



DETECTION OF OCHRATOXIN A IN STORED BARLEY USING NEAR-INFRARED (NIR) HYPERSPECTRAL IMAGING

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Near-Infrared (NIR) hyperspectral imaging system operated in the wavelength region of 1000 to 1600 nm was used to detect ochratoxin A contaminated barley kernels. Five concentration levels of ochratoxin A contaminated barley kernels, five infection periods of non-ochratoxin A producing *Penicillium verrucosum* infected barley kernels, and sterile control samples were subjected to single kernel imaging. The acquired image data were processed and subjected to Principal Component Analysis (PCA) to identify significant wavelengths. Linear and quadratic discriminant pair-wise, two-class, and six-class models were developed and tested with statistical and histogram features extracted from significant wavelengths. The significant wavelength 1480 nm was identified only in the ochratoxin A contaminated samples, and not identified in the non-ochratoxin A producing *P. verrucosum* infected samples. The significant wavelengths 1310, and 1360 nm were found in both ochratoxin A contaminated samples and non- ochratoxin A producing *P. verrucosum* infected ones. The classification accuracy of more than 97% was achieved in discriminating between different concentration levels of ochratoxin A contaminated samples and a classification accuracy of 100% was achieved in discriminating between sterile control samples, and ochratoxin A contaminated samples.

Key words: *Penicillium verrucosum*, Near-Infra Red (NIR), wavelengths, significance, Principal Component Analysis (PCA), Ochratoxin A, detection

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the major cereal grains with an annual world production of 144 Mt (FAOSTAT, 2014). 70 % of world barley production is used as animal feed, and 30% as malt (Akar et al., 2004). Barley is the most preferred cereal grain for malt production than other cereal grains such as wheat and rye for its quality, nearly 90% of the malt is utilized by breweries and the remaining 10% as food substitutes to make breakfast products, biscuits and desserts (Akar et al., 2004; Bamforth and Barclay, 1993). The barley end use varies across the world, developed countries utilize more than 80% of their barley production as animal feed and some developing countries utilize most of their barley production for food; Morocco per capita consumption is 88 kg/ year (Bhatty, 1986). Canada produced 8.2 Mt of barley in 2015 (Statistics Canada, 2015), and Canada exports 0.5 Mt of malt and is ranked second among malt exporters (Agriculture and Agri-Food Canada, 2015). It is important to preserve the quality of produced barley before it reaches the consumers.

Fungi are one of the major pests causing spoilage in stored grains. The fungal infection in stored barley reduces germination, discolours grains, reduces market value, and can cause mycotoxin contamination and eventual livestock poisoning. Germination and protein content are major factors for assessing the quality of malting barley, and quality changes can result in total rejection and huge economic loss to the farmers. Lower moisture content and temperature levels can reduce the fungal growth in the stored grains, but some localized areas with higher moisture content and temperature can occur inside the storage structure due to temporal variability inside grain caused by external environmental factors. The localized areas with higher moisture content and temperature can result in significant spoilage of stored grains.

Mycotoxins are naturally occurring secondary metabolites of fungi and can cause serious health problems to consumers. The major mycotoxins found in food products are aflatoxin, deoxynivalenol, fumonisins, ochratoxin A, T-2 toxin, and zearalenone. The field level mycotoxins like deoxynivalenol (DON),

zearalenone (ZEN), fumonisins, and T-2 toxin are produced by *Fusarium* spp. The presence of aflatoxin in Canadian stored products are not reported in the literature, but are reported to be found in imported food products (CFIA, 2014). Ochratoxin A is the major storage mycotoxin found in wheat, barley, rye and oats in Canada. Regulations stipulating maximum limits for ochratoxin A in food products are proposed by Health Canada at 0.5 to 7 µg/ kg, which is currently under consultation (Health Canada, 2009; 2012).

