



# NIR absorbance characteristics of deoxynivalenol and of sound and *Fusarium*-damaged wheat kernels

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The near infrared (NIR) absorption spectra of deoxynivalenol (DON) and single wheat kernels with or without DON were examined. The NIR absorption spectra of 0.5–2000 ppm of DON in acetonitrile were recorded in the 350–2500 nm range. Second derivative processing of the NIR spectra and spectral subtractions showed DON absorption bands at 1408 nm, 1904 nm and 1919 nm. NIR spectra of sound and *Fusarium*-damaged kernels were also acquired using two instruments. Subtraction of average absorption spectra and second derivative spectra were evaluated to identify different NIR signatures of the two types of kernel. Differences in peak height and positions of the NIR absorption bands of the kernels were noted. At 1204 nm, 1365 nm and 1700 nm, the differences were in the heights of the absorption peaks. Such differences may be attributed to changes in the levels of grain food reserves such as starches, proteins and lipids and other structural compounds. Shifts in absorption peak positions between the two types of kernels were observed at 1425–1440 nm and 1915–1930 nm. These differences may arise from other NIR active compounds, such as DON, which are not common for the two types of kernel. Since the NIR absorption of DON may have contributed to the shifts between sound and *Fusarium*-damaged kernels, this study indicates the potential for NIR spectrometry to evaluate *Fusarium* damage in single kernels based on the DON levels.

**Keywords:** NIR, wheat, *Fusarium* head blight, deoxynivalenol, *Fusarium*-damaged kernels

## Introduction

Deoxynivalenol [DON; 12,13-epoxy-3,7,15-trihydroxy-trichothec-9-en-8-one; CAS no. 51481-10-8] is a trichothecene mycotoxin accumulated in wheat and other small cereal grains as a result of *Fusarium* head blight (FHB) caused by *Fusarium graminearum* (*Gibberella zeae*) or *F. culmorum*.<sup>1</sup> FHB causes yield loss by causing sterility in wheat heads or by producing kernels which are shrivelled and light in weight. These FHB-infected kernels or *Fusarium*-damaged kernels (FDKs) have a chalky white or pink colour and are often referred to as scabby or tombstone kernels. Further, FHB affects the quality of food, feed, malting and brewing products

due to the presence of DON, which has been implicated in mycotoxicoses in both humans and farm animals.<sup>2</sup> FHB incidence has reached epidemic proportions in the USA and in many other wheat growing areas in the world.<sup>3,4</sup> It has been estimated that the cumulative direct economic losses from FHB in wheat and barley in nine US wheat growing states were \$2.5 billion from 1993 through to 2001. The combined direct and secondary economic losses for all the crops were estimated at \$7.7 billion.<sup>5</sup> Development of resistant cultivars is regarded as one of the most economical methods for controlling FHB damage in wheat.

Many analytical methods have been developed for the detection and quantification of DON and other trichothecenes in cereals, such as thin-layer chromatography, liquid, gas and supercritical fluid chromatography and immunochemical methods.<sup>2</sup> Though quite accurate, these methods are expensive, time-intensive and destructive to the sample. Evaluating the resistance of wheat breeding germplasm to FHB could be greatly expedited if breeders had access to rapid, non-destructive methods for identifying FDKs and for estimating their DON levels. Non-destructive methods such as optical sorting,<sup>6</sup> image-based machine vision<sup>7</sup> and density separation techniques<sup>8</sup> have been developed to identify FDKs of wheat to assess the damage caused by FHB disease. However, such methods are unable to detect FDKs based on their DON levels.

*Fusarium*-infected grains, including both FDKs and DON containing asymptomatic grains, have varying levels of DON in response to the FHB infection. The DON levels of FDKs depend on many factors such as the virulence of the strain of the *Fusarium* pathogen, resistance of the cultivar to FHB, the time of infection relative to the growth and developmental stage of the wheat head and the climatic conditions before and during the period of infection.<sup>9–13</sup> Other fungi and insect damage can also cause scabby kernels that may not contain DON. Moreover, the presence of DON in asymptomatic kernels has also been reported,<sup>13–15</sup> probably as a result of DON translocation within various tissues in the infected wheat head.<sup>16</sup> This limits the feasibility of techniques such as colour sorting, machine vision or density separation to detect *Fusarium*-infected kernels that are asymptomatic or to differentiate scabby kernels without DON. When a large number of small samples are evaluated, rapid, single-kernel, non-destructive methods for detecting FDKs based on DON levels become very important. Rapid, single-kernel, near infrared spectrometric methods have been developed that can successfully identify FDKs and estimate their DON levels with a standard error of 44 ppm.<sup>17</sup>

Improvements to NIR spectrometric techniques may be realised if DON has unique NIR spectral signatures and, hence, influences the NIR absorption of the FDKs. Ruan *et al.*<sup>18</sup> demonstrated that NIR absorbance is influenced by DON levels of grains and showed that NIR spectroscopy could be used to determine DON levels in barley using a neural network model.

