



Detection of different stages of fungal infection in stored canola using near-infrared hyperspectral imaging



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ABSTRACT

Near-infrared (NIR) hyperspectral imaging system was used to detect different stages of fungal infections in stored canola. Artificially infected canola seeds (Fungi: *Aspergillus glaucus* and *Penicillium* spp) were subjected to hyperspectral imaging in the range between 1000 and 1600 nm at 61 evenly distributed wavelengths. Four wavelengths 1100, 1130, 1250 and 1300 nm were identified as significant wavelengths and were used in statistical discriminant analysis. Pair-wise, two-class and six-class classification models were developed to classify the healthy and different stages of fungal infected samples. Linear, quadratic and Mahalanobis discriminant classifiers were used to classify healthy, five stages of *A. glaucus* and five stages of *Penicillium* spp infected canola seeds. All the three classifiers classified healthy and fungal infected canola seeds with a classification accuracy of more than 95% for healthy canola seeds and more than 90% for the initial stages of *A. glaucus* and *Penicillium* spp infected canola seeds. The classification accuracy increased to 100% with increase in fungal infection level (length of time since inoculation). All the samples subjected to imaging were tested for seed germination and free fatty acid value (FAV). The germination decreased with increase in amount of fungal infection, whereas FAV increased with increase in amount of fungal infection.

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1. Introduction

Canada produced an average of 15 million tonnes (Mt) of Canola (*Brassica napus* L. and *Brassica campestris* L. cultivars of low erucic acid oil and low glucosinolates) and exported an average of 8 Mt of canola seeds per year in the last five years (2010–2014). The economic value of canola in Canada is CAD \$20 billion and it is the most valuable crop in Canada by surpassing wheat in recent years (Statistics Canada, 2015). It is important to store the canola properly before it reaches the consumers. The major cause of spoilage in canola is fungi because of high moisture content also the high oil content (40–45%) restricts other pests like insects to multiply. Postharvest fungi can develop inside storage facilities and cause considerable damages to seeds under favorable conditions even after eliminating preharvest fungi by drying to safe moisture levels before storage.

The fungal infection in canola can reduce seed germination, oil quality, create unacceptable odours and can increase free fatty acids

and ergosterol content in seeds. The presence of fungi on the stored canola also helps mites to survive when they consume mold on canola seeds. The quality and quantity reduction due to the presence of fungi in canola results in reduced financial returns to the producers and if undetected can cause serious food safety issues to the consumers. The major fungal genera causing damage to canola in Canada are *Aspergillus* and *Penicillium*. *Aspergillus glaucus*, *Aspergillus candidus* and *Penicillium* spp are predominantly found in stored canola in Canada (Pronyk et al., 2004; White et al., 1982). *A. glaucus* can thrive, multiply and decompose canola even at moderate relative humidity levels while *A. candidus* and *Penicillium* spp require higher seed moisture contents (Pronyk et al., 2004; Sauer et al., 1992).

The detection of fungal infection at an early stage can reduce further damage to stored canola by applying suitable physical or chemical treatments. The traditional methods used for fungal detection such as microscopic culture methods and instrumental methods (gas chromatography, liquid chromatography, mass spectrometry) involve longer incubation time and tedious extraction procedures. There are various techniques developed to detect the fungal infection such as soft-X-rays (Narvankar et al., 2009),

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thermal imaging (Chelladurai et al., 2010), which are all non-invasive methods and require very short time to detect fungal damage; however, these can detect fungal infection only at advanced stages of deterioration. Polymerase Chain Reaction (PCR) methods (Hayat et al., 2012) which can even identify fungal species requires 24 h for DNA extraction and further processing.

Near-infrared (NIR) hyperspectral imaging is a combination of conventional imaging and spectroscopy which can provide spatial and spectral data of a given sample. NIR hyperspectral imaging system operates in the range from 700 to 2500 nm of the electromagnetic spectrum. Every material due to their physical and chemical nature reflect, transmit, absorb and emit energy in a particular manner at different wavelengths; hyperspectral imaging technique utilizes these effects to collect desirable data. The data acquired by NIR hyperspectral imaging can identify different chemical constituents of a material and it is also called chemical imaging. NIR hyperspectral imaging technique is a non-invasive or non-damaging method, and requires very limited sample preparation prior to imaging. Hyperspectral imaging was first utilized in remote sensing applications to map mineral resources and now it is extensively used in many sectors of agriculture, pharmaceuticals, medicine, environment, and food processing (Vadivambal and Jayas, 2015; Mahesh et al., 2015). NIR hyperspectral imaging used in the food processing sector involves many food quality and food safety parameters assessment applications.