The presence of fungal infection and mycotoxin contamination should be detected at an early infection stage to avoid complete deterioration of grains, and to take corrective measures. The traditional methods like chromatographic and microscopic cultural methods take long time to detect the presence of fungal infection and mycotoxin contamination in food products. NIR hyperspectral imaging system is a unique combination of both spectroscopy and conventional imaging and is widely used to detect the quality of food products. NIR hyperspectral imaging system was used to detect fungal infection in canola (Senthilkumar et al., 2012; 2015) and wheat (Singh et al., 2007; Senthilkumar et al., 2016), and ochratoxin A contamination in stored wheat (Senthilkumar et al., 2016). Application of NIR hyperspectral imaging system to detect Ochratoxin A contamination in barley grains has not been reported in the literature. Thus, the objectives of this study were to:

1. assess the potential of NIR hyperspectral imaging system to detect ochratoxin A contamination in barley kernels;
2. determine the classification accuracy between sterile control samples and ochratoxin A contaminated barley kernels; and
3. compare the spectral patterns of ochratoxin A contaminated barley (artificially infected with ochratoxin A producing *Penicillium verrucosum* strain) kernels and non-ochratoxin A contaminated barley kernels (artificially infected with non-ochratoxin A producing *P. verrucosum* strain).

MATERIALS AND METHODS

The procedure for sample preparation, data collection, and analysis has been reported earlier (Senthilkumar et al., 2012; 2015; 2016) and reproduced briefly here for easy reference.

Sample preparation

Fifty one kg of mixed variety barley grains were (13.7% moisture content -wet basis; 2013 crop year) obtained from Agriculture and Agri-Food Canada and were divided as 50 kg for ochratoxin A study and 1 kg as sterile control sample. The 50 kg barley was conditioned to 21.5% moisture content by adding a calculated amount of distilled water. The barley grains conditioned to 21.5% were autoclaved at 120°C for 20 min to remove the biological contaminants present in the barley grains. Ten kg of conditioned and sterilized barley grains were divided into two main samples each weighing 5 kg and labelled as sample 1, and sample 2, and the remaining 40 kg of barley grains were used as buffer samples.

The sample 1 was artificially infected with ochratoxin A producing *P. verrucosum* (strain number: C1343-1, Canadian Grain Commission) and sample 2 was infected with non-ochratoxin A producing *P. verrucosum* (strain number: C618-2). The samples 1 and 2 were further divided equally into 5 subsamples each (OTA 1 to 5) and (Non-OTA 1 to 5). All subsamples were placed between 2 kg of buffer sample on the top and 2 kg of buffer sample at the bottom, inside a 20L plastic pail with known amount of KOH solution with a determined specific gravity. The buffer samples and the KOH solution were used to maintain the moisture content of subsamples throughout the study. The five subsamples infected with ochratoxin A producing *P. verrucosum* and five subsamples infected with non-ochratoxin A producing *P. verrucosum* were placed in two different environmental chambers maintained at 20±2°C and 70±4% RH to avoid cross contamination. The remaining 1kg of barley sample was conditioned to 17% moisture content and autoclaved at 120°C for 20 min to be used as sterile control sample.

NIR hyperspectral imaging system

The NIR hyperspectral imaging system was comprised of NIR camera equipped with Indium Gallium Arsenide (InGaAs) detector (Model No. SU640-1.7RT-D, Sensors Unlimited Inc., Princeton, NJ, USA) to capture image data, Liquid Crystal Tunable Filters (LCTFs) to tune on to the desired wavelength range (VariSpec, Cambridge Research and Instrumentation Inc., Woburn, MA, USA), tungsten-halogen lamps to emit light in the desired wavelength range, and data processing unit with Lab VIEW program (version 7.1, National Instruments Corp., Austin, TX, USA) to capture images and MATLAB software (The

