Estimating Deoxynivalenol Content of Ground Oats Using VIS-NIR Spectroscopy

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ABSTRACT

The potential of VIS-NIR spectroscopy as a rapid screening method for resistance of *Fusarium*-inoculated oats to replace the costly chemical measurements of deoxynivalenol (DON) was investigated. Partial Least Squares (PLS) regression was conducted on 2nd derivative spectra (400-2350 nm) of 166 DON-contaminated samples (0.05-28.1 ppm, mean= 13.06 ppm) using separate calibration and test set samples. The calibration set had 111 samples and the test set had 55 samples. The best model developed had 3 PLS components and a root mean square error of prediction (RMSEP) of 3.16 ppm. The RPD value of the prediction model was 2.63, an acceptable value for the purpose of rough screening. Visual inspection and the VIS spectra of the samples revealed that high-DON samples tended to be darker in colour and coarser in texture compared to the low-DON samples. The second derivative spectra showed that low-DON samples tended to have more water and fat content than high-DON samples. With an RMSEP value of 3.16 and RPD of value of 2.63, it seems possible to use VIS-NIR spectroscopy to semi-quantitatively estimate DON content of oats and discard the worst genotypes during the early stages of screening.

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Abbreviations

DON- Deoxynivalenol, FDK- Fusarium Damaged Kernels, FHB- Fusarium Head Blight, PLS- Partial Least Squares, RMSECV- Root Mean Square Error of Cross Validation, RMSEP- Root Mean Square Error of Prediction, RPD- ratio of standard error of prediction to standard error of the measured values, SKNIRS- Single Kernel Near Infra-Red Spectroscopy.

INTRODUCTION

Fusarium head blight (FHB) is an important disease of small grain cereals such as wheat, barley, and oats. It is caused by several *Fusarium* species, but the most important and frequently isolated ones in northern and central Europe are *F. graminearum, F. culmorum* and *F. avenaceum* (Bottalico and Perrone, 2002; Parry et al., 1995). The disease is favoured by warm weather accompanied by prolonged moist conditions at and around flowering (McMullen et al., 1997; Parry et al., 1995).

Fusarium head blight leads to floret sterility and light-weight scabby kernels causing overall yield reduction (Dexter et al., 1996; Goswami and Kistler, 2004; Parry et al., 1995). In addition to yield reduction, FHB is also associated with reductions in malting quality of barley (Schwarz et al., 2001) and milling and baking quality of wheat (Dexter et al., 1996; Wang et al., 2005). *Fusarium*-infected kernels show weaker gluten strength, reduced loaf volume and deterioration in pasta colour (Dexter et al., 1996; Dexter et al., 1997). Increase in reducing sugars and non-starch lipids and decreases in cellulose, hemicellulose, glutenin and water-extractable protein were reported in *Fusarium* damaged kernels (FDK) (Boyacioglu and Hettiarachchy, 1995).

Mycotoxins produced by *Fusarium* spp. are of major concern due to the associated health risks to humans and animals (Bergsjø et al., 1993; Ciegler, 1978; Parry et al., 1995). Symptoms can be manifested as severe vomiting, bloody diarrhoea, extensive haemorrhaging, decreased weight gain, feed refusal, dermatitis, reproductive disorders and death (Bergsjø et al., 1993; Ciegler, 1978; D'Mello et al., 1999). Therefore, the European Commission (EC) has set maximum permissible limits for *Fusarium* mycotoxins in processed and unprocessed food and feed to ensure consumer safety (Scudamore et al., 2007). The EC limit of deoxynivalenol (DON) in unprocessed wheat and oats is set at 1750 ppb, and at 500 ppb in finished cereal based food items (Scudamore et al., 2007).