There has been no attempt to study the NIR absorbance of DON in pure form and as a component in grains. Because this NIR absorption may be used to detect DON in FDKs and evaluate *Fusarium* damage based on DON levels, it is important to study these NIR absorption spectra. This manuscript presents a characterisation of the NIR absorption spectra of pure DON and sound and FDKs.

## Materials and methods

### NIR scanning of DON

The NIR spectra of DON were collected using an ASD QualitySpec Pro spectrometer (ASD Inc, Boulder, CO, USA).

DON in pure crystalline form (Sigma Aldrich) was dissolved in acetonitrile (C<sub>2</sub>H<sub>3</sub>N) to prepare a 2000 ppm stock solution. It was serially diluted with acetonitrile to prepare a series of solutions ranging down to 0.5 ppm DON. The transmission spectra of these solutions were acquired in IR quartz cuvettes (10 mm path length) in the 350–2500 nm region. Three different spectra were collected for each DON concentration.

### NIR scanning of sound and FDKs

The NIR spectra of wheat (Variety Wheaton) were collected using 15 kernels each of sound kernels and FDKs. The kernels were extracted from uninfected and artificially inoculated wheat heads. For artificial inoculation, the heads were inoculated at anthesis by injecting a single central floret with 10 µL macroconidia ( $1 \times 10^5 \text{ mL}^{-1}$ ) of *F. graminearum* isolate Z-3639 (USDA-ARS Culture Collection accession NRRL 29169) and covering with plastic bags for 48 h. From these wheat heads, previous unpublished single kernel DON measurements by gas chromatography-mass spectrometry showed that sound kernels from uninoculated healthy heads had non-detectable DON levels, but FDKs of the artificially inoculated wheat heads had DON levels ranging from 33–1008 ppm.

Wheat kernels were scanned using a single-kernel NIR (SKNIR) system (Pertin Instruments, Stockholm, Sweden), which is used for automatic sorting of kernels and a high resolution VIS-NIR ASD QualitySpec Pro spectrometer. The SKNIR system coupled with a 256-element InGaAs detector with a resolution of 3.125 nm per diode scanned kernels in the 950–1650 nm range and recorded spectra in 5 nm intervals. The ASD QualitySpec Pro spectrometer with a spectral resolution of 3 nm at 700 nm and 10 nm at 1400 nm and 2100 nm had a scanning range of 350–2500 nm with a sampling interval of 1.4 nm for the 350–1000 nm region and 2 nm for the 1000–2500 nm region and the associated spectral software interpolated the spectral data collected at 1 nm intervals. Data on each kernel were acquired twice in two different positions. The SKNIR system automatically feeds kernels into the viewing bucket and orients them by vibrating the kernels during manual mode scanning. For scanning with the ASD QualitySpec Pro spectrometer, kernels were manually positioned between the tip of the fibre-optic probe (ASD Chem Reflectance Probe/135320, ASD Inc, Boulder, CO, USA) and the Spectralon standard, spaced 8 mm apart and coupled to the irradiating/collecting points of the ASD spectrometer. The fibre-optic probe had 33 illuminating and 4 collecting 200 micron core fibres.

### Analysis of spectral data

The SKNIR scans were automatically saved as GRAMS/AI, Version 8.0 (Thermo Fisher Scientific, Waltham, MA, USA) .spc files. The ASD scans saved as .asd files were converted to .spc format using the RS3 (ASD Inc, Boulder, CO) and the GRAIMS/AI software packages. Second derivative processing of the spectral data was carried out with the Savitsky–Golay procedure of the GRAMS/AI software, which used a second-degree

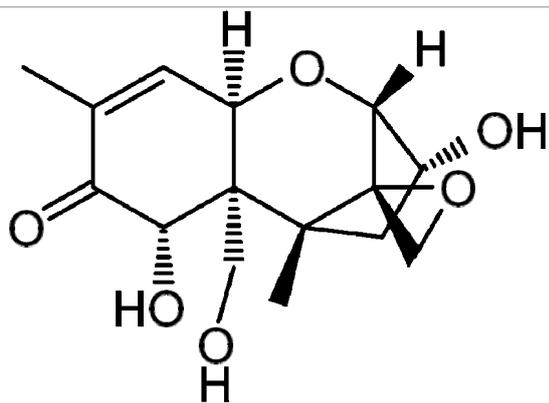


Figure 1. The structure of deoxynivalenol [(3 $\alpha$ ,7 $\alpha$ )-3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one]

polynomial with 15 data points to identify peak positions in the composite spectra.<sup>19</sup>