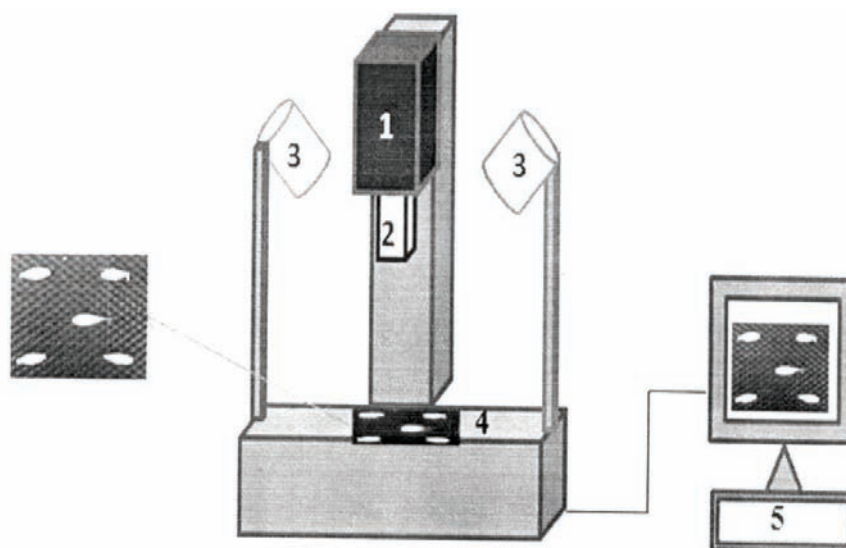


Fig. 1. Schematic representation of NIR hyperspectral imaging system (1. NIR camera, 2. LCTF filters, 3. Tungsten-halogen lamps, 4. Barley sample, 5. Data acquisition and processing system)

Mathworks, Inc., Natick, MA, USA) to process the captured image data (Senthilkumar et al., 2012; 2015; 2016) (Fig. 1).

Image acquisition and data analysis

Three hundred kernels from each of the 10 subsamples were subjected to single kernel imaging in wavelength range from 1000 to 1600 nm at 10 nm interval, by placing five barley kernels in the field of view of NIR camera without touching each other on a black board. Three hundred kernels from the OTA 1 and 300 kernels from the Non-OTA 1 were randomly selected and subjected to single kernel imaging after 18 weeks post inoculation, and the remaining subsamples (OTA 2 to 5 and Non-OTA 2 to 5) were subjected to single kernel imaging after 20, 22, 24, and 26 wk post inoculation.

Three dimensional (hypercube) acquired image data with intensity values were transformed into two dimensional data with reflectance values at 61 wavelengths in the range from 1000 to 1600 nm. The transformation was done by first changing the hypercube data into image format and then to two dimensional binary format. The 2D reflectance data were subjected to Principal Component Analysis (PCA) to identify most significant wavelengths based on highest factor loadings. Statistical features mean, median, standard deviation, variance, maximum, and minimum; and 10 histogram features corresponding to significant wavelengths were extracted and used in

linear and quadratic statistical classification models developed using Statistical Analysis Software (PROC DISCRIM, Version 9.1.3, SAS Institute Inc., Cary, NC) to discriminate between sterile control barley kernels and ochratoxin A contaminated barley kernels. The statistical classification models used 80% of barley kernels out of the 300 kernels from each subsample (240 kernels) as a training set and 20% (60 kernels) as a testing set.

Moisture content of barley kernels in each subsample and control sample were determined every 2 wk by placing 10 g of barley samples in triplicates in a hot air oven for 20 hr at $130 \pm 1^\circ\text{C}$ (ASABE, 2012). The germination of barley kernels were determined every two wk by placing 25 barley kernels on a filter paper (Whatman No 3) soaked with 5.5 mL of distilled water and placed in a petri dish (9 cm dia) (Wallace and Sinha, 1962) and germination was determined after 7 d of incubation. The ochratoxin A concentrations in *P. verrucosum* infected barley kernels were tested at Central Testing Laboratory, Winnipeg, MB using an ELISA testing kit.

