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Application of near infrared spectroscopy to detect aflatoxigenic fungal contamination in rice

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ABSTRACT

The objective of this research was to apply the near infrared spectroscopy (NIRS), with a wavelength range between 950 and 1650 nm, to determine the percentage of fungal infection found in rice samples. The total fungal infection and yellow-green *Aspergillus* infection, which is often indicative of aflatoxigenic fungal infection, are the focus of this research. Spectra were obtained on 106 rice samples, by reflection mode, including 90 naturally contaminated samples, and 16 artificially contaminated samples. Calibration models for the total fungal infection were developed using the original and pretreated absorbance spectra in conjunction with partial least square regression (PLSR). The statistical model developed from the untreated spectra provided the greatest accuracy in prediction, with a correlation coefficient (r) of 0.668, a standard error of prediction (SEP) of 28.874%, and a bias of −0.101%. For yellow-green *Aspergillus* infection, the most accurate predictive statistical model was developed using a pretreated (maximum normalization) NIR spectra, with the following statistical characteristics (r = 0.437, SEP = 18.723% and bias = 4.613%). Therefore, the result showed that the NIRS could be used to detect aflatoxigenic fungal contamination in rice with caution and the technique should be improved to get better prediction model. However, there is an evident from NIR spectra that the moisture and starch content in rice affects the overall extent of fungal infection.

1. Introduction

Aflatoxin B_1 (AFB_1) is a naturally occurring mycotoxin that can result in detrimental side effects if consumed by humans or animals. AFB_1 is produced by filamentous fungi corresponding to *Aspergillus* section *Flavi* particularly *Aspergillus flavus* (Godet & Munaut, 2010). AFB_1 is found in a wide range of agricultural products including rice, with greater prevalence in tropical and humid climates (Reddy, Reddy, & Muralidharan, 2009). The International Agency for Research on Cancer (IARC) has classified AFB_1 as a carcinogen for animals and humans (IARC, 1993) and many countries have strict legal regulations concerning AFB_1 contamination in food and agricultural products. Indeed, the European Commission (EU) has fixed the maximum limit for AFB_1 contamination in cereals, grains, beans and groundnuts at 2 μg/kg, while for corn, rice and spices it is set at 5 μg/kg (Regulation (EU) No 165/2010).

The early detection of aflatoxigenic fungal infection in post-harvest or stored cereals and grains represents a key opportunity to reduce the risk of AFB_1 infection from the food chain. As such, various methods have been developed and utilized to determine fungal contamination in food. Traditionally, mycotoxigenic fungal infection in food has been detected using microbiological methods in a laboratory setting. This includes fungal enumerating using plate-counting or direct plating techniques, isolating in appropriate media and identifying the genus and species level by morphological characterization, including macroscopic characters (color, size, colony appearance) and microscopic characters (conidia, conidiophore, conidial heads) (Samson, Hoekstra, & Frisvad, 2004, p. 64, 2006; Pitt & Hocking, 2009, p. 19). However, these methods are time consuming, requiring a well equipped laboratory and skilled laboratory staff. There are also difficulties regarding assay standardization and errors arising from contamination (Atkins & Clark, 2004). Currently, the polymerase chain reactions (PCR) method is considered a good alternative option for fungal molecular diagnostics due to high specificity and sensitivity (Niessen, 2007; Paterson, 2006). PCR techniques have been used for classification and identification of *Aspergillus* (Geiser et al., 2007; Samson, Hong, & Frisvad, 2006) and for the detection of these strains from

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agricultural samples (Sartori et al., 2006). However, PCR-based techniques have some technical limits due to protocol complexities, reagent costs and the choice of specific primers for each species (Santos, Fraga, Kozakiewicz, & Lima, 2010). Thus, cost effective methods are still highly desirable.

Near-infrared spectroscopy (NIRS) is a powerful technique for characterizing the chemical composition of very complex materials including food and agricultural products. It is a fast, nondestructive, environmental friendly and highly accurate method that requires little expert training. It has been used to analyze proteins in rice (Zhang et al., 2007), sugar content in fruits (Camps & Christen, 2009), pectin in Japanese pears (Sirisomboon, Tanaka, Fujita, & Kojima, 2007) and to detect microorganisms in fruit juices (Al-Holy, Mengshi, Cavinato, & Rasco, 2006). This technique has been successfully applied to detect mycotoxins and mycotoxigenic fungal contamination in agricultural products, including the studies of deoxynivalenol content in wheat kernels (Pettersson & Aberg, 2003), aflatoxins and ochratoxin A in red paprika (Hernández-Hierro, García-Villanova, & González-Martín, 2008) and AFB_1 in maize and barley (Fernández-Ibañez, Soldado, Martínez-Fernández, & de la Roza-Delgado, 2009). Berardo et al. (2005) described the application of NIR to assess maize, detecting the fungal contamination *Fusarium verticillioide*, which can lead to the production of the toxin fumonisin. Singh, Jayas, Paliwal, and White (2012) used a short-wave NIR hyperspectral imaging system in the range of 700–1100 nm to detect storage fungi in wheat, including *Penicillium* spp., *Aspergillus glaucus* and *Aspergillus niger*.