## Results and discussion

### NIR absorption of DON

The DON molecule (Figure 1) has a number of –OH, –C=O and –CH functional groups. The overtone frequencies of the stretching and bending vibrations of those functional groups fall in the NIR region. Therefore, the NIR transmission spectra of DON solutions were investigated to identify specific absorption bands. The absorbance spectra (in OD units) or log [1/*T*] spectra of solutions having 0.5 ppm to 2000 ppm of DON in

acetonitrile are presented in Figure 2. There were no visible absorption bands in the spectral region below 1000 nm. The region after 2200 nm is also typically noisy because of the poor sensitivity of the sensor. Therefore, ASD spectrometer spectral data in figures are presented only between 1000–2100 nm. The strong absorption band with a peak around 1717 nm showed noise due to a high OD above 2.0. This strong absorption band was due to the –CH stretch first overtone of the –CH<sub>3</sub> group of the acetonitrile molecule.

Changes in NIR absorption due to differences in the DON concentration were subtly visible in the 1390–1440 nm and 1880–1950 nm regions of the OD spectra (Figure 2). To amplify those differences, each spectrum was subtracted from the 2000 ppm spectrum and the resulting difference spectra are presented in Figure 3. These difference spectra clearly showed the position and strength of the NIR absorption bands of DON. The absorption band peaked at 1414 nm (Table 1) is probably due to the first overtone of –OH groups of DON while the ~10-fold stronger band at 1906 nm may be due to the second overtone of –C=O and R–OH vibrations of the DON molecules. Second derivatives of the spectra were analysed to further resolve these two band positions. Spectral subtraction of the second derivative spectra identified three DON absorption bands with peaks at 1408 nm, 1904 nm and 1919 nm (Figure 4, Table 1). The band at 1408 nm may arise from the first overtone of the –OH and the two bands at 1904 and 1919 nm may be due to the second overtones of the –C=O group.<sup>20</sup> These results indicate that DON has distinct NIR absorption bands.

### NIR absorption of sound and FDKs

The average absorption spectra for the two types of kernel and the difference spectrum are shown in Figure 5. The difference