Spectroscopic methods have gained attention as rapid tools for semi-quantification of DON contamination and detection of scab damage in wheat and barley (Beyer et al., 2010; Delwiche, 2003; Delwiche and Hareland, 2004; Dowell et al., 1999; Peiris et al., 2010; Ruan et al., 2002). Most of these studies focus on classification of kernels as scabby or sound with the aim of reducing DON contamination by removing the scab-damaged kernels. For example, single kernel near-infrared spectroscopy (SKNIRS) successfully classified single wheat kernels as scabby or sound. More DON-contaminated kernels were identified by this method than by visual inspection of the samples (Dowell et al., 1999). The SKNIRS method also semi-quantitatively classified wheat kernels as low-DON (< 60 ppm) or high-DON kernels (> 60 ppm) with 96% accuracy (Peiris et al., 2010). Sorting *Fusarium*-infected wheat samples by a commercial high-speed bichromatic sorter using 675 nm and 1480 nm reduced FDK level significantly (Delwiche et al., 2005). The ratio of DON concentration of the 'accepts' and the 'rejects' to that of the unsorted was 51% and 650%, respectively. Successive passes through the sorter were successful at further reducing DON contamination (Delwiche et al., 2005).

Overall shape of NIR spectra of sound and scab-damaged wheat kernels was found to be similar, but scab-damaged kernels had lower spectral absorption compared to sound kernels. This difference was suggested to be the result of the higher optical density and moisture content of the sound kernels (Delwiche, 2003). Further investigation showed that the slope of the short wavelength side of a broad carbohydrate absorption band centred at 1,200 nm was effective in discriminating sound and scab-damaged kernels with test set accuracies of 95%. The accuracy was further improved by including kernel mass in the model (Delwiche and Hareland, 2004) as scab-damaged kernels have lower kernel mass compared to sound ones.

The most economical and environmentally friendly option of managing FHB is the use of resistant cultivars. Identifying and selecting resistant cultivars involves testing and evaluating thousands of genotypes every year. This is a highly labour intensive and

economically demanding process (Mesterhazy, 2003; Steffenson, 2003). Therefore, rapid methods such as NIR spectroscopy which have previously been reported to successfully identify FDK and semi-quantitatively determine DON contamination in wheat and barley could facilitate resistance breeding programs by reducing the cost of chemical analysis. Therefore, the objectives of this study were *i)* to identify and characterize wavelength regions which are important in estimating DON content of *Fusarium*-infected oats and *ii)* to test the feasibility of NIR spectroscopy as a rapid method in quantifying DON contamination of oats originating from *Fusarium*-inoculated nurseries.

MATERIALS AND METHODS

Samples

One hundred sixty six DON-contaminated oat samples (minimum = 0.05 ppm, maximum = 28.1 ppm, mean= 13.06 ppm, and StDev= 8.40 ppm) were assembled from a large sample collection to cover a range of DON contamination relevant for evaluating FHB resistance of oat genotypes. Most of the samples (n = 135) originated from FHB resistance trials conducted from 2007- 2010 at the Vollebekk Research Farm, Norwegian University of Life Sciences. These samples consist of more than 60 genotypes from Nordic breeding companies. The FHB nurseries were inoculated with *F. graminearum*-infected oat kernels (Skinnes et al., 2010) at Zadoks 31/32 stage (Zadoks et al., 1974). Experimental plots were 0.45 m x 2.0 m spaced 0.3 m apart. Plants were mist irrigated every evening following inoculation until 10 days after the last flowering date to facilitate *Fusarium* infection. A few samples (n = 31) collected from farmers' fields were included to increase the frequency of low-DON samples. These samples were of the cultivar Belinda which was grown in 2010. The DON distribution of the samples is shown in Fig. 1.

Figure 1 comes here.

Grinding, Scanning, and Mycotoxin Analyses

The samples from the FHB nurseries were ground for two minutes using a Stein Laboratories Mill (Model M-2, Stein Laboratories Inc., Atchison, Kansas) and the samples from farmers' fields were milled into 2 mm particle size by a Perten laboratory mill (Model 3303, Perten Instruments AB, Segeltorp, Sweden). Although grinding is considered as a labour and time consuming step, scanning whole kernels was avoided to minimize noise that may arise from non-uniformity in scab damage and DON-contamination among the kernels. Kernels from *Fusarium*-affected fields are neither uniformly DON-contaminated nor infected but rather are mixtures of kernels with a spectrum of infection and DON levels (Liu et al., 1997).

All samples were scanned using XDS Rapid Content Analyser™ (FOSS Analytical, Hillerød, Denmark) measuring in the 400-2500 nm wavelength region at 0.5 nm intervals. The sample (~ 5 g) was placed in a sample holder (4 cm i.d.), covered with a disposable cup-back and was placed in the spectrophotometer. Spectra were recorded as log (1/R) using the FOSS NIR systems Vision® software. Three parallels from each sample were scanned and the spectra were averaged for each sample prior to analyses.

