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Monitoring fungal growth on brown rice grains using rapid and non-destructive hyperspectral imaging

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ABSTRACT

This research aimed to develop a rapid, non-destructive, and accurate method based on hyperspectral imaging (HSI) for monitoring spoilage fungal growth on stored brown rice. Brown rice was inoculated with a non-pathogenic strain of *Aspergillus oryzae* and stored at 30 °C and 85% RH. Growth of *A. oryzae* on rice was monitored using viable colony counts, expressed as colony forming units per gram (CFU/g). The fungal development was observed using scanning electron microscopy. The HSI system was used to acquire reflectance images of the samples covering the visible and near-infrared (NIR) wavelength range of 400–1000 nm. Unsupervised self-organizing map (SOM) was used to visualize data classification of different levels of fungal infection. Partial least squares (PLS) regression was used to predict fungal growth on rice grains from the HSI reflectance spectra. The HSI spectral signals decreased with increasing colony counts, while conserving similar spectral pattern during the fungal growth. When integrated with SOM, the proposed HSI method could be used to classify rice samples with different levels of fungal infection without sample manipulation. Moreover, HSI was able to rapidly identify infected rice although the samples showed no symptoms of fungal infection. Based on PLS regression, the coefficient of determination was 0.97 and root mean square error of prediction was 0.39 log (CFU/g), demonstrating that the HSI technique was effective for prediction of fungal infection in rice grains. The ability of HSI to detect fungal infection at early stage would help to prevent contaminated rice grains from entering the food chain. This research provides scientific information on the rapid, non-destructive, and effective fungal detection system for rice grains.

1. Introduction

Rice is one of the world's most important staple foods (Pitt et al., 1994). During storage, rice grains are often contaminated with spoilage or toxigenic fungi, with *Aspergillus* being the most frequent (Braghini et al., 2009; Reddy et al., 2008; Tonon et al., 1997). Changing climate and unseasonal rainfall may facilitate fungal colonization of rice grains (Pitt et al., 1994; Russell et al., 2010). Fungal contamination in rice grains constitutes quantitative and qualitative losses, including discoloration, mustiness, biochemical changes, and undesirable odor and appearance, resulting in lowered quality and market value of the grains (Narvankar et al., 2009; Park et al., 2005). Moreover, fungal infection in rice grains may also cause health hazards due to the occasional production of mycotoxins, which are hazardous to humans and animals and constitute a factor for economic losses in food products (Kumar et al., 2008; Miller, 1995). Using moldy materials as food ingredients gives low or unacceptable product quality and most importantly, may result in mycotoxins to the final products (Reddy et al., 2009; Russell et al., 2010; Tanaka et al., 2007).

A number of methods have been explored to detect contamination caused by spoilage and pathogenic fungi, including enzyme-linked immunosorbent assay (ELISA) (Muthomi et al., 2008), fluorescence polarization immunoassay (Chun et al., 2009), and polymerase chain reaction (Levin, 2012). These detection methods are reliable, specific, and sensitive, however, most of these methods are technically complicated, difficult, and expensive. Hence, efforts have been made to develop rapid, inexpensive, and non-destructive techniques to detect fungal contamination in cereal grains.

Hyperspectral imaging (HSI) is an emerging technique that integrates spectroscopy and imaging to provide both spectral and spatial information on the distribution of the components of an object (Gendrin et al., 2007; Li et al., 2014). When compared with conventional spectroscopic techniques that are considered as pointed-based scanning instruments, HSI technique can acquire spectral data not only from a single point but also on each pixel of an image. Therefore, the main advantage of hyperspectral imaging is in the spatial feature since the examination of a large area on the sample surface is possible (Panagou et al., 2014). Hyperspectral images comprise hundreds of contiguous wavebands for each spatial position of a target studied. Hence, each pixel in a hyperspectral image contains the spectrum of that specific position. The resulting spectrum can be thought of as a fingerprint,

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which can be used to characterize the chemical composition of that particular pixel (Panagou et al., 2014).

HSI offers many advantages over conventional analytical spectroscopic methods and is gaining increasing interest for food quality evaluation (Li et al., 2014; Panagou et al., 2014). HSI has also been employed for detection of spoilage and pathogenic fungi in cereals, including the detection of *Fusarium* in wheat (Bauriegel et al., 2011), the investigation of fungal development in maize kernels (Williams et al., 2012), and the early detection of toxigenic fungi on maize (Del Fiore et al., 2010). HSI has been proven to be a rapid and non-destructive multi-component analytical technique enabling several determinations simultaneously without extensive sample preparation (Bauriegel et al., 2011; Li et al., 2014; Panagou et al., 2014). HSI analysis often generates a large amount of informative high-dimensional data, which require effective chemometric approaches to extract and interpret crucial and valuable information from the hyperspectral images (Li et al., 2014; Panagou et al., 2014).

Self-organizing map (SOM), known as Kohonen network, is an unsupervised learning chemometric method that is usually used for data exploratory purposes. The SOM provides an individual visual identity for each sample by reducing the dimension of the original data, while preserving the comprehensive information of the original data and allowing multivariate explorative comparisons between samples, facilitating sample-to-sample comparison by direct visual inspection (Wirth et al., 2012). Therefore, SOM can be used to visualize and interpret high-dimensional data by projecting them onto a two-dimensional neuron map. SOM can also be used for grouping complex data without *a priori* knowledge of the groups present (Kittiwachana et al., 2013; Wirth et al., 2012).

