

Comparison of Sample Preparation Methods on the Infected Corn Seed Detection by NIR Spectroscopy

Thawatchai Phetkaeo^{1,2,4*}, Rungnapha Klaithin^{2,4}, Parichat Theanjumpol^{2,4},
Kaewalin Kunasakdakul^{2,3,4}, Sa-nguansak Thanapornpoonpong^{1,2,4}
and Suchada Vearasilp^{1,2,4}

¹Department of Plant Science and Natural Resources, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

²Postharvest Technology Research Institute, Chiang Mai University, Chiang Mai 50200, Thailand

³Department of Entomology and Plant pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

⁴Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10400, Thailand

*Corresponding author. E-mail: veloma_99@hotmail.com

ABSTRACT

*The purpose of this study was to compare the sample preparation for detection of the corn seed infected with *Aspergillus flavus* by NIR (near infrared) spectroscopy. There were two kinds of samples: whole grain corn, and ground corn seed. The spectra of non-infected (normal seed) and infected seeds at the ratio of 5, 10, 15 and 20% w/w were scanned by using NIRSystem 6500 in the wavelength range 1100-2500 nm. The ground sample was prepared by laboratory mill, then its spectrum was scanned using the same process as the whole infected grain. The mathematical techniques such as second derivative, multiplicative scatter correction and second derivative were used to transform the NIR spectra. The calibration equations to predict the infected quantity of infected seeds of whole grain and ground samples were developed by partial least squares regression (PLSR). It was found that the calibration equation for the infected seed prediction of ground sample provided the higher value of the correlation coefficient (R) and ratio of standard deviation of reference data in validation set to SEP (RPD) than whole grain samples which were 0.98, 5.36 and 0.80, 1.74 respectively. Meanwhile, the standard errors of calibration (SEC), the standard errors of prediction (SEP) and the averages of difference between actual and NIR values (Bias) of ground sample were lower than those from whole grain sample. There were 4.18, 4.08, 0.54% and 1.17, 1.32, 0.38% respectively. Therefore, ground sample is suggested before subject to NIR spectroscopy to detect the infected maize seed.*

Key words: Corn, Near infrared spectroscopy, *Aspergillus flavus*

INTRODUCTION

A. flavus produces aflatoxin as a secondary metabolite in the seeds of a number of crops both before and after harvest (Klich, 2007). Corn is an important food and forage crop. (Krishnamurthi, 1969). During corn storage, grains undergo quantitative and qualitative losses. The losses occur mainly because of improper storage. A large number of pathogenic fungi, bacteria, viruses and insects, infecting corn grain cause combined worldwide annual losses of 9.4% (Shurtleff, 1980). *A. flavus* is a minor pathogen of corn, peanuts and cotton (Taubenhaus, 1920). In corn, *A. flavus* may be brought to the surface of developing seeds by insects or by colonizing the silks and growing down to the seed area. The ear is colonized from the tip down. Although, the fungus does not infect the kernel until the kernel is nearly mature (Payne, 1998). Fungi affect the quality of grain through increase in fatty acid, reduction in germination, mustiness and finally spoilage of grain (Hiscocks,

1965). Many commercial cereals are analyzed routinely for quality components (Williams et al., 1984, 1985). Some NIRS equations have been developed for detection of mold in grain and forages: Roberts et al. (1987) have been used to estimate mold in alfalfa (*Medicago sativa* L.) and used to relate the presence of glucosamine to visible mold scores in contaminated barley and to develop an equation for barley glucosamine (Roberts et al., 1991). Phetkiao et al. (2011a) used VIS/NIR spectroscopy to specify the identify of *A. flavus* and *A. niger* isolated from maize seed, and reported that VIS/NIR spectroscopy could detected the differential quantity of maize seed infected with *A. flavus* (Phetkiao et al., 2011b). Klaithin et al. (2011) reported that near infrared spectroscopy could detect the *A. flavus* infected milled rice. Kaewcheenchai et al. (2010) compared the performance of NIR calibration models for total oil content prediction in corn kernels by different corn preparations. It was found that different sample preparation methods affected the performance of the models. Furthermore, there were some approaches in evaluating NIRS calibrations by varying method of sample preparations in order to achieve a significant improvement result over previous methods. The purpose of this experiment was to estimate accurately mycelia contamination, so that the data could be used with the near-infrared spectroscopy (NIRs) to develop a prediction equation for fungal contamination in corn and to compare the different sample preparations in detection of infected corn seed by NIR spectroscopy. Thus, this experiment aimed to evaluate near infrared technique alternative to chemical analysis.