DON contents of the samples from the FHB nurseries were analysed at the University of Minnesota, using GC-MS (detection limit of 0.05 ppm) following the protocol described by Mirocha et al (1998) and Fuentes et al (2005), while the samples from the farmers' fields were analysed using LC-MS/MS (detection limit of 0.04 ppm) at the Finnish Food Safety Authority, EVIRA following the protocol described by Kokkonen and Jestoi (2009).

Data Analysis

Prior to analysis, the samples were split into two classes; every third sample (according to DON level) was picked out to be in the test set. This gave a calibration set of 111 samples and a test set of 55 samples. Data were analysed using The Unscrambler X, ver. 10.1 (CAMO software AS, Oslo, Norway). Spectra were pre-treated using the 2^{nd} derivative and 2^{nd}

polynomial order option of the Savitzky Golay derivatives (Savitzky and Golay, 1964) using 51 smoothing points. The smoothing was done in two separate windows (400 - 1098 nm and 1101 - 2350 nm) due to the detector shift at 1100 nm in the spectrophotometer. Partial least squares (PLS) regression (Martens and Næs, 1989) was performed on the calibration set, using full cross-validation (Efron, 1983) and the developed model was used to predict the test set samples.

RESULTS AND DISCUSSION

General Properties of the Spectra

The overall shape and general features of the spectra of the low-DON and high-DON samples were similar. However, differences in texture, density and colour in the samples influenced the light scattering properties and these differences were seen as varying apparent absorption levels in the spectra. Low-DON samples tended to have lower absorption at the VIS region and higher absorption at the NIR region than the high-DON samples (Fig. 2). Visual inspection of our samples showed that low-DON samples tended to be lighter in colour and finer in texture while high-DON samples tended to be darker in colour and coarser in texture. Brownish discoloration of the hulls and the caryopses is a common symptom in *Fusarium*-infected oat kernels (Tekle et al., 2012) and removal of such kernels has been shown to decrease level of mycotoxin contamination (Scudamore et al., 2007).

Figure 2 comes here.

Calculating the 2nd derivatives of the original spectra resolved the broad peaks in the original spectra and removed most of the light scattering effects that seemed to be relevant in classifying the samples. However, the resolved peaks in the 2nd derivative spectra were able to segregate the samples into 2- 3 classes (Fig. 3), revealing differences in chemical composition of the high- and low-DON samples. The peaks centred at ~ 492 nm and ~ 638 nm classified

the samples into three classes where as the peaks centred at ~1210 nm, ~1432 nm, and ~1924 nm classified the samples into two classes (Fig. 3). The peaks at ~1432 nm and ~1924 nm are most likely water O-H bands (Segtnan and Isaksson, 2004; Segtnan et al., 2001;Williams, 2001), indicating that low-DON samples tended to have more water than high DON samples. The fat doublets at ~1701 nm and ~1725 nm also seem to be important for calibration with low-DON samples displaying more fat content than high-DON samples (Fig. 3).

Figure 3 comes here.

Previous studies have shown that *Fusarium*-damaged and sound wheat kernels differ in their major food reserves and water content (Boyacioglu and Hettiarachchy, 1995; Delwiche, 2003; Jackowiak et al., 2005; Wang et al., 2005). Therefore, spectral differences between sound and scabby kernels were attributed to differences in major seed constituents rather than DON content *per se.* This was because of the extremely low concentration of the toxin and due to overlap of bands in the NIR region from major seed constituents (Girolamo et al., 2009; Liu et al., 2009; Siuda et al., 2008). Even though it was difficult to attribute specific spectral features to DON, the overall effects of *Fusarium* infection on the physical, chemical and structural attributes of kernels was correlated to DON contamination and was used to estimate DON level of samples (Girolamo et al., 2009; Liu et al., 2009; Siuda et al., 2008).

