Rapid detection of aflatoxin B_1 in paddy rice as analytical quality assessment by near infrared spectroscopy

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Abstract: A rapid identification method for aflatoxin B_1 in paddy rice samples was developed by using near infrared spectroscopy under a wavelength range of 1 000-2 500 nm. Eighty paddy rice samples were collected from both natural and artificial infection with aflatoxin B_1 to build the calibration models based on the partial least square regression method. The best predictive model to detect aflatoxin B_1 in paddy rice was obtained using standard normal variate detrending spectra, with a correlation of 0.850, and a standard error of prediction of 3.211%. Therefore, the result showed that near infrared spectroscopy could be a useful instrumental method for determining aflatoxin B_1 in paddy rice. The near infrared spectroscopy methodology can be applied to the monitoring of aflatoxin fungal contamination in postharvest paddy rice during storage and may become a powerful tool for the safety of grain and grain products.

Keywords: near infrared spectroscopy, rapid detection, quality assessment, aflatoxin B_1, paddy rice, partial least square regression

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1 Introduction

Mycotoxins are toxic fungal metabolites that can have harmful side effects when consumed by humans or animals[1-3]. The most commonly known mycotoxins are the aflatoxins. These mycotoxins are naturally produced by *Aspergillus flavus* and *Aspergillus parasiticus*[4]. Aflatoxins have been recognized as group I carcinogens by the International Agency for Research on Cancer[5,6]. Aflatoxin B_1 (AFB_1) is the most potent naturally occurring mycotoxin with potential damaging effects on health of humans and animals. The European Commission (EU) has set maximum residue levels of AFB_1 in corn, rice and spices at 5 μg/kg[7].

Due to factors affecting storage temperature and moisture, paddy rice is susceptible to contamination by moulds during storage. Certain moulds can produce mycotoxins, including the very harmful AFB_1, which is common in paddy rice. Therefore, it is necessary to establish a rapid, simple and effective method for detection of AFB_1 in paddy rice.

A variety of well-established methodologies for analyzing aflatoxins in cereal grains have been tested by American Association of Cereal Chemists (AACC) and the United States Department of Agriculture (USDA)[8,9]. These existing methods include thin layer chromatography, high performance liquid chromatography and over-pressured layer chromatography. Other methods include the immunosorbent assay (ELISA), immunochromatographic strips, and immuno-affinity columns[10-15]. These chemical-based laboratory methods have the advantage of being precise and accurate.

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However, they are time-consuming, laborious, expensive, and require a well-equipped laboratory and skilled laboratory personnel to perform and interpret these tests. The lengthy process precludes rapid and real-time detection of aflatoxins in grains. Near-infrared spectroscopy (NIRS) is an excellent candidate for measurements of the chemical components in very complex materials including cereals, fruits and vegetables[16]. It has been used to measure the content of water, oil, fibre, starch, and protein in cereals and grains[17]. More recently, NIRS has also been successfully applied to predict fumonisin B_1 in maize and deoxynivalenol in wheat[18,19]. Hernandez-Hierro et al.[20] noted that NIRS was a powerful technique for measuring AFB_1 and total aflatoxins in red paprika. The results of spectroscopic models developed have demonstrated that NIRS technology is an excellent alternative for fast AFB_1 detection in maize and barley[21]. Tripathi and Mishra[22] observed that Fourier transform near-infrared spectroscopy could be used for rapid, non-destructive quantification of AFB_1 in red chili powder. Sirisomboon et al.[23] found that NIRS could accurately detect the incidence of rice infected by aflatoxigenic fungi.

However, very few studies have applied NIRS technology to the detection and quantification of AFB_1 contamination in paddy rice. Therefore, the objectives of the present research were to assess the feasibility of using NIRS to detect AFB_1 concentration in naturally and artificially contaminated paddy rice samples and to explore the potential of the method for the prediction of contamination in unknown paddy rice samples.