MATERIALS AND METHODS

The experiment was conducted at Postharvest Technology Research Institute, Chiang Mai University. Thirty kilograms corn seed var. DK 888 were divided into two lots, which were: non-infected lot and the lot which was mixed with *A. flavus* in various ratios. The first lot, the surface of non-infected grain was disinfected by dipping in a 0.1% sodium hypochlorite solution for 1 min, rinsing in sterile water and drying on paper. The infected corn were prepared by inoculation with 1.4 ml of *A. flavus* spore suspension, of which their concentrations referred to of approximately 10^6 spores/ml. Later on, it was placed on potato dextrose agar (PDA) and incubated at 37°C for 4 days. When the mycelium was presented on the infected corn seed, it was used to mix into the second corn seed lot at the ratio of 0, 5, 10, 15 and 20% w/w. There were two methods: the method using whole grain sample and ground grain sample. The numbers of 30 samples in each mixing ratio were used. The samples were contained in the pasting cell (the sample weight ~ 200 g per samples and 30 samples per treatments) the whole grain sample from seed non-infected (normal seed) and the infected seeds were scanned to reflectance spectra by using NIRSystem 6500 (FOSS NIRSystem, Silver Spring, USA) in the wavelength range 1100-2500 nm. After that, all samples were ground by the sample mill instrument. The ground samples were scanned with the same process as the whole grain samples. The mathematical techniques were used to transform the NIR spectrum with the Unscrambler® version 7.6 (Camo, Oslo, Norway).

RESULTS AND DISCUSSION

The original spectra of whole grain found peaks at 1436, 1756, 1930, 2334 and 2372 nm (Figure 1). Whereas the ground corn found peaks at 1436, 1758, 2332 and 2368 nm (Figure 2).

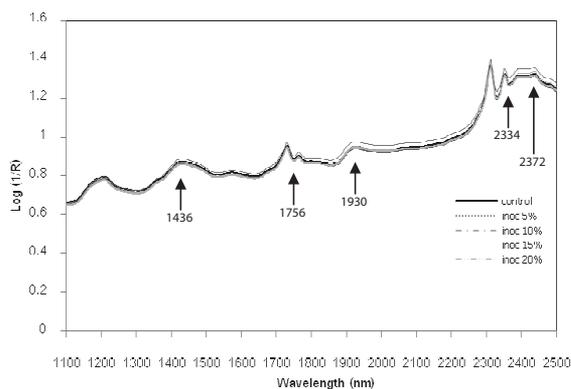


Figure 1. Original spectrum of whole grain (normal seed) and mixture of whole grain infected with *Aspergillus flavus* in wavelength range 1100-2500 nm.

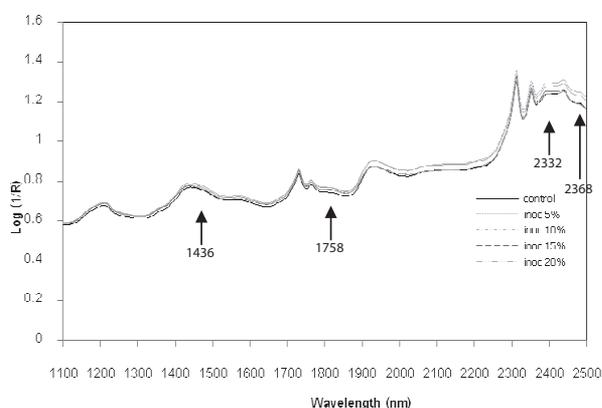


Figure 2. Original spectrum of ground corn (normal seed) and mixture of ground corn infected with *Aspergillus flavus* in wavelength range 1100-2500 nm.