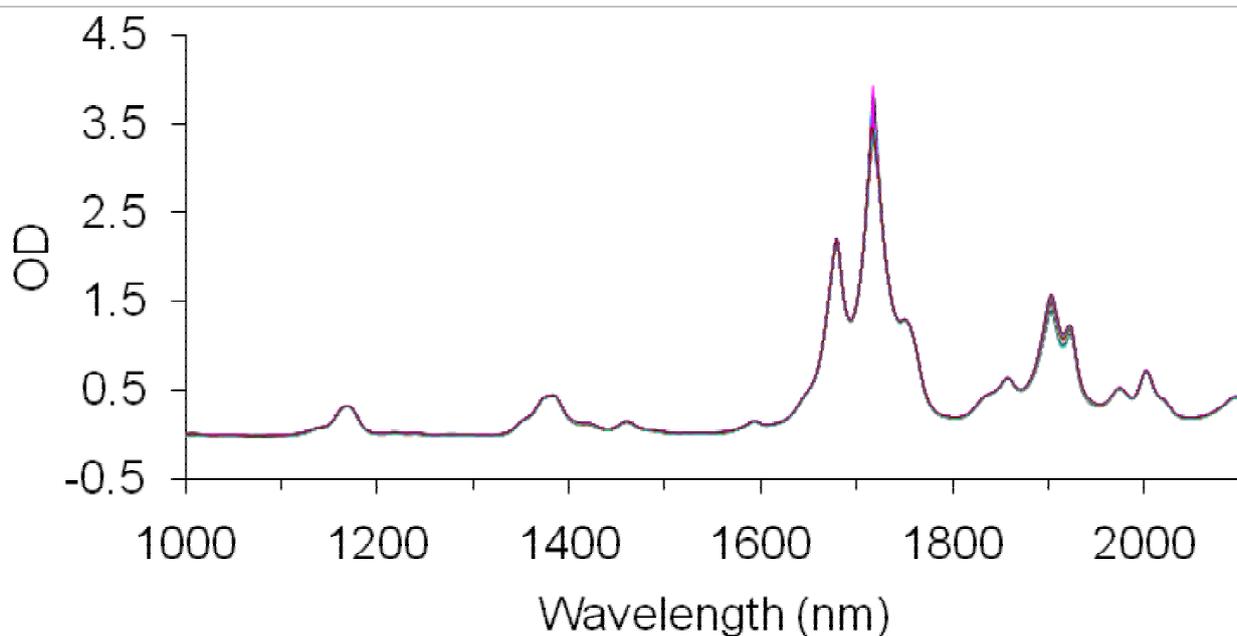
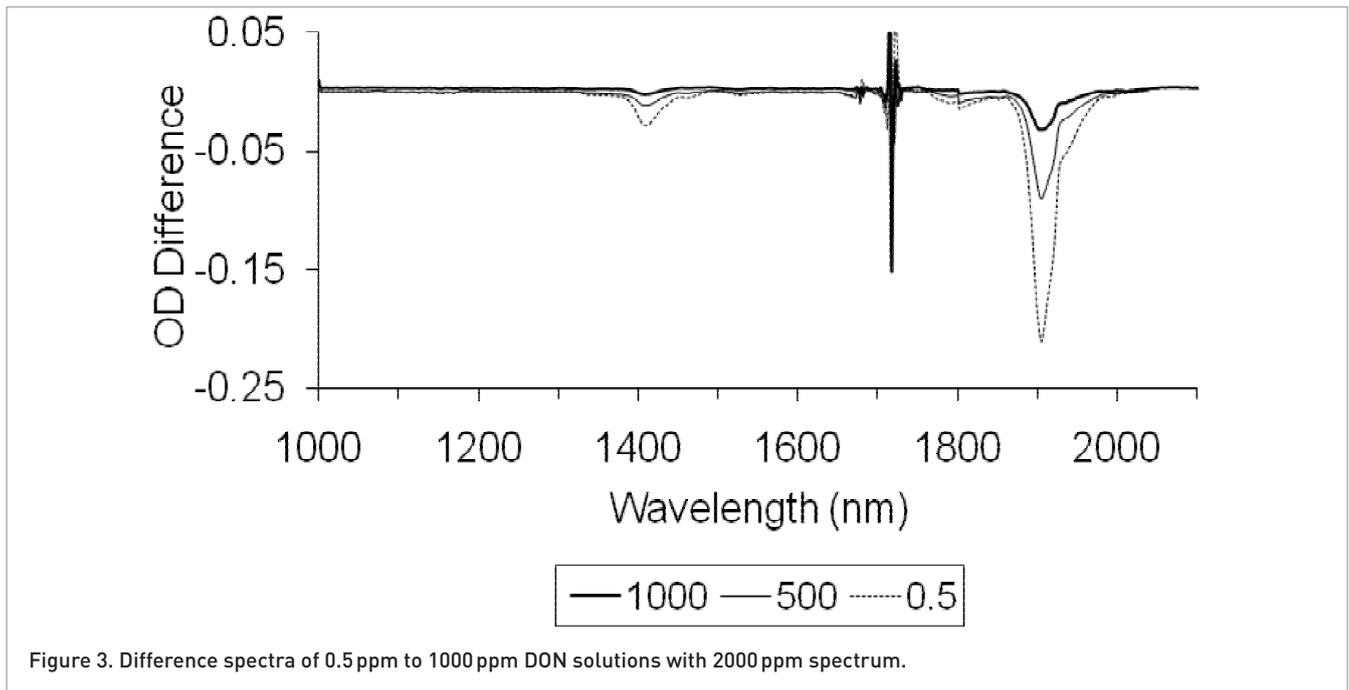
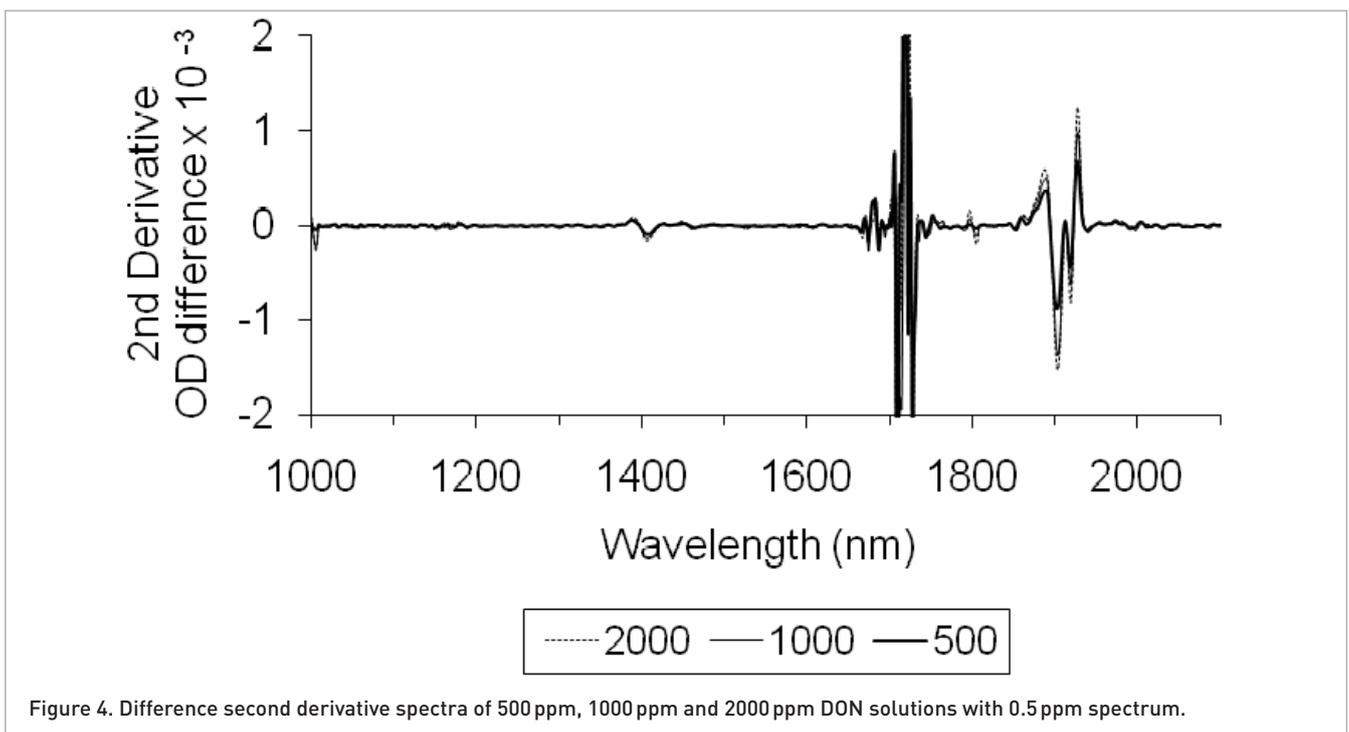