2 Materials and methods

2.1 Naturally infected paddy rice samples

In this study, approximately 3 kg paddy rice samples were collected at the experiment station of the Northeast Agricultural University, Harbin, China.

2.2 Artificially induced infection in paddy rice samples

Storage experimentations were carried out to obtain AFB_1 contaminated samples. Paddy rice samples were classified as positive when the AFB_1 content was higher than 20 μg/kg and negative if the AFB_1 content was below 20 μg/kg. Artificially contaminated paddy rice samples containing different moisture levels (10%–22%) (w.b.) were obtained by adding water to induce the growth of AFB_1 for three months in a room at a controlled temperature of (30±2)℃ [24-26]. Moisture content of paddy rice samples was measured based on the AOAC (1995b) official method (oven dry method, 135℃, 3 h). All tests were performed in triplicate. Data were expressed as mean of triplicate determinations.

2.3 Near-infrared scanning

Eighty samples (30 naturally and 50 artificially contaminated) were selected. The set of samples was divided into two subsets. The larger set (60 samples) was used to calibrate the NIRS analysis and to cross-validate the derived equation. The smaller set (20 samples) was used to test the goodness of fit of the calibration model. All paddy rice samples were measured in duplicate. The paddy rice samples were first used for Near-infrared (NIR) analysis. Then these samples were ground with a laboratory mill, passed through a 1.0 mm sieve and used in the reference method. Each scan consisted of a sample of 45 g being placed on a platform and the samples were then scanned using a Fourier transform near infrared spectrometer (ANTARIS Ⅱ type, Thermo Nicolet, Madison, USA) in diffuse reflection mode. The NIR was in a range of wave numbers of 10 000-4 000 cm^{-1} (1 000-2 500 nm). Spectra of each sample were automatically recorded as Absorbance (A) corresponding to log(1/R). The scanning time of each sample was approximately 90 s. Each sample was scanned three times and the average spectrum of the sample was employed for data analysis.

2.4 Reference method

The detection of AFB_1 was based on a competition-type ELISA. Briefly, to obtain the sample solution, 20 g of ground paddy rice sample was added to 100 mL of methanol/water (1:1, V/V). The mixture was then shaken vigorously for 3 min with a shaker. The supernatant was obtained by centrifugation for 10 min at 5 000 r/min. The final 400 μL supernatant was mixed with 600 μL sample diluent. Fifty microliters of the diluted sample mixture were used in the ELISA test kit

assay. The detection of the test kit assay was based on the method of Zheng et al.[13]. The 50 μL sample extract or AFB_1 standard substance were each mixed with 50 μL of enzyme standard substance in individual wells. The plate was incubated at room temperature for 15 min, then washed 5 times with a washing concentrate, followed by addition of 100 μL enzyme substrate to each well and incubating for an additional 15 min. Then the 50 μL stop solution was added to individual wells and the intensity of the resulting yellow color was measured at a wavelength of 450 nm. The ELISA readers used in the study were EL301 microwell readers (Bio-Tek Instruments Inc., Vermont, USA). This was a quantitative test able to detect AFB_1 levels between 0 and 81.00 μg/kg.

2.5 NIRS data analysis

All spectral analyses were conducted using the Unscrambler 10.3 (Camo Technologies, Woodbridge, NJ, USA) and SPSS software (Ver. 8.2, SPSS Institute, INC., Cary, NC). The spectral data were analyzed using partial least square regression (PLSR) with various preprocessing techniques. Then, the reference data were combined with NIR reflection spectra. The total spectra data were divided into calibration and prediction sets in a 3:1 ratio. The calibration sets with and without spectral pretreatment were used to develop the PLSR models. In this study, a chemometric model for the AFB_1 in paddy rice was generated.

Six spectral pretreatments were applied using the following methods: smoothing, normalizing, baseline offset, standard normal variate (SNV), SNV + detrending (SNVD), and multiplicative scatter correction (MSC).