The bands at 1436, 1756, 1758 and 1930 correspond to O-H stretching modes of water (Iwamoto et al., 1995; Ozaki, 2002; Williams and Norris, 2001; Büning-Pfaue, 2003; Osborne et al., 1993). On account of the fact that water was a main composition chemical element of Agricultural material. At peaks 2332 and 2334 nm related to C-H stretching and C-H deformation combination mode of CH₂ and starch. At peaks that 2368 and 2372 nm related to C-N-C symmetric stretching 1st overtone of protein (Osborne et al., 1993; Shenk et al., 2001; Williams and Norris, 2001). Whereas their bonds involving hydrogen have higher anharmonicity and stronger overtone absorptions, they could absorb NIRS result in overlapping with the peak of the molecular components. The absorbance of the whole grain was higher than the ground corn that relationship with the scattering of light. Similar to the result from Manley et al. (1994), reported that the log 1/R values of reflectance spectra of whole grain wheat higher than NIR reflectance spectra of ground grain wheat samples. Norris and Williams (1984) showed the log 1/R values of reflectance spectra of ground grain samples which were affected the particle size, with coarser samples having higher absorption and higher log 1/R values. When comparing the NIRs absorbance between the non infected whole grain (normal seed) and the infected whole grain seed and ground grain (normal seed) and the ground infected seed, it was found that absorbance of non-infected samples decreased more than fungal mixed samples.

The calibration equations to predict the infected quantities of infected seed were developed by partial least squares regression (PLSR). The spectral data for PLSR model development of whole

grain were transformed with second derivative (5 nm averaging for left and right side). PLSR model of whole grain obtained the value of the correlation coefficient (R), the standard errors of calibration (SEC) and the standard errors of prediction (SEP) of 0.80, 4.18%, 4.08% respectively (Table 1). Whereas the spectral data for PLSR model development of ground corn were transformed with multiplicative scatter correction and second derivative (5 nm averaging for left and right side). The best calibration equation PLSR of ground corn obtained the value of the correlation coefficient (R), the standard errors of calibration (SEC) and the standard errors of prediction (SEP) of 0.98, 1.17%, 1.32% respectively (Table 2).

Table 1. PLSR calibration results for detection whole grain infected by *Aspergillus flavus*.

Pre-treatment	Wavelength region (nm)	F	R	SEC	SEP	Bias	RPD
MSC	1100-2500	4	0.79	4.35	4.36	1.11	1.63
2 nd Derivative 5	1110-2450	5	0.80	4.18	4.08	0.54	1.74
MSC + 2 nd Derivative 5	1550-2488	6	0.81	1.15	4.58	0.44	1.55

MSC: multiplicative scatter correction, 2nd Derivative: second derivative, F: latent variables or number of factors used in the calibration equation, R: multiple correlation coefficients, SEC: standard error of calibration, SEP: standard error of prediction, Bias: average of difference between actual value and NIR value, RPD: ratio of standard deviation of reference data in validation set to SEP.

Table 2. PLSR calibration results for detection ground corn infected by *Aspergillus flavus*.

Pre-treatment	Wavelength region (nm)	F	R	SEC	SEP	Bias	RPD
MSC	1100-2500	5	0.95	2.18	2.08	0.46	3.40
2 nd Derivative 10	1150-2480	7	0.98	1.22	1.34	0.43	5.28
MSC + 2 nd Derivative 5	1110-2450	8	0.98	1.17	1.32	0.38	5.36

Regression coefficient of the calibration equation of whole grain and ground corn samples found various peaks 1470, 1522, 1806, 1898, 2110, 2144, 2176, 2362 nm and 1482, 1900, 2096, 2146, 2174, 2360 nm respectively (Figure 3 and 4). At peaks at 1470, 1482, 1806, 2144, 2146, 2174 and 2176 nm related to fungal infection, could be assigned to the first overtone of OH stretching modes of glucose, NH in most amino acids and CH combination bands in cis unsaturation (Berardo et al., 2005; Fernández-Ibañez et al., 2009). At peak 1522 nm corresponding to the third overtone of OH deformation hydroxyl group CO stretching of primary amides, which could be ascribed to cutin and β -glucan (Berardo et al., 2005). The bands of pure ergosterol were observed at peaks 1898 and 1900 nm corresponding at the first overtone attributed to CH stretching mode corresponding at the CH₂ group and the third overtone rings deformation mode attributed to -CH=CH₃ groups of benzene and the first overtone of O-H stretching O-H deformation-combination modes corresponding to hydroxyl-CH=CH-, -CH=CH₂- and the third overtone deformation mode of CH (Berardo et al., 2005). At peaks 2096, 2110, 2360 and 2362 nm present peaks of glucosamine were fungal cell wall polymers (Roberts et al., 1987, 1991). Therefore, ground sample is suggested before subject to NIR spectroscopy to detect the infected corn seed.