Unsupervised chemometric methods are ideal for a preliminary examination of the data but do not directly aid in the formation of predictive models. For this application, supervised methods such as multiple linear regression (MLR), principal component regression (PCR), and partial least squares (PLS) regression are often used. PLS regression is a chemometric technique that is useful in predicting one or more dependent variables simultaneously from a set of several predictors (independent variables). PLS has been used widely as a regression model due to its ability to analyze highly collinear data and provide good generalization (Wold et al., 2001).

Although HSI technique has been employed for detection of fungal infection in cereal grains, it has never been studied to monitor fungal contamination in rice grains. Moreover, this research appears to be the first attempt to implement HSI integrated with SOM to aid in visualization and classification of healthy and fungally infected rice grains. This research thus aimed to develop a rapid, non-destructive, and accurate method based on hyperspectral imaging for monitoring fungal growth on brown rice. It also aimed to visualize sample classification using SOM and to determine the correlation between hyperspectral reflectance and conventional colony counts using PLS regression. Brown rice was used in this study because, due to its nutritive value (the bran is kept intact), brown rice has become popular as a healthy food, but at the same time, it is prone to be infected by fungi. *Aspergillus* was used in this study to represent the most common spoilage fungi found in stored rice grains since Aspergilli are the major contaminants in rice seeds stored under high humidity conditions (Braghini et al., 2009; Reddy et al., 2008; Tanon et al., Tonon et al., 1997). However, the proposed method is expected to be applicable as a practical method for assessment of spoilage and pathogenic fungal contamination.

2. Materials and methods

2.1. Fungal inoculation

Aspergillus was used in this study to represent the most prevalent spoilage fungal genus found in stored rice grains. For safety considerations, a non-toxigenic *Aspergillus* strain was used to minimize the exposure of the researchers and environment to mycotoxigenic fungi, which are hazardous and considered to be potentially carcinogenic.

Aspergillus oryzae (TISTR 3018) was obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand. *A. oryzae* was maintained on potato dextrose agar (Sigma-Aldrich, Sigma Chemical Co., St. Louis, MO, USA) and incubated at 25 °C as recommended by the Thailand Institute of Scientific and Technological Research. Inoculum suspension was prepared from fresh, mature, 5-day-old cultures grown on potato dextrose agar. The colonies were covered with sterile distilled water, and spores were washed from the agar surface by gently rubbing the colonies with a sterile loop. The spore suspension was shaken with a vortex mixer and then filtered through layers of sterile cheesecloth to remove mycelium. The specific inoculum concentration (spores/ml) was adjusted by microscopic enumeration with a Neubauer Improved haemocytometer (C-Chip, DHC-N01, Digital Bio, Tokyo, Japan) before inoculation into brown rice to obtain the desired number of spore forming units on the rice. Preliminary experiments were conducted to determine the population of spores necessary in the spore suspension to result in an initial population of ~4 log (spores/g) on rice grains.

Thai Hom Mali or Jasmine brown rice (*Oryza sativa* L. cv. KDML105) was obtained from Surin province, Thailand. Rice was inoculated with the fungal suspension to obtain the specific spore concentration on rice grains. Thirty-five grams of inoculated samples was stored in a 90 mm × 15 mm sterile polystyrene Petri dish and incubated at 30 °C and 85% RH in a program incubator (IN801, Yamato Scientific Co., Ltd., Tokyo, Japan). All sample groups (treatments), including non-inoculated sample (Non) and fungal inoculated rice after storage for 0 (d0), 2 (d2), 4 (d4), 6 (d6), 8 (d8), and 10 (d10) days, were analyzed using viable mold counts, scanning electron microscopy (SEM), and hyperspectral imaging. This process was repeated 30–40 times. Therefore, a total of 210 hyperspectral images (30 images for each of 7 sample groups) of bulk samples was acquired.

2.2. Microbiological analysis

Twenty-five grams of brown rice from each sample group was homogenized in 225 ml of 0.1% phosphate buffer for 2 min using a Stomacher Lab-blender (Pro-media SH-001, Elmex Ltd., Tokyo, Japan). Serial decimal dilutions were prepared using the same diluent and each of the serial dilutions was inoculated in duplicate on 3 M Petrifilm Yeast/Mold Count Plates (3 M, St. Paul, MN, USA). All plates were incubated at 30 °C for 72 h. The colonies were counted as soon as possible after growth was apparent. Plate counts were recorded as colony forming units per gram (CFU/g).

2.3. Scanning electron microscopy

Rice grains were randomly selected from each sample group. The samples were fixed with 2% OsO_4 for 1 h at room temperature (25 °C) and washed with distilled water before dehydration. Sample dehydration was undertaken in a graded ethanol series (30%, 50%, 70%, and 95%) for 10 min for each concentration and in 100% ethanol for 5 min with 3 repetitions. The samples were then critical-point dried in a critical point dryer (Quorum model K850, UK). The samples were mounted with double-adhesive tape on specimen stubs and a gold coating applied using a sputter coater (Balzers model SCD 040, Balzers Union Ltd., Furstentum, Liechtenstein). Surface examination was performed on the endosperm part and embryo (germ) side of grain surface using a scanning electron microscopy (JEOL JSM-5410 LV, Tokyo, Japan) at an accelerating voltage of 15 kV.