The best discriminant model for paddy rice was selected by using the highest coefficient of determination (R^2), lowest standard error of calibration (*SEC*), and lowest standard error of prediction (*SEP*). Both, the ratio of the standard deviation of reference values in the validation set to the standard error of prediction (*RPD*), and the ratio of the range to the *SEP* (*RER*) were employed to evaluate the performance of each model.

3 Results and discussion

3.1 Characteristics of reference data sets

The reference data obtained from natural infection and artificial infection are presented in Table 1. The paddy rice samples (n=80) were distributed in the AFB_1 content range of 6.90-54.82 μg/kg with a mean value of 22.33 μg/kg and a standard deviation of 11.72 μg/kg. Table 1 showed the statistical values of AFB_1 (μg/kg) within the calibration and prediction sets. The distribution of the AFB_1 content in samples had a mean of 22.58 μg/kg for the calibration set and 21.57 μg/kg for the prediction set. As shown in Figure 1, the indices of skewness and kurtosis and the normal distribution line reported in the histogram, permitted comparison of AFB_1 content with a normal distributed variable. Furthermore, few samples with extremely high AFB_1 values were observed, due to the low moisture content of paddy rice sampled during natural infection and artificial infection with moisture level below 13%. The uneven distribution of the data was helpful in improving the prediction performance of the model.

Table 1 Aflatoxin B_1 in samples of paddy rice

	Calibration set (60 samples)	Prediction set (20 samples)
	Aflatoxin B_1/μg·kg^{-1}	Aflatoxin B_1/μg·kg^{-1}
Minimum	7.05	6.90
Maximum	54.82	48.96
Mean	22.58	21.57
Standard	11.94	11.27

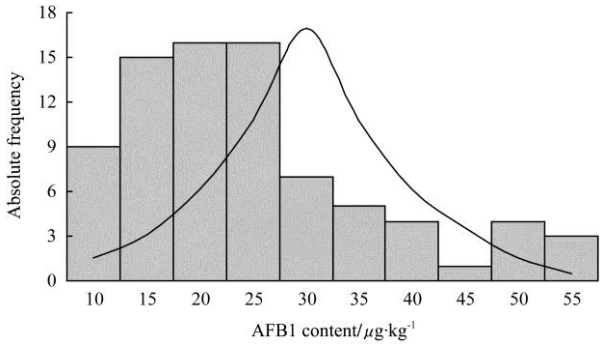

Figure 1 Frequency distribution and normal curve of aflatoxin B_1 content

Reference data results obtained when analyzing paddy rice samples indicated that presence of AFB_1 in paddy rice with moisture between 16% and 22% was detected in quantities >20 μg/kg, while AFB_1 was low in naturally infected paddy rice samples. NIRS was unfit for the direct detection of AFB_1 in paddy rice with concentrations lower than 20 μg/kg. This survey

highlights the potential of NIRS methodology for detectability of AFB_1 at 20 μg/kg level. The AFB_1 content of the eighty paddy rice samples was in a range from 6.90 μg/kg to 54.82 μg/kg, while the average absorbance of all the wavelength points of each paddy rice sample was in a range of 0.467-0.656. The sensitivity of the method for the detection of AFB_1 was calculated according to the following formula:

sensitivity = (maximum of average absorbance – minimum of average absorbance)/(maximum of reference value – minimum of reference value) (1)

Sensitivity was 0.004 $(\mu g/kg)^{-1}$. This result agrees with what was reported by other researchers[21]. The moisture content of paddy rice is one of the most important factors governing the fungal growth and their mycotoxin production[24]. Abdullah et al.[27] also noted that the critical moisture content for rice grains maintained at 25℃ without fungal growth was 13%. Therefore, the low-moisture-content storage can be recognized as a safe storage system[28].