NIR hyperspectral imaging has been used in detecting various quality parameters associated with raw grains such as prediction of oil and oleic acid concentrations in corn kernels (Weinstock et al., 2006) classification of sound and stained wheat kernels (Berman et al., 2007), wheat class classification using Linear Discriminant Analysis (LDA), Quadratic Discriminant Analysis (QDA), and Artificial Neural Network (ANN) Models (Mahesh et al., 2008), wheat class classification at different moisture levels (Mahesh et al., 2011), wheat class classification using wavelet texture features (Choudhary et al., 2009), identification of vitreous and non-vitreous durum wheat kernels (Shahin and Symons, 2008), classification of maize based on kernel hardness (Williams et al., 2009), evaluation of maize endosperm texture (Manley et al., 2009), detection of insect damaged wheat kernels (Singh et al., 2009), identification of pre-germinated barley (Arngren et al., 2011), classification of oat and groat kernels (Serranti et al., 2013), identification of rice seed cultivar (Kong et al., 2013) and classification of contaminants from wheat (Ravikanth et al., 2015).

NIR hyperspectral imaging techniques have also been used to detect pre- and post-harvest fungal infection in raw grains, detection of scab and other mold damaged wheat kernels (Delwiche, 2003), detection of scab damaged hard red spring wheat kernels (Delwiche and Hareland, 2004), detection of *A. glaucus*, *Aspergillus niger* and *Penicillium* spp infected wheat kernels (Singh et al., 2007), classification of sound and *Fusarium* damaged wheat kernels (Peiris et al., 2009), detection of fungal-infected corn kernels (Tallada et al., 2011), fungal development in maize kernels (Williams et al., 2012a), quantification of ergot bodies in cereals (Vermeulen et al., 2012), differentiation between species and strains of members of the genus *Fusarium* (Williams et al., 2012b), detection of *Fusarium* head blight in wheat kernels (Barbedo et al., 2015) and detection of *Fusarium* damaged oats (Tekle et al., 2015).

Potential of NIR hyperspectral imaging technique has not yet been studied in any great detail for quality analysis in oilseeds. The study done by Senthilkumar et al. (2012) was a preliminary study which explored the potential of NIR hyperspectral imaging to classify healthy and heavily fungal infected high oil content canola seeds. The study used only *A. glaucus* infected canola samples. The classification accuracy between healthy and *A. glaucus* infected canola samples were more than 95% using LDA and QDA classifiers.

NIR hyperspectral imaging technique to determine classification accuracies between healthy and artificially fungal infected canola by different species of fungi has not been reported. The objectives of this study were:

1. to determine the classification accuracy between healthy, *A. glaucus* and *Penicillium* spp infected mixed variety canola seeds using NIR hyperspectral imaging, and
2. to determine the classification accuracy between different stages (two, four, six, eight and tenth weeks after inoculation) of fungal infected canola seeds.

2. Material and methods

2.1. Sample preparation

Mixed variety canola seeds of the 2013 crop year were obtained from a grain elevator in Manitoba with a moisture content of 8.5%. Eleven kilograms of mixed variety canola were conditioned to 14% (wet basis) moisture content to help fungi grow and multiply by mixing a calculated amount of distilled water and were surface sterilized with 1% sodium hypochlorite solution to remove any pre-existing contaminants on canola seeds, then rinsed with distilled water and were then wiped using paper towels to remove excess water. The modified and sterilized canola seeds were divided into three subsamples, two subsamples of canola seeds were artificially infected with *A. glaucus* and *Penicillium* spp cultures obtained from the Cereal Research Centre, Agriculture and Agri Food Canada, Winnipeg, Manitoba and one subsample was preserved as healthy canola seeds (Senthilkumar et al., 2012). Both artificially infected subsamples were further divided equally and placed into five separate porous bags for each subsample. We placed the samples in between buffer samples on shelves in pails (20 L capacity) having KOH solution at the bottom to maintain 14% moisture content and to avoid fluctuations in the moisture content of canola (Sathya et al., 2009; Sun et al., 2014) (Fig. 1). We also avoided cross-contamination by sealing the pails and by placing the three replicates in three different environmental chambers maintained at 30 °C. There were 10 fungal-infected sample bags each weighing one kilogram and one healthy sample bag weighing one kilogram in each of the environmental chambers. The samples were labeled based on fungal species as follows; *A. glaucus* bag 1 and *Penicillium* spp bag 1 (Set 1), *A. glaucus* bag 2 and *Penicillium* spp bag 2 (Set 2), *A. glaucus* bag 3 and *Penicillium* spp bag 3 (Set 3), *A. glaucus* bag 4 and *Penicillium* spp bag 4 (Set 4), *A. glaucus* bag 5 and *Penicillium* spp bag 5 (Set 5), and healthy sample bag 1 (Set 6).

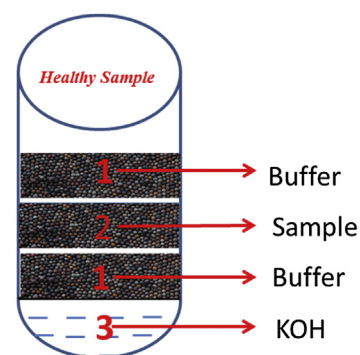


Fig. 1. Illustration of canola seed placement between the buffer samples and KOH solution inside the 20 L pails.

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