The Models

The models developed using the VIS, NIR, and the whole wavelength region are shown in Table 1. In addition, the models for the 400-1098 nm and 1101-2350 nm regions were also calculated by dividing the spectra at the detector shift. The best model was calculated by using the whole wavelength region. This model had only 3 PLS components, an RMSECV and RMSEP value of 4.06 ppm and 3.16 ppm, respectively (Table 1). The RPD value of value of 2.63 is considered poor, but is acceptable for rough screening of resistance at the early stages

of breeding programs (Williams, 2001). A slight non-linearity was observed in the measured versus predicted DON level plot. The error distribution was uniform along the different DON-levels. The low-DON samples tended to be over- estimated while the high-DON samples tended to be under estimated (Fig. 4). The residual validation variance remained low after the recommended 3 PLS components demonstrating the stability of the calibration model. The other calibration models that used only portions of the whole wavelength region were poorer than the model developed using the whole wavelength region, as displayed by their higher RMSEP and lower RPD values (Table 1).

Table 1 comes here

Figure 4 comes here

The accuracy of spectroscopic methods in estimating DON contamination is highly dependent on the relationship between degree of scab damage and the actual DON level of the investigated samples (Siuda et al., 2008). Apparently healthy looking kernels can be contaminated with considerable levels of DON while kernels with visible scab damage are not necessarily contaminated with corresponding high levels of DON (Dexter and Nowicki, 2003). Varietal differences and impact of growing season and location on the bulk chemistry of samples can weigh over the effect of scab damage. These factors can explain the relatively high RMSEP and the poor RPD values of our models. Therefore, it is not possible to use our model in the milling industry where much lower DON levels than the RMSEP value are relevant and a much higher RPD values than what we found are required.

CONCLUSIONS

The objective of this study was to test the feasibility of VIS-NIR spectroscopy in estimating DON contents of ground oat genotypes to evaluate their resistance to FHB. It was

possible to develop stable calibration models using the VIS and/or NIR regions; and the best model was developed by using the whole wavelength region (400-2350 nm). The model had 3 PLS components an RMSECV and RMSEP value of 4.06 and 3.16 ppm. The RMSEP was not low enough to implement the model in the milling industry. However, the model was good enough for screening purposes as the prediction error was low enough to discard the worst genotypes during successive selections as high DON levels are common in artificially inoculated nurseries. It is important to update the calibration model with new samples from different growing seasons, locations, and genotypes in order to maintain its stability and be able to predict new samples with acceptable level of prediction errors.

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Table 1: Prediction performance data for the calibration set (cross-validated results; $R^2_{(xv)}$, RMSECV and $RPD_{(xv)}$) and the test set ($R^2_{(ts)}$, RMSEP and $RPD_{(ts)}$), PLS-C gives the number of PLS components used in the different models, The calibration set consisted of 111 samples, and the test set consisted of 55 samples.

Wavelength regions	PLS-C	$R^2_{(xv)}$	$R^2_{(ts)}$	RMSECV	RMSEP	$RPD_{(xv)}$	$RPD_{(ts)}$
400- 780	5	0.72	0.79	4.47	3.8	1.9	2.19
400- 1098	6	0.74	0.82	4.28	3.49	1.98	2.38
1101- 2350	4	0.76	0.82	4.18	3.58	2.03	2.33
780.5- 2350	4	0.75	0.82	4.22	3.52	2	2.36
400- 1098 & 1101- 2350	3	0.77	0.86	4.06	3.16	2.09	2.63

PLS-C-Partial least squares components
RMSECV-Root mean square error of cross validation
RMSEP-Root mean square of prediction
$RPD_{(xv)}$- ratio of standard error of calibration to standard deviation of measured values of the calibration set
$RPD_{(ts)}$- ratio of standard error of prediction to standard deviation of measured values of the test set