3.2 Spectral characteristics

Six spectral outliers were found by Unscrambler 10.3 and removed from the raw absorption spectra. Figure 2 shows the average NIRS (raw absorption spectra) of the paddy rice samples obtained over a wave number between 10 000 and 4 000 cm^{-1}. Different NIR raw spectra from samples of paddy rice: non-contaminated (a), moderately contaminated with AFB_1 (b) and highly contaminated with AFB_1 (c) are shown in Figure 3. The average NIRS of paddy rice pre-treated by applying the first derivative method of Savizkye-Golay at AFB_1 are presented in Figure 4.

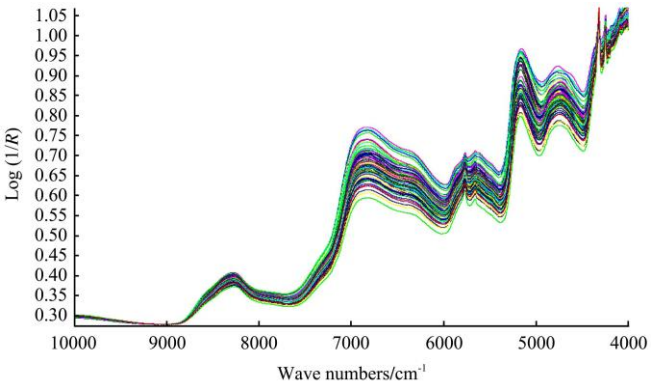

Figure 2 NIR raw spectra of samples of paddy rice, based on the aflatoxin B_1 infection in the sample

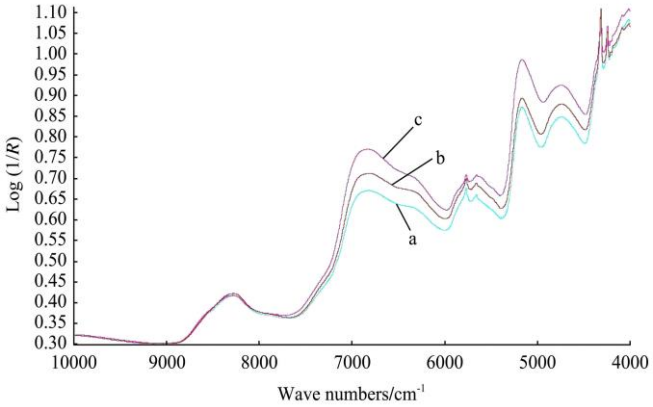

Figure 3 Different NIR raw spectra for non-contaminated (a), moderately contaminated with aflatoxin B_1 (b) and highly contaminated with aflatoxin B_1 (c)

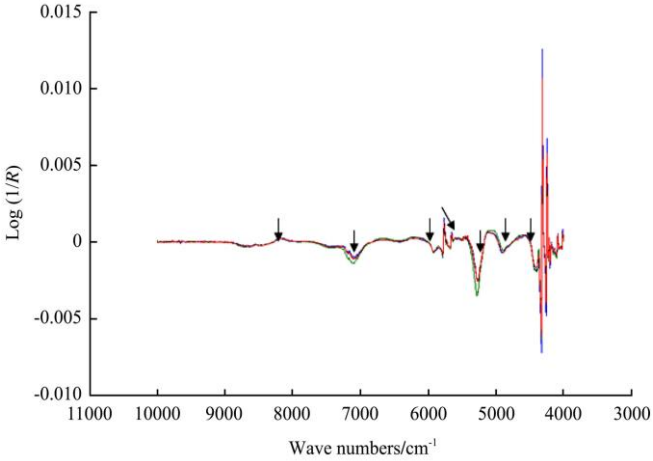

Figure 4 First derivative NIR spectra of different paddy rice samples

From the comparison between NIR raw spectra (Figure 2 and Figure 3) and the first derivative spectra (Figure 4), it can be observed that treatment by the first derivative allowed the overlapping peaks of the spectra to be distinctly separated and to show more details in the spectral characteristics of the paddy rice samples. Treatment with the derivative for NIRS improved the spectral resolution and exposed the corresponding positions of absorption peaks for all functional groups. The first derivative spectra of the raw spectra are presented in Figure 4. The minimum value in the first derivative spectra corresponds to the position of the absorption peak in the raw absorption spectra. The positions of absorption peaks for the main functional groups (Figure 4) were determined by comparing NIR absorption spectra between Figure 2 and Figure 3.