Figure 2. ASD spectra of DON solutions from 0.5 ppm to 2000 ppm. Path length = 10 mm.



spectrum indicated two broad bands around 1205 nm and after 1400 nm. These bands were further resolved by examining second derivative spectra (Figure 6), which showed two clear absorption peaks at 1195 nm and 1425 nm. The band at 1195 nm, which was found in both types of kernel, is in the CH second overtone region and may result from differences in the levels of stored food reserves such as carbohydrates, proteins

and lipids. *Fusarium* infection depletes food reserves for fungal growth and the kernels shrink and become lighter.<sup>21,22</sup> In the 1400 nm region, the absorption band for sound kernels had its peak at 1430 nm while that of the FDKs was at 1445 nm. The difference peak was at 1425 nm. The higher wavelength position of the peak in the FDK spectra, relative to the sound kernels, may be due to the influence of DON found in FDKs.



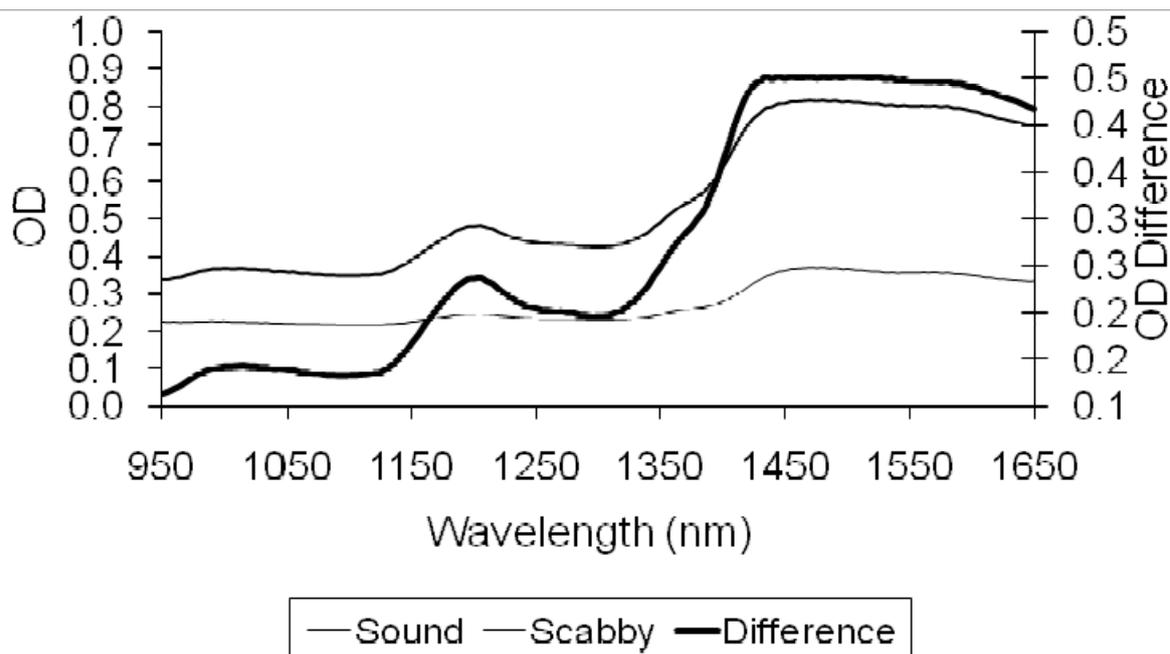


Figure 5. Average SKNIR spectra of sound and FDKs (scabby) and the difference spectrum.

