending 3rd overtone, primary amides; C-N stretching 3rd overtone amides with no N substitution. C-H in phase deformation 3rd overtone, CHO groups.
f	1816	-0.55	O-H deformation 3rd overtone, secondary alcohols.
g	1896	-0.53	O-H deformation 3rd overtone, primary alcohols.
h	1954	0.74	N-H , stretching 3rd overtone, *trans*- secondary amides; O-H stretching 1st overtone O-H defomation combination hydroxyl.
i	2032	-0.68	C=O stretching 2nd overtone primary amide.
l	2178	0.67	N-H , bending 2nd overtone, *trans*- secondary amides; C-H stretching 1st overtone *cis* unsaturation, combination.
m	2282	-0.67	N-H , bending 2nd overtone, *cis*- secondary amides; C = O stretching 3rd overtone primary alcohols; $-CH_2-$; 2nd overtone C-H asymmetrical deformation.
n	2378	0.70	N-H bending 2nd overtone, primary amides; C-O symmetrical vibrations 2nd overtone.



C) Ergosterol

Wavelength	r(2nd derivative)	chemical bond
a	1018	-072	-H stretching 2nd overtone, internal OH bonds, single bridge.
b	1190	-075	C-H stretching 2nd overtone, CH_3 groups.
c	1240	-0.71	" " "
d	1360	0.63	C-H combination band CH_3 groups, OH stretching 1st overtone alcohol.
e	1384	-0.64	C-H combination band CH_2 groups, OH stretching 1st overtone.
f	1436	0.70	C-H combination band CH_2 groups.
g	1692	-0.77	C-H stretching 1st overtone, CH_2 groups –CH= CH_3 groups; benzene rings deformation 3rd overtone.
h	1730	0.57	C-H stretching 1st overtone, CH_2 groups –CH=; -CH_2 ; CH asymmetrical deformation 3rd overtone.
i	1818	-0.56	O-H deformation 3rd overtone alcohols.
l	1910	0.70	O-H stretching / O-H defomation 1st overtone combination; hydroxyl -CH=CH-: -CH=CH_2 ; CH deformation 3rd overtone.
m	1996	-0.74	O-H deformation 3rd overtone alcohols.
n	2106	0.37	C-H stretching 1st overtone, sketal in-plane deformation, combination.
o	2382	0.62	C-H stretching 2nd overtone aliphatic compounds.

Figure 3. Spectral/chemical relationship of (**A**) total fungal infection, (**B**) *F. verticillioides,* and (**C**) ergosterol, in standards samples (*X*-axes = wavelength; *Y*-axes = *r*). Correlations are based on refs *14*, *19*, and *28*.

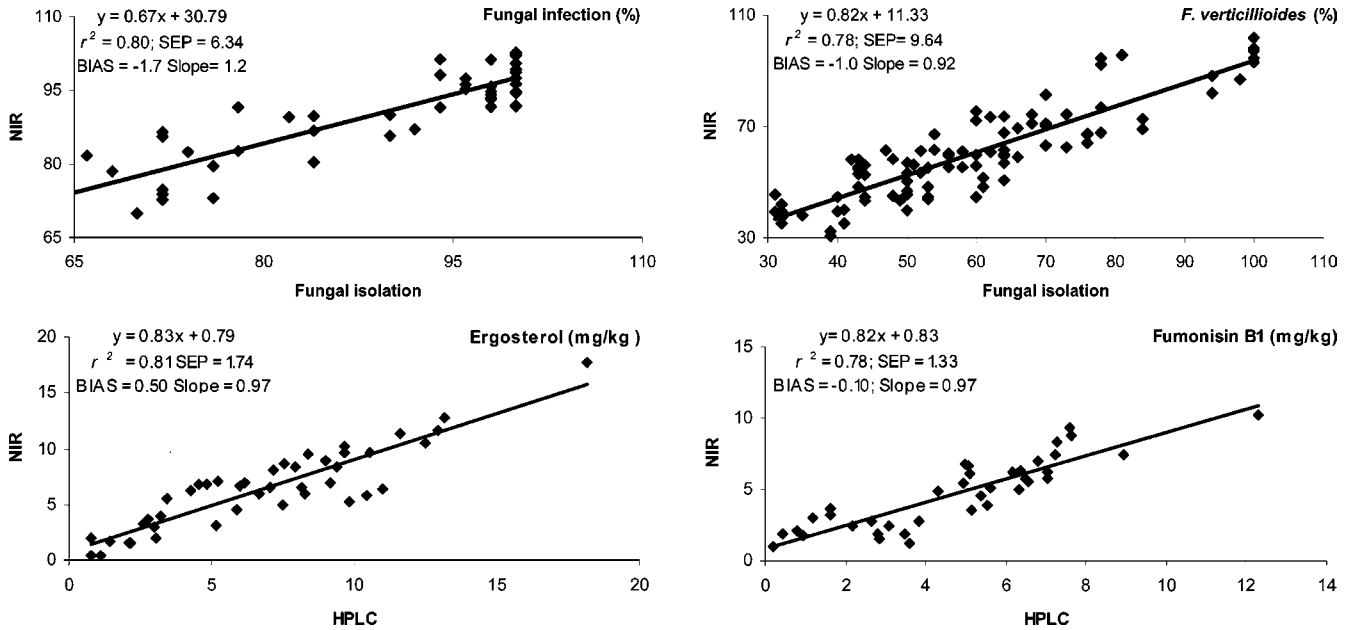


Figure 4. Scatter plot of fungi and *F. verticillioides* infection (percent) versus NIR in maize kernel samples and HPLC versus NIR content in ergosterol and fumonisin B_1 in maize flour samples. Coefficient of simple correlation (r^2); SEP, standard error of prediction; bias, average difference between NIR values and corresponding laboratory values.

model utilizing meals ($r^2 = 0.66$ and SECV = 5.92) and kernels ($r^2 = 0.79$ and SECV = 9.40), respectively. This predictive performance was confirmed by scatter plot (**Figure 4**) of measured total fungi and *F. verticillioides* infection versus NIR-predicted values of independent set of maize kernel samples ($r^2 = 0.80$ and 0.78; SEP = 6.34 and 9.64, respectively).

For the metabolites detected, SEC values ranged from 1.31 for ergosterol to 1.49 for total FE; R^2 varied from 0.72 for FE to 0.88 for ergosterol. In the cross-validation, the r^2 values ranged from 0.56 for FE to 0.84 for ergosterol, and SEC(V) values ranged from 1.41 for FB1 to 1.84 for FE. The best predictive ability of the model, developed for ergosterol content

in maize meal, was confirmed by scatter plot of measured ergosterol versus NIR-predicted values of the independent set of maize meal samples; r^2 and SEP were 0.81 and 1.74, respectively, with magnitude similar to that of the calibration set, and bias of 0.50 (**Figure 4**).

From the results reported here, it appears that NIR could accurately predict the percentage of total fungi and *F. verticillioides* infection in maize kernels and the content of ergosterol and FB1 in meals. Currently available methods to quantify toxin levels (i.e., HPLC) involve high costs and are time-consuming. These methods are unsuitable for use at harvesting on the threshing-floor (farmyard) or terminal grain (silos, storage bin). The NIR methodology can be applied for monitoring mold contamination in postharvest maize to distinguish contaminated from clean grain lots and may become a powerful tool for monitoring the safety of our food supply. A further development of this work will be to improve the calibration equations, using a wider database of samples analyzed with traditional methods, to make the model more stable and to validate it with another separate set of maize samples infected by fungi. The limits of detection of this method need to be determined, even if the detection limit usually recognized is ~0.1% of the considered analyte.

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