RESULTS AND DISCUSSION

Moisture content, germination and ochratoxin A concentrations

The moisture content of ochratoxin A producing *P. verrucosum* and non-ochratoxin A producing *P. verrucosum* infected barley kernels (10 subsamples)

were maintained in the range from 21.5±0.3% to 21.8±0.4% throughout the study period of 26 wk. The buffer samples and KOH solution helped the subsamples to maintain the moisture content throughout the study (Nithya et al., 2011; Senthilkumar et al., 2016). The initial germination of all the subsamples was at 92.3±0.6% and at the end of 4 wk it reached 0% due to more mycelial growth and eventual damage caused to the kernels. The ochratoxin A concentrations in barley kernels infected with ochratoxin A producing *P. verrucosum* strain after 18, 20, 22, 24, and 26 wk were 85, 160, 273, 472, 618 ppb, respectively.

Significant wavelengths

The wavelengths 1310, 1360 and 1480 nm corresponding to ochratoxin A producing *P. verrucosum* infected samples were identified to be significant based on the highest factor loadings in the first PCA. The wavelengths 1260, 1310, and 1360 nm from the non-ochratoxin A producing *P. verrucosum* infected samples were identified as significant based on the highest factor loadings in the first PCA. The wavelength 1480 nm was identified as significant only in samples infected with ochratoxin A producing *P. verrucosum* strain (Senthilkumar et al., 2016). The wavelength 1310 nm can be attributed to *P. verrucosum* infection (both ochratoxin A and non-ochratoxin A producing) (Singh et al., 2007; Teena et al., 2014; Senthilkumar et al., 2015; 2016).

Pair-wise classification models

Five pair-wise models were developed to discriminate: (i) between sterile control barley kernels and different concentration levels of ochratoxin A contaminated barley kernels; (ii) between sterile control barley kernels and non-ochratoxin A producing *P. verrucosum* infected barley kernels; (iii) between different concentration levels of ochratoxin A contaminated barley kernels; (iv) between different infection periods of non-ochratoxin A producing *P. verrucosum* infected barley kernels; and (v) between different concentration levels of ochratoxin A contaminated barley kernels, and different infection periods of non-ochratoxin A producing *P. verrucosum* infected barley kernels. Both the linear and quadratic discriminant models of the first and second pair-wise classification models differentiated sterile control barley samples, different concentration levels of ochratoxin A contaminated barley kernels, and different infection periods of non-ochratoxin A producing *P. verrucosum* infected barley kernels with a classification accuracy of 100%. The third linear and quadratic pair-wise models discriminated different concentration levels

of ochratoxin A contaminated barley kernels with classification accuracies between 97.3 and 100 % (Table 1). The fourth linear and quadratic pair-wise model discriminated different infection periods of nonochratoxin A producing *P. verrucosum* infected barley kernels with classification accuracies between 98.4 and 100% (Table 2). The fifth linear and quadratic pair-wise model discriminated between different concentration levels of ochratoxin A contaminated barley kernels and different infection periods of non-ochratoxin A producing *P. verrucosum* infected barley kernels with classification accuracies between 99.1 and 100% (Table 3).

Two-class classification models

Three two-class models were developed to discriminate (i) between sterile control barley kernels (300 kernels) and combined kernels from five concentrations levels of ochratoxin A contaminated kernels (300 kernels) and combined kernels from five infection periods of non-ochratoxin A producing *P. verrucosum* infected kernels (5x300 kernels), and (iii) between sterile control kernels (300 kernels) and

Table 1. Pair-wise linear and quadratic classification accuracies between five concentration levels of ochratoxin A contaminated barley kernels (OTA).

Pair-wise model	Sample*	Classification accuracies	
		Linear	Quadratic
OTA 1 vs OTA 2	OTA 1	98.2	98.7
	OTA 2	97.3	98.4
OTA 1 vs OTA 3	OTA 1	100	100
	OTA 3	100	100
OTA 1 vs OTA 4	OTA 1	100	100
	OTA 4	100	100
OTA 1 vs OTA 5	OTA 1	100	100
	OTA 5	100	100
OTA 2 vs OTA 3	OTA 2	100	100
	OTA 3	100	100
OTA 2 vs OTA 4	OTA 2	100	100
	OTA 4	100	100
OTA 2 vs OTA 5	OTA 2	100	100
	OTA 5	100	100
OTA 3 vs OTA 4	OTA 3	100	100
	OTA 4	100	100
OTA 3 vs OTA 5	OTA 3	100	100
	OTA 5	100	100
OTA 4 vs OTA 5	OTA 4	100	100
	OTA 5	100	100

*sample size- 300 kernels.