The NIR spectra of sound and FDKs collected with the ASD spectrometer were of kernels scanned in the 350–2500 nm range; however, the spectra are presented for the 1000–2100 nm range, since most of the clear absorption peaks are present within this range. The raw absorption spectra show a number

of bands. The difference spectrum of the averaged spectra of sound kernels and FDKs (Figure 7) highlighted the bands with peaks around 1208 nm, 1440 nm, 1905 nm and 2001 nm (Table 1). The second derivative spectra (Figure 8) were used to resolve the absorption peaks of the sound kernels and FDKs.

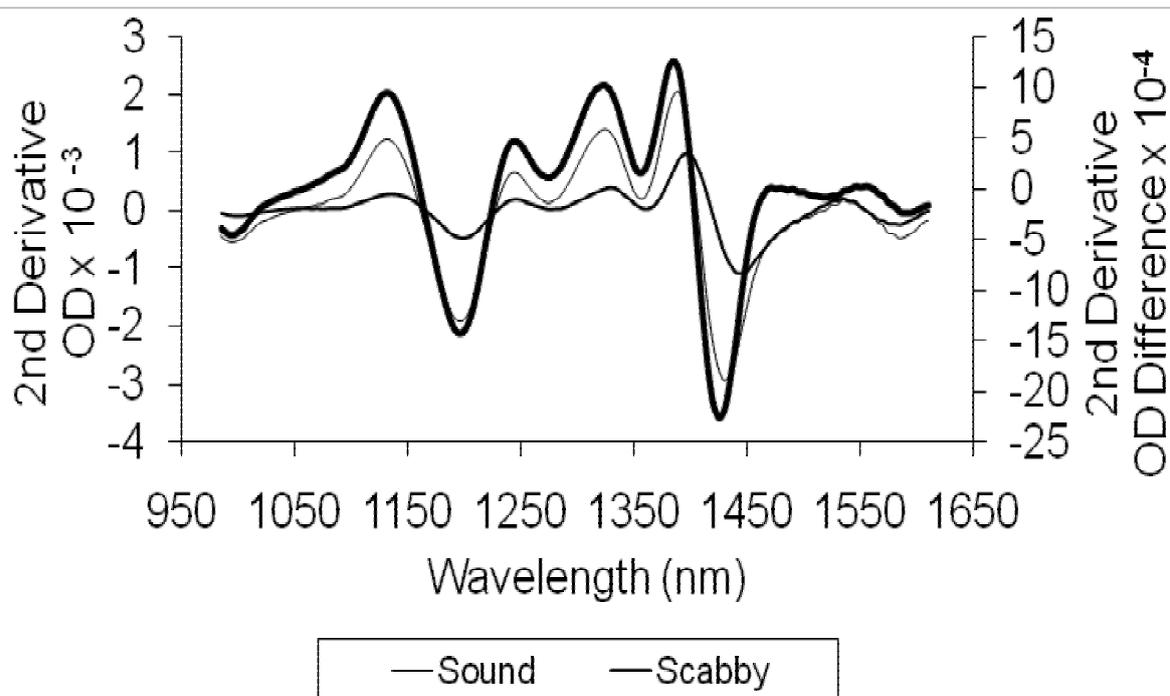
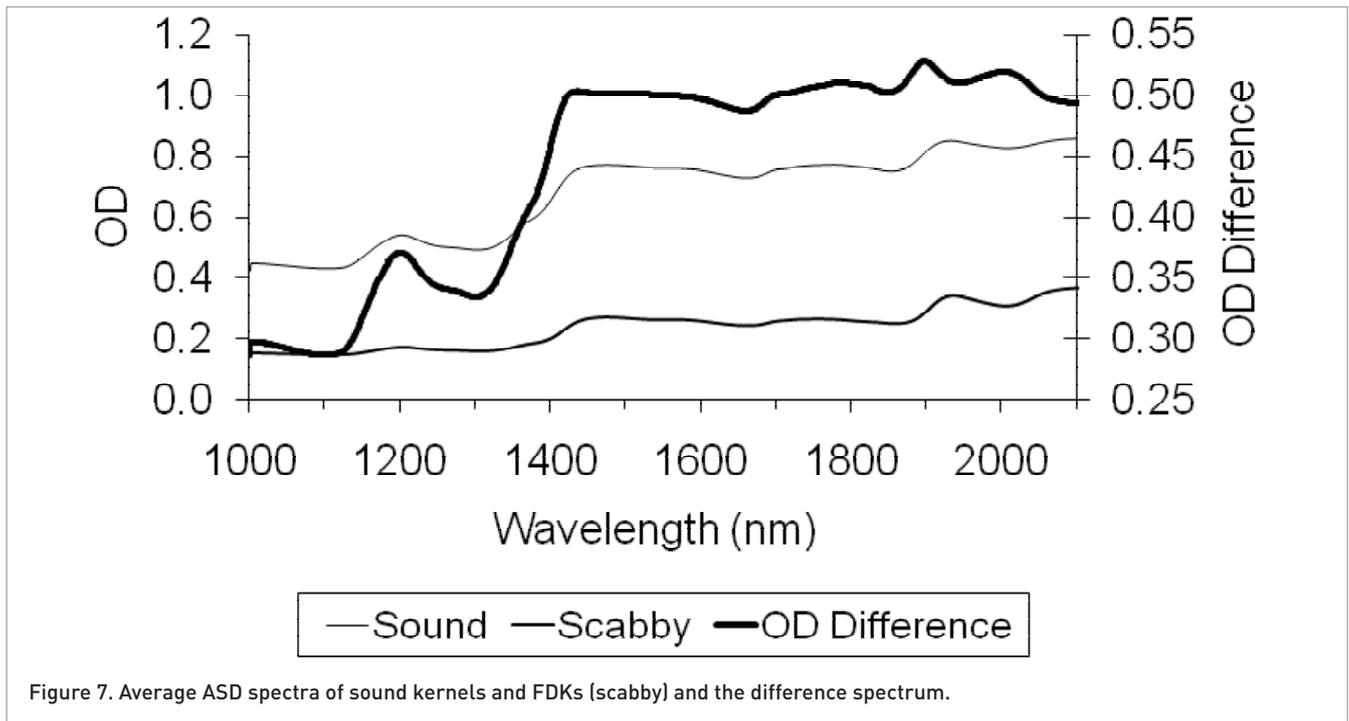