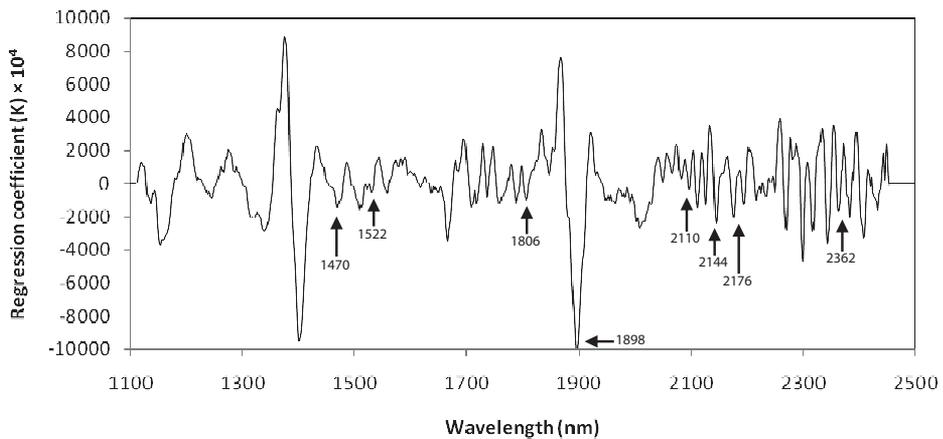


Figure 3. Regression coefficient plots for whole grain samples infected by *Aspergillus flavus* calibration equation.

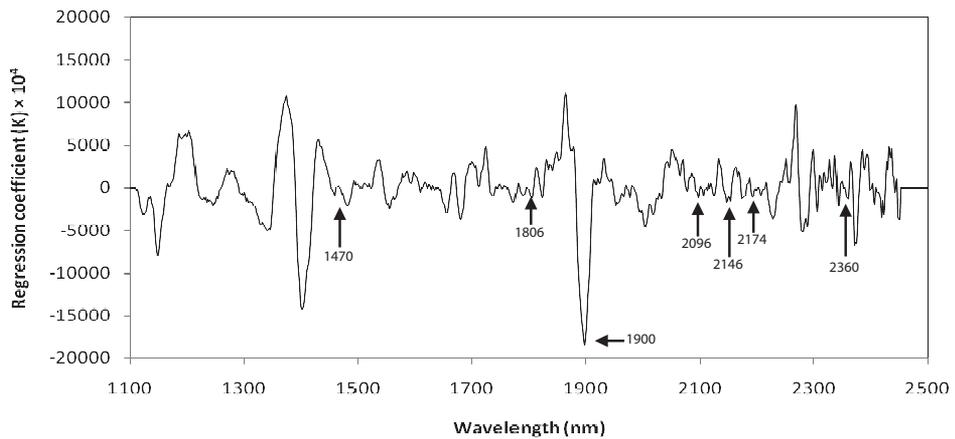


Figure 4. Regression coefficient plots for ground grain sample infected by *Aspergillus flavus* calibration equation.

CONCLUSION

The experiment described effectively that NIR reflectance can be successfully applied to the analysis of detection of *A. flavus* infected corn seed. In addition, whole grain sample had a significant response to NIR reflectance spectra. Therefore, particle size and sample size may introduce variation into absorbance spectra and reduce the accuracy of analysis. Whole grain sample had response spectra in the NIR region less than ground corn. Data pretreatments such as MSC, first or second derivatives and the first or second derivatives with MSC could compare of different corn preparations. Moreover, the best methods of whole grain corn sample were second derivative (5 nm averaging for left and right side) and ground corn seed sample were multiplicative scatter correction and second derivative (5 nm averaging for left and right side). However, ground sample is suggested before subject to NIR spectroscopy to detect the infected corn seed.

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