Table 2. Pair-wise linear and quadratic classification accuracies between five infection periods of non-ochratoxin A producing *P. verrucosum* infected barley kernels (Non-OTA)

Pair-wise model	Sample*	Classification accuracies	
		Linear	Quadratic
Non-OTA 1 vs Non-OTA 2	Non-OTA 1	98.9	99.1
	Non-OTA 2	98.4	99.0
Non-OTA 1 vs Non-OTA 3	Non-OTA 1	100	100
	Non-OTA 3	100	100
Non-OTA 1 vs Non-OTA 4	Non-OTA 1	100	100
	Non-OTA 4	100	100
Non-OTA 1 vs Non-OTA 5	Non-OTA 1	100	100
	Non-OTA 5	100	100
Non-OTA 2 vs Non-OTA 3	Non-OTA 2	100	100
	Non-OTA 3	100	100
Non-OTA 2 vs Non-OTA 4	Non-OTA 2	100	100
	Non-OTA 4	100	100
Non-OTA 2 vs Non-OTA 5	Non-OTA 2	100	100
	Non-OTA 5	100	100
Non-OTA3 vs Non-OTA 4	Non-OTA 3	100	100
	Non-OTA 4	100	100
Non-OTA 3 vs Non-OTA 5	Non-OTA 3	100	100
	Non-OTA 5	100	100
Non-OTA 4 vs Non-OTA 5	Non-OTA 4	100	100
	Non-OTA 5	100	100

*sample size- 300 kernels.

Table 3. Pair-wise linear and quadratic classification accuracies between five concentration levels of ochratoxin A contaminated barley kernels (OTA), and five infection periods of non-ochratoxin A producing *P. verrucosum* infected barley kernels (Non-OTA)

Pair-wise model	Sample*	Classification accuracies	
		Linear	Quadratic
OTA 1 vs Non-OTA 1	OTA 1	99.1	99.2
	Non-OTA 1	99.4	99.7
OTA 2 vs Non-OTA 2	OTA 2	100	100
	Non-OTA 2	100	100
OTA 3 vs Non-OTA 3	OTA 3	100	100
	Non-OTA 3	100	100
OTA 4 vs Non-OTA 4	OTA 4	100	100
	Non-OTA 4	100	100
OTA 5 vs Non-OTA 5	OTA 5	100	100
	Non-OTA 5	100	100

*Sample size- 300 kernels

combined kernels from five concentrations levels of ochratoxin A contaminated kernels and five infection periods of non-ochratoxin A producing *P. verrucosum* infected kernels (10x300 kernels). The classification accuracies for all the three two-class models were 100% for sterile control barley kernels, combined kernels from five concentrations levels of ochratoxin A contaminated samples, and combined kernels from five infection periods of non-ochratoxin A producing *P. verrucosum* infected kernels.

Six-class classification models

Two six-class models were developed to discriminate (i) between sterile control barley kernels and five concentrations levels of ochratoxin A contaminated kernels; and (ii) between sterile control kernels and five infection periods of non-ochratoxin A producing *P. verrucosum* infected kernels. The first linear and quadratic six-class models differentiated sterile control kernels with classification accuracies of 100%, concentration level of ochratoxin A contaminated (OTA 1, 85 ppb) barley kernels with a classification accuracy of 98.3 for linear classifier and 98.5% for quadratic classifiers, and the remaining four concentration levels (OTA 2 to OTA 5) with a classification accuracy of 100% for both linear and quadratic classifiers (Table 4).