The spectra of AFB_1 infection were measured and

analyzed to demonstrate correlation to some of the bands observed in the infected samples (Figure 4). Several bands in Figure 4 were suitable for the determination of AFB_1 in the paddy rice sample distinguished from the uninfected one. The peak at 8 304 cm^{-1} can be assigned to the first overtone from the C-H stretching mode corresponding to CH_2 and CH_3 groups[23]. The peak at 6 838 cm^{-1} and 4 022 cm^{-1} can be attributed to combination bands of C-H stretching mode of fatty acids and carbohydrates. The peak at 5 665 cm^{-1} can be assigned to the second overtone from the C-H stretching mode corresponding to a CH_2 group. The peak at wavelengths between 5 222 and 5 102 cm^{-1} corresponds to the overtone from the O-H stretching mode of water and glucose[18]. The peak at wavelength of 4 747 cm^{-1} corresponds to a second overtone of C=O stretching modes of intermolecular esterification, and NH in amino acids[29]. From Figure 4, this particular absorbance region correlates most strongly with the AFB_1 infection. This appears to confirm that the moisture and starch content in paddy rice affects the overall extent of AFB_1 infection.

3.3 Spectral preprocessing and PLSR model building

PLSR for AFB_1 infection was presented in Table 2. The best preprocessing method and calibration model were selected on the basis of the highest value of correlation coefficient of determination (R^2) and lowest *SEC* and *SEP* values. Table 2 shows that preprocessing greatly improved the quality of a model. The best model obtained used the SNVD pretreated spectra. The values obtained for R^2 and *SEC* were 0.841 and 3.215%, respectively. The R^2 and *SEP* found on the independent prediction set were 0.850 and 3.211%, respectively. *RPD* values are often used as a key target for determining the prediction capacity of a model. The higher *RPD* values indicate better NIRS predictions[30]. The *RPD* and *RER* values for the prediction set were 1.968 and 2.679, respectively.

Researchers have focused on the possibility of developing an NIR method to detect mycotoxins and mycotoxigenic fungi in grains and grain products. Berardo et al.[18] determined that the optimal predictive ability for the percentage of total fungal infection of *F.verticillioides* was obtained by a calibration model for the maize kernels (r^2=0.750 and SECV=7.430) and maize meal (r^2=0.790 and SECV=10.950), respectively. The NIR methodology can also be applied for detecting the contamination of the fumonisin producer *F. verticillioide* in maize. Fernández-Ibañez et al.[21] found that specific wavelengths in the near infrared regions (1 100–2 500 nm) were related to fungal infection, which agreed with the results from this research. Sirisomboon et al.[23] observed that NIRS could accurately detect the incidence of rice infected by aflatoxigenic fungus, with a wavelength range of 950-1 600 nm.

Table 2 Regression results using PLSR discriminant models for paddy rice contaminated with aflatoxin B_1

Pretreatment	Calibration		Prediction			
	R^2	*SEC*	R^2	*SEP*	*RPD*	*RER*
Raw spectra	0.819	3.453	0.815	3.269	1.811	3.562
Smoothing	0.818	3.451	0.821	3.259	1.872	2.998
Normalizing	0.838	3.222	0.852	3.231	2.165	3.019
Baseline offset	0.801	3.596	0.790	3.621	1.416	2.697
SNV	0.821	3.423	0.850	3.208	2.091	3.541
SNVD	0.841	3.215	0.850	3.211	1.968	2.679
MSC	0.797	3.648	0.819	3.523	1.883	3.321

Note: *SEC*, standard error of calibration; *SEP*, standard error of prediction; *RPD*, ratio of performance to deviation; *RER*, ratio of the range to the *SEP*; SNV, standard normal variate; SNVD, standard normal variate detrending; MSC, multiplicative scatter correction.

