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Detection of fungal infection in five different pulses using near-infrared hyperspectral imaging

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ABSTRACT

The five major pulse crops grown in Canada are: chick peas, green peas, lentils, pinto beans and kidney beans. Potential causes of fungal infection in these pulses are *Aspergillus flavus* and *Penicillium commune*. Early stages of fungal infections in pulses are not detectable with human eyes and traditional microbial methods require significant time to detect fungal infection. Near-infrared (NIR) hyperspectral imaging system is an advanced technique widely being assessed for detection of insect infestation and fungal infection in cereal grains and oilseeds. The primary objective of this study was to assess the feasibility of the NIR hyperspectral imaging system to identify fungal infections in pulses. Hyperspectral images of healthy and fungal infected chick peas, green peas, lentils, pinto beans and kidney beans were acquired and features (six statistical and 10 histogram) were used to develop classification models to identify fungal infection caused by *A. flavus* and *P. commune*. Images of healthy and fungal-infected kernels were acquired at 2 week intervals (0, 2, 4, 6, 8 and 10 weeks from artificial inoculation). Six-way (healthy vs the five different stages of infection) and two-way (healthy vs every stage of infection) models were developed and classifications were done using linear discriminant analysis (LDA) and quadratic discriminant analysis (QDA) classifiers. The LDA classifier identified both types of fungal infections with 90–94% accuracy while using the six-way model, and with 98–100% accuracy when using the two-way models for all five types of pulses. The QDA classifier also showed promising results as it gave 85–90% accuracy for the six-way model and 96–100% accuracy for the two-way models. The two fungal species could not be differentiated by the hyperspectral imaging.

1. Introduction

Pulses belong to the family Leguminosae and are a major source of human nutrition because of their high protein content. Over the past 20 years, Canada has emerged as the world's largest exporter of lentils and one of world's top five exporters of beans. These pulses contribute more than $2 billion to the Canadian economy. The major types of pulses grown in Canada are: chick peas (*Cicer arietinum* L.), green peas (*Pisum sativum* L.), lentils (*Lens culinaris* Medikus), pinto beans (*Phaseolus vulgaris pinto* L.) and kidney beans (*P. vulgaris* L.) with the major production coming from Saskatchewan which accounts for 79.3% of the total pulses in Canada (Statistics Canada, 2011). Pulses have high amounts of aspartic acid, glutamic acid, leucine, lysine, and arginine and also possess functional properties like water solubility, foaming and fat binding capacity (Joyce et al., 2010). Storage losses in pulses are a major problem because of fungal infection and insect infestation.

Fungal species especially *Aspergillus* spp. and *Penicillium* spp. can grow on many food commodities under suitable conditions and produce secondary metabolites, mycotoxins, which are toxic to humans and animals (Del Fiore et al., 2010). Food and Agricultural Organization (FAO, 2014) of the United Nations reported that more than 25% of world food production is affected by fungal infection. Fungal presence can damage a product, produce discolouration, release volatile compounds and release toxins. Hence, they need to be identified at the initial stages of infection so that quick corrective actions can be implemented. There are many traditional methods available for identifying fungi but they are costly and time consuming. Hence, there is a need for a rapid, non-destructive, and accurate method for the detection of fungal infection.

One such method is the use of hyperspectral imaging which allows the characterization of a sample's spectral (spectroscopic

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component) and spatial (imaging component) characteristics and is also a rapid and non-destructive method (Gowen et al., 2007). Hyperspectral imaging produces a hypercube, which can facilitate the analysis of extrinsic and intrinsic properties of samples. Several studies involving the application of hyperspectral imaging for detecting insect infestation and fungal infection in cereals and oilseeds have been reported in the literature and few of these are summarized in the following text. Singh et al. (2009) studied insect infestation levels in wheat using a NIR hyperspectral imaging system. The wheat seeds were artificially infested with *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), *Cryptolestes ferrugineus* (Stephens), and *Tribolium castaneum* (Herbst) insects and images were scanned in the wavelengths of 1000–1600 nm using the NIR hyperspectral imaging system and the significant wavelengths were selected using the multivariate image analysis. Six statistical image features (maximum, minimum, mean, median, standard deviation and variance) and 10 histogram features were extracted from the images at 1101 nm and 1305 nm and were used in statistical discriminant classifiers (linear, quadratic). Linear and quadratic discriminant analysis classifiers classified healthy and insect-damaged wheat seeds with 85–100% accuracy.

The potential of NIR hyperspectral imaging system to identify fungal infection in wheat was studied by Singh et al. (2007). Wheat kernels were artificially infected with *Aspergillus glaucus* group and *Aspergillus niger* Van Tieghem and images were taken in the wavelength range of 1000–1600 nm. Multivariate image analysis was used to reduce the data size and two-class and four-class models were developed by k-means clustering discriminant (linear, quadratic and Mahalanobis) analyses. The two-class models were able to classify 100% of healthy seeds and 97.8% of infected seeds and the four-class models were able to accurately identify 91.7% of healthy seeds and 95% of the infected seeds. *Aspergillus glaucus* infection in high oil content canola (Senthilkumar et al., 2012) and *A. glaucus* and *Penicillium* spp. infections in mixed variety canola (Senthilkumar et al., 2015) were detected using NIR hyperspectral imaging system with a classification accuracy of more than 90%.