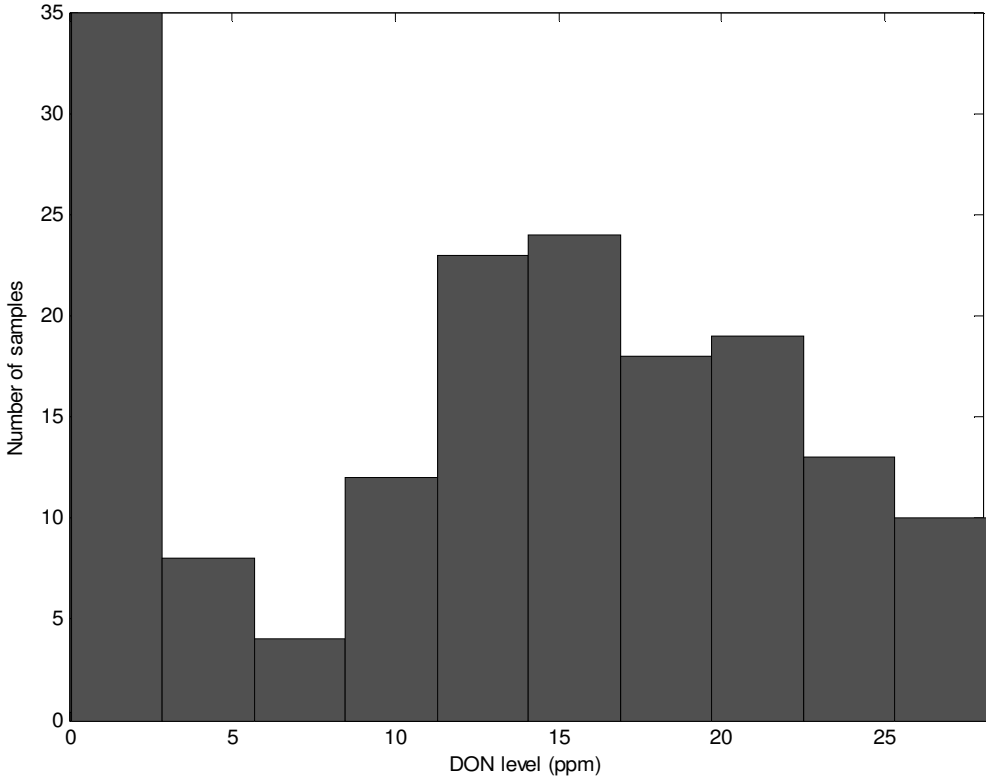

Figure 1: Frequency plot for DON levels of the 166 samples used to develop and test VIS-NIR prediction model.

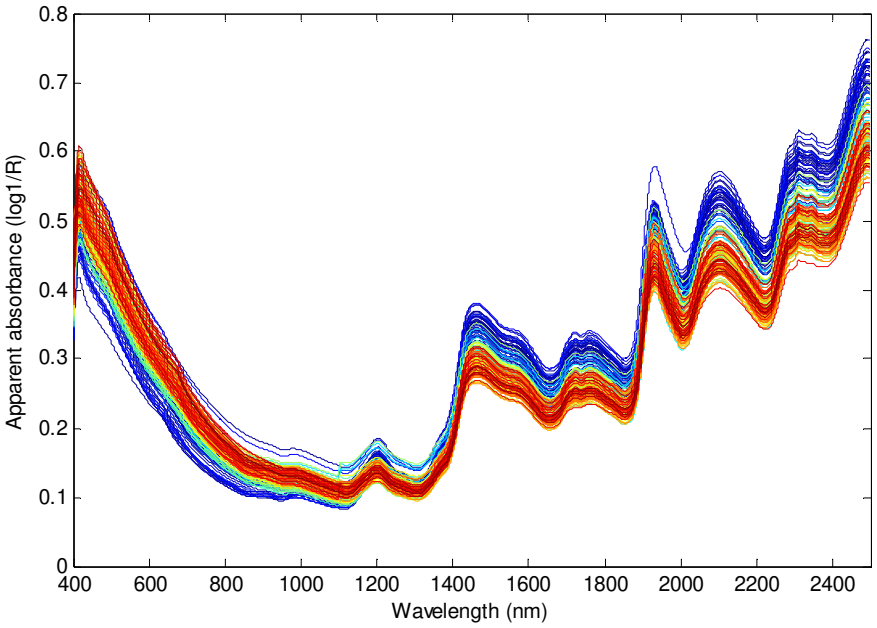

Figure 2: Original spectra of ground oat samples (*n=166*) contaminated with different levels of deoxynivalenol. Spectra are coloured according to DON levels (blue= low, green= medium, red= high).