The second linear and quadratic six-class models discriminated sterile control kernels with classification accuracies of 100%, first infection period of non-

ochratoxin A producing *P. verrucosum* infected kernels (Non-OTA 1) with a classification accuracy of 98.4 for linear classifier and 98.9 for quadratic classifier, and the remaining four infection periods of non-ochratoxin A producing *P. verrucosum* infected kernels with a classification accuracy of 100% for both linear and quadratic classifiers (Table 5). The classification accuracies between different samples increased with increase in fungal infection periods (Teena et al., 2014).

CONCLUSION

The ochratoxin A contaminated barley kernels can easily be discriminated from sterile control kernels and non-ochratoxin A producing *P. verrucosum* infected kernels. The ochratoxin A contaminated kernels with concentrations as low as 85 ppb can be differentiated from sterile control samples with a classification accuracy of 100%. The classification accuracy increased with fungal infection periods. The NIR hyperspectral imaging system can easily differentiate between concentration levels of ochratoxin A contamination with classification accuracies between 97.3 and 100%, and between infection periods of non-ochratoxin A producing *P. verrucosum* infected kernels with classification accuracies between 98.4 and 100%. The time needed for the NIR hyperspectral imaging system to acquire image data in the range between 1000 and 1600 nm at 10 nm interval, and to process the image data require less than 2 min for 5 barley kernels. The

Table 4. Six-class linear and quadratic classification accuracies (%) between sterile and concentration levels of ochratoxin A contaminated barley kernels (OTA)

Statistical classifiers	Sample*	Sterile	OTA 1	OTA 2	OTA 3	OTA 4	OTA 5
Classification accuracies							
Linear	Sterile	100.0	0.0	0.0	0.0	0.0	0.0
	OTA 1	0.0	98.3	1.7	0.0	0.0	0.0
	OTA 2	0.0	0.0	100	0.0	0.0	0.0
	OTA 3	0.0	0.0	0.0	100	0.0	0.0
	OTA 4	0.0	0.0	0.0	0.0	100	0.0
	OTA 5	0.0	0.0	0.0	0.0	0.0	100
Quadratic	Sterile	100	0.0	0.0	0.0	0.0	0.0
	OTA 1	0.0	98.5	1.5	0.0	0.0	0.0
	OTA 2	0.0	0.0	100	0.0	0.0	0.0
	OTA 3	0.0	0.0	0.0	100	0.0	0.0
	OTA 4	0.0	0.0	0.0	0.0	100	0.0
	OTA 5	0.0	0.0	0.0	0.0	0.0	100

*sample size- 300 kernels.

Table 5. Six-class linear and quadratic classification accuracies between sterile control and infection periods of non-ochratoxin A producing *P. verrucosum* infected barley kernels (Non-OTA).

Statistical classifiers	Sample*	Sterile	Classification accuracies				
			Non-OTA 1	Non-OTA 2	Non-OTA 3	Non-OTA 4	Non-OTA 5
Linear							
	Sterile	100	0.0	0.0	0.0	0.0	0.0
	Non-OTA 1	0.0	98.4	1.6	0.0	0.0	0.0
	Non-OTA 2	0.0	0.0	100	0.0	0.0	0.0
	Non-OTA 3	0.0	0.0	0.0	100	0.0	0.0
	Non-OTA 4	0.0	0.0	0.0	0.0	100	0.0
	Non-OTA 5	0.0	0.0	0.0	0.0	0.0	100
Quadratic							
	Sterile	100	0.0	0.0	0.0	0.0	0.0
	Non-OTA 1	0.0	98.9	1.1	0.0	0.0	0.0
	Non-OTA 2	0.0	0.0	100	0.0	0.0	0.0
	Non-OTA 3	0.0	0.0	0.0	100	0.0	0.0
	Non-OTA 4	0.0	0.0	0.0	0.0	100	0.0
	Non-OTA 5	0.0	0.0	0.0	0.0	0.0	100

*sample size - 300 kernels.

significant wave lengths corresponding to ochratoxin A contaminated kernels were different from significant wave lengths corresponding to non-ochratoxin A producing *P. verrucosum* infected kernels.

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