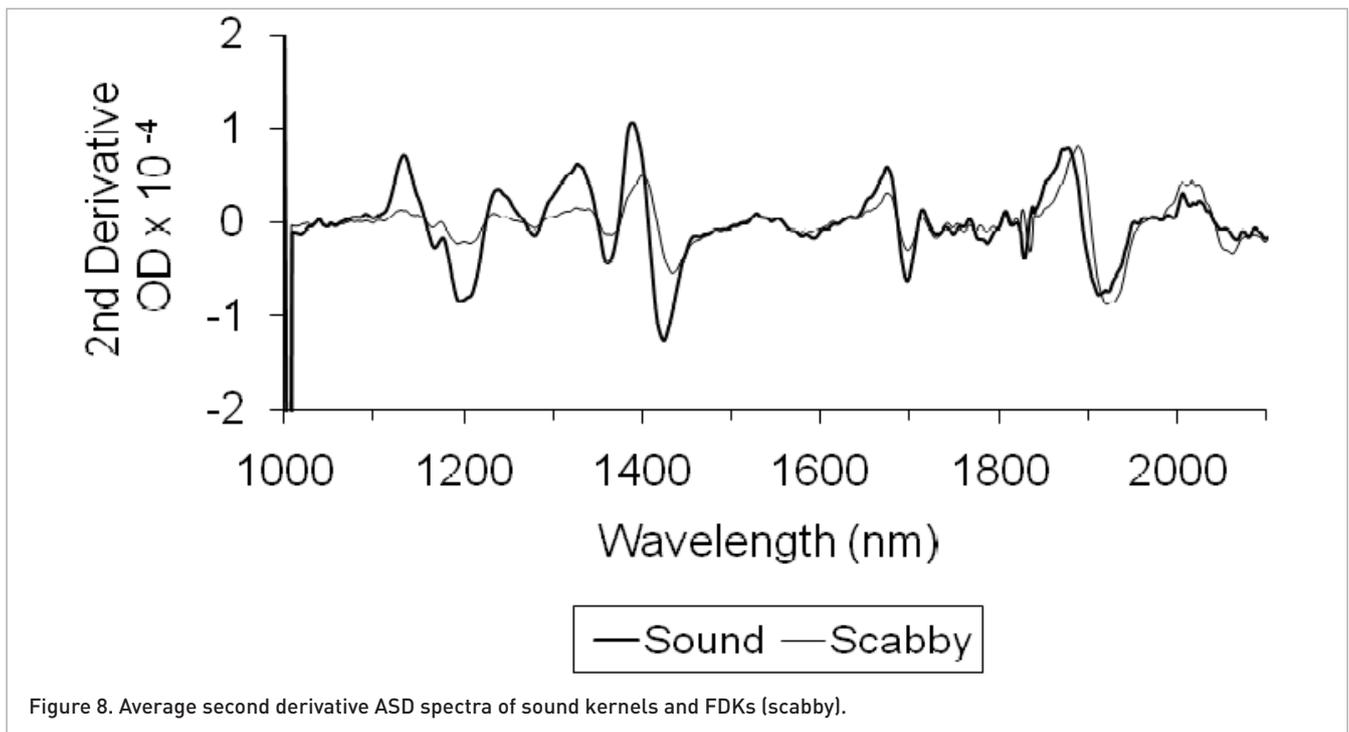
Figure 6. Average second derivative SKNIR spectra of sound and FDKs (scabby) and the difference spectrum.