The linear regression plots of the calibration and prediction data sets for the best model (SNVD preprocessing) showing measured versus predicted AFB_1 content were presented in Figure 5 and Figure 6, respectively. A correlation plot was drawn, as shown in Eq. (2) with a coefficient of determination (R^2) of 0.852, from the actual values and calibration set.

$$y = 0.9077x + 3.7749 \quad (2)$$

Eq.(2) shows good performance in predicting AFB_1 content of the paddy rice samples in the range of 6.90–54.82 μg/kg. The equation of the straight line for correlation plots of the prediction data sets was as Eq. (3).

$$y = 0.8917x + 1.149 \quad (3)$$

where, the coefficient of determination (R^2) was 0.811.

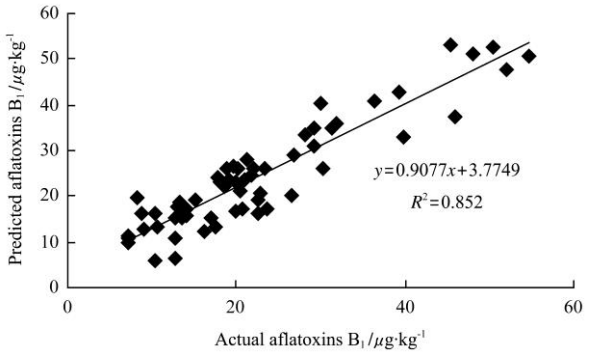

Figure 5 Linear regression plot of measured versus predicted aflatoxin B_1 content of the calibration data set

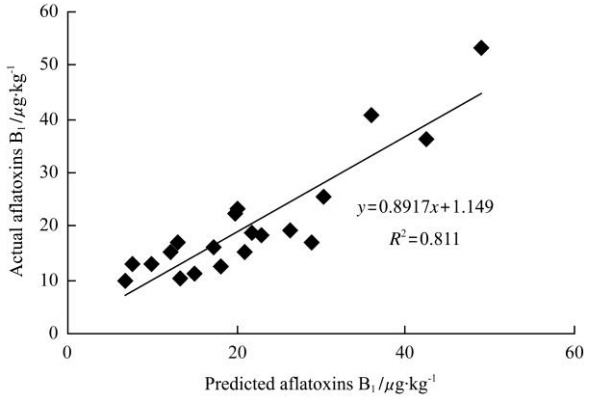

Figure 6 Linear regression plot of measured versus predicted aflatoxin B_1 content of the prediction data set

4 Conclusions

A feasible method applied to the detection of AFB_1 in paddy rice by NIRS was studied in this research. Compared with traditional techniques, the NIRS method may predicate the quantification of AFB_1 in paddy rice with rapid, simple and non-destructive advantages. The NIRS technique may have practical applications for monitoring aflatoxin and fungal contamination in postharvest paddy rice during storage. The present research studied the potential of NIRS methodology as a fast and non-destructive tool for detection of AFB_1 at 20 μg/kg level. The sensitivity of the near infrared spectroscopy methodology for the detection of AFB_1 in the paddy rice was 0.004 $(\mu g/kg)^{-1}$.

The *RPD* values of the chemometric models presented in this study are low. It appears that the prediction performance of chemometric models is not very useful. Consequently, to improve the accuracy of the model, additional paddy rice samples should be considered. For example, more paddy rice samples naturally contaminated with AFB_1, inoculated samples, as well as normally uncontaminated samples of different origins and varieties from grain depots and local markets of different regions should be further assessed. The presented results offer wide opportunities for further improving the model for detection of other types of aflatoxins in paddy rice.

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