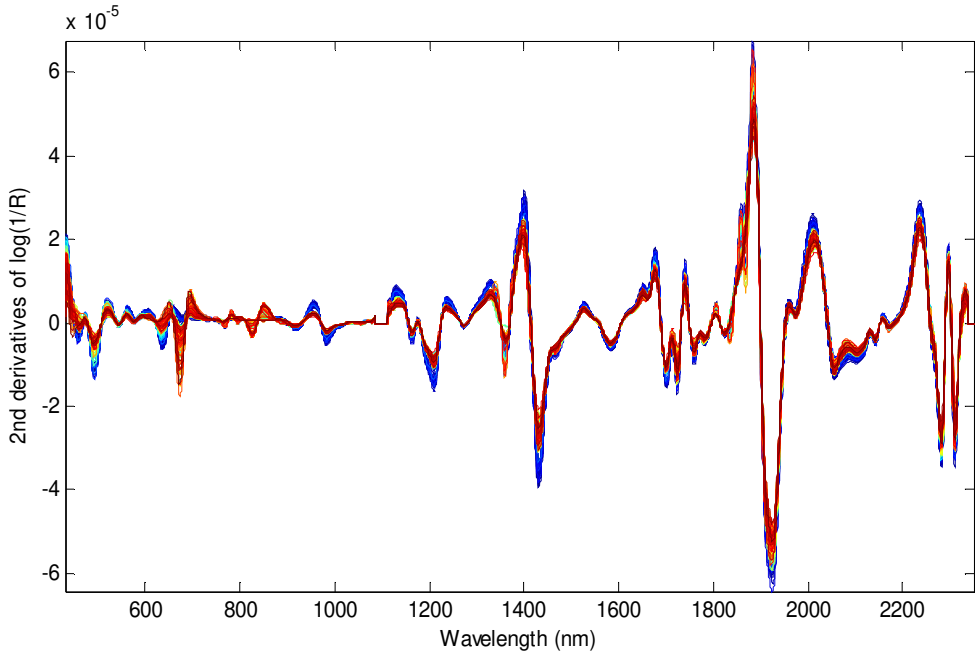

Figure 3: Second derivative spectra of ground oat samples (n=166) contaminated with different levels of deoxynivalenol. Spectra are coloured according to DON levels (blue= low, green= medium, red= high).

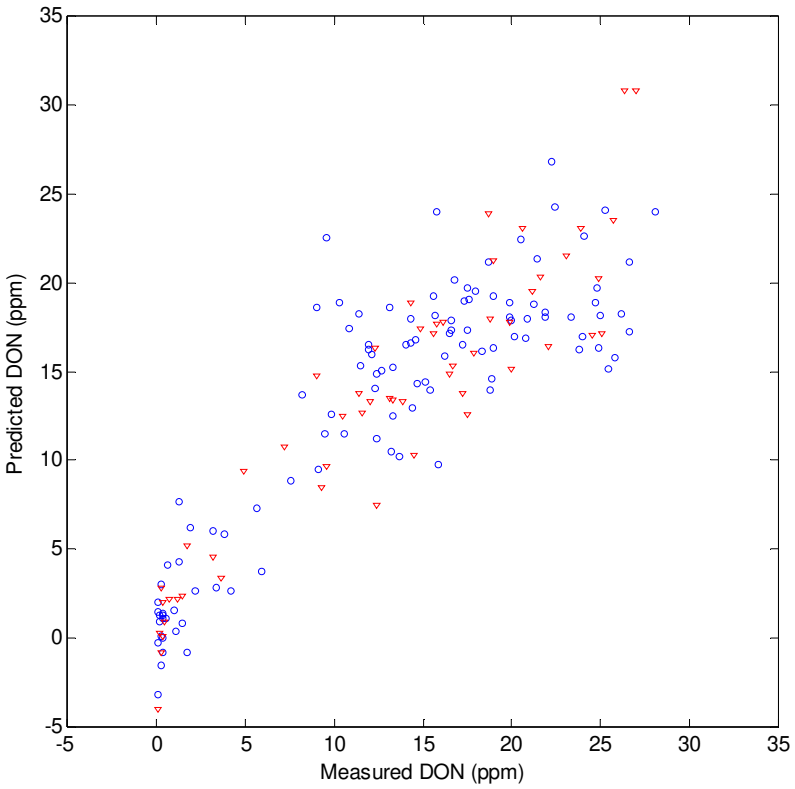

Figure 4: Reference deoxynivalenol values plotted against the predicted values using a 3-component PLS model based on the spectral region 400-2350 nm. Blue circles are cross-validated results for the calibration set (111 samples). Red triangles are test set results (55 samples).