The studies described above collectively show the potential for the development of a NIR method for the detection of mycotoxins and mycotoxigenic fungi in agricultural products, particularly cereals and cereal products. However, the application of NIRS for detection of aflatoxins and aflatoxigenic fungi in rice has not been reported so far. Thus, the objective of this research was to apply the near infrared spectroscopy (NIRS) to detect the total fungal infection (%) and yellow-green *Aspergillus*, potentially aflatoxigenic fungi, infection (%) in rice samples.

2. Materials and methods

2.1. Samples

2.1.1. Natural infection

Between 1 and 2 kg of rice samples were purchased from different markets and supermarkets in and around Bangkok, as well as at local mills in central Thailand. Ninety separate rice samples were purchased in total, consisting of 30 Jasmine rice samples, 30 white rice samples and 30 brown rice samples.

2.1.2. Artificial infection

Sixteen artificially contaminated rice samples were created by adding droplets of a fungal suspension containing 10^2, 10^4, 10^6 and 10^8 spores/ml of *A. flavus* M3T8R4G3 aflatoxigenic strain isolated from rice. The strain was supplied by the culture collection of the Department of Microbiology, Faculty of Science, Chulalongkorn University, Thailand. The concentration of fungal suspension of 10^2, 10^4, 10^6 and 10^8 spores/ml provided the ranges of percentage of fungal infection of 0–4%, 2–10%, 20–52% and 92–100%, respectively, which was covered the minimum to the maximum of the percentage of fungal infection (0–100%).

2.2. Near-infrared scanning

The 106 separate rice samples (90 natural and 16 artificially contaminated) were subjected to NIR scanning. Each scan consisting of a sample of 150 g of rice being placed in an open cup with diameter of 75 mm, up to a depth of 25 mm and the sample was then scanned using NIR with a wavelength range of 950–1650 nm by a NIR spectrometer (DA 7200 NIR Diode Array Analyzer, Perten Instruments AB, Sweden) in diffuse reflection mode. Each rice sample was analyzed in triplicate (318 sub-samples). The scanning was done three times on each sub-sample and the average reflection spectra were used for data analysis.

2.3. Reference method

The direct plating method is considered one of the most effective techniques for detecting, enumerating and isolating fungi from particulate food such as grains and nuts (Pitt & Hocking, 2009, p. 19; Samson et al., 2004, p. 64). Thus, the total fungi contamination and yellow-green fungi contamination (representative *Aspergillus* section *Flavi* as aflatoxin B_1 producer) were also determined using this technique for all the rice samples under investigation in this study.

The standard protocol recommended by the International Commission of Food Mycology (ICFM) was used. Fifty rice grains were randomly selected from each rice sample and aseptically put directly onto the surface of a DG18 (Dichloran 18% glycerol agar) culture medium (10 grains/plate). The plates were incubated at 25 °C for 5–7 days. After incubation, the number of infected rice grains was counted and the results were expressed as a percentage. Yellow-green spore-producing filamentous fungi were isolated and identified using the identification key of Samson et al. (2004: p. 64) and Pitt and Hocking (2009: p. 19).

2.4. Data analysis

Prior to the development of the NIR chemometric models, the total fungal infection (%) and the yellow-green *Aspergillus* infection (%) of the 318 reference data were checked for outliers using Eq. (1) (Sirisomboon, Tanaka, Kojima, & Williams, 2012)

$$\frac{x-\bar{x}}{\mathrm{SD}} \geq 3 \tag{1}$$

which is the *Z* score and when the Z score is ≥3, it means that the *x* value is outside the ±3SD range where 99.7% of data is. Then the *x* value will be considered as outlier. *x* is the reference value of total fungal infection or yellow-green *Aspergillus* infection, $\bar{x}$ is the average total fungal infection or yellow-green *Aspergillus* infection, and SD is the standard deviation.

All spectral analyses were carried out using The Unscrambler 9.8 (Camo, Norway). The NIR reflection spectra were transformed to absorption spectra and subjected to principal component analysis (PCA) to detect spectrum outliers. Then, the reference data were merged with NIR reflection spectra. The total spectra data were separated into two sets: calibration and prediction sets in ratio of 7:3. The calibration set, both with and without spectral pretreatment, was used to develop partial least square regression (PLSR) models. Two separate models were generated, the first on the total fungal infection (%) and the second on the yellow-green fungal infection (%).

Pretreatment was performed using one of the following methods: multiplicative scatter correction (MSC), normalization (mean, maximum, range), first derivative according to Savizky–Golay (11 and 21 wavelength points with polynomial order of 2), second derivative according to Savizky–Golay (11 and 21 wavelength points with polynomial order of 2), standard normal variate (SNV), SNV + detrending (SNVD) and baseline offset.

Independent validation of the calibration models was achieved using the prediction set. The model with the highest correlation (*r*), lowest standard error of calibration (SEC), standard error of prediction (SEP), and bias was selected as being optimal. The