Kaliramesh et al. (2013) used hyperspectral imaging to detect insect infestation in mung bean (*Vigna radiata* L.) R. Wilczek using images in the near-infrared region. They collected images in the wavelengths of 1000 nm–1600 nm at 10 nm intervals and used the most significant wavelengths, which were 1100, 1290 and 1450 nm, for classification. From each of these wavelengths, six statistical features namely maximum, minimum, mean, median, variance and standard deviation were extracted and used as inputs to the statistical LDA and QDA classifiers, which gave classification accuracies of 85% and 82% for identifying uninfested and infested mung bean kernels, respectively.

In another study, Kaliramesh et al. (2014) determined the main constituents in mung beans using near-infrared hyperspectral imaging. Images of mung beans were acquired at 960 nm–1700 nm at 10 nm intervals and seventy five NIR reflectance intensities were extracted from each sample. They used principal components regression (PCR) and partial least squares regression (PLSR) models for prediction. For moisture, protein, and starch content predictions of mung beans, PLSR models demonstrated better performances than PCR models. Hyperspectral imaging has been used to detect insect infestation in pulses (Kaliramesh et al., 2013), but there are no reported studies dealing with the detection of fungal infection in pulses using NIR hyperspectral imaging technique.

Therefore, the objectives of this study were: to develop an algorithm to classify healthy and fungal-infected pulses; to use this algorithm to identify fungal infection in five pulse types namely, chick peas, green peas, green lentils, kidney beans, and pinto beans; and to compare the classification accuracies between different fungal infection stages (different weeks after inoculation).

2. Materials and methods

2.1. Fungal sample preparation

The fungal species, namely *Penicillium commune* Thom, C. and *A. flavus* Link, J. were acquired from the Cereal Research Centre and Food Science Department, University of Manitoba, Winnipeg, Canada. The fungal species were made to grow in large numbers by placing infected seeds on filter paper saturated with 7.5 mL aqueous NaCl solution in petri dishes. Seven days later, pure fungal lines were placed for one week at 30 °C on potato dextrose agar. These agar moulds were then placed in 200 mL sterilized water and shaken in a plastic spray bottle mixed with 1 drop of Tween 20. The five pulse types namely chick peas, green peas, green lentils, pinto bean and kidney beans were obtained from the Saskatchewan Pulse Growers, Saskatoon, Canada. Each of the pulse types were moisturized to 17% moisture content (wet basis) by adding predetermined quantities of distilled water and mixing in a rotating drum. The moisturized pulses were surface sterilized by soaking in 1% sodium hypochlorite solution for 2 min. These were then rinsed thoroughly using sterilized water and dried on a paper towel for 2 h. Seeds were then sprayed with fungus-inoculated water until all seeds were wet. The control samples were maintained at a moisture content of 15% (wet basis, no inoculation).

2.2. Hyperspectral imaging system

2.2.1. Hardware

The system used for this research is the same as described by Senthilkumar et al. (2012) and Kaliramesh et al. (2013) and is briefly described here. A short wave near infrared hyperspectral imaging system (SWNIR) consisted of a FFT-CCD area-scan image sensor (Model No. C7042, Hamamatsu Photonics, Hamamatsu, Japan) working in the 900–1700 nm range with image size of 640 × 480 pixels. The camera sensor was made up of indium-gallium-arsenide (InGaAs). Two liquid crystal tunable filters (LCTFs), which allowed wavelengths only at specified values, were mounted on the camera and offered excellent wavelength selection and imaging quality.

Data acquisition was done using the PCI data acquisition card (National Instruments PCI-1422., Austin, TX). The card could acquire images from a wide range of digital cameras. The image acquisition card was compatible with the RS-422 signals provided by the InGaAs camera and the camera was mounted on a copy stand. This stand contained a pair of 300 W halogen lamps (one on each side of the camera system, mounted at 45° angle). Halogen lamps emit light in the visible-near infrared (400–2500 nm) region of the electromagnetic spectrum suitable for spectroscopic imaging applications (Ushio Lighting Inc., Cypress, CA).

The whole system (Fig. 1) was connected by data cables that served two purposes: the first to provide and receive vital signals from the camera and second to provide all the DC power requirements. The LCTF cable linked the LCTF to the LCTF controller and the COM cable linked the PC to the LCTF controllers.

2.2.2. Software

The software provided a user interface, which had different modes of operation. The mode of operation was selected by the user and was shown in the current mode indicator. There was an option for co-adds which must be picked prior to collection of spectroscopic data. After this the dark count image was collected which represented the inherent noise that was subtracted from the collected spectroscopic data. The dark count indicator was turned ON once the dark count was collected; it remained constant for that