Two types of kernel showed different patterns in NIR absorption spectra. The noise at 1000 nm and 1830 nm was due to the detector cut-off.

The second derivative spectra showed prominent absorption band peaks around 1204 nm, 1365 nm and 1700 nm for both sound kernels and FDKs with sound kernel peaks having

higher intensity. These bands may be due to second overtone, combination and first overtone vibrations of  $-CH$  groups<sup>20</sup> of the structural and food reserve components of grains, such as starch and other carbohydrates, lipids and proteins, which are present at different levels in FDKs and sound kernels. The absorption bands appearing at 1425 nm and 1915 nm in sound



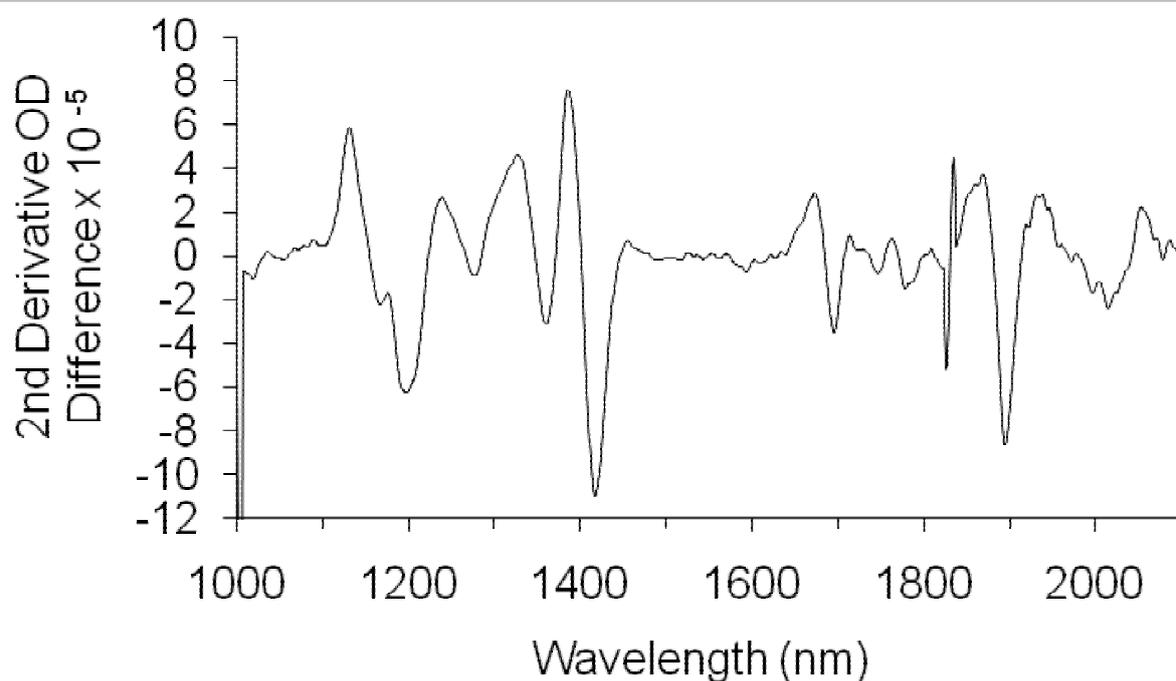


Figure 9. The difference spectrum between average second derivative spectra of sound kernels and FDks (scabby).

kernels were shifted by approximately 15 nm in FDks and positioned at 1440 nm and 1930 nm. It is probable that these shifts may be due to compounds that are not common to both types of kernel, such as DON.

The difference of the second derivative spectra of sound kernels and FDks is presented in Figure 9. Notable difference bands were observed with peaks at 1202 nm, 1363 nm, 1420 nm, 1698 nm and 1896 nm. The strongest difference bands, with peaks at 1420 nm and 1896 nm, may arise from the DON NIR absorption bands at 1408 nm, 1904 nm and 1919 nm (Table 1).

The difference in spectral bands around 1200 nm and 1420 nm (Figures 6 and 9) were common to spectra collected from both instruments. However, these results showed the ASD spectrometer can get high-resolution, noise-free spectra up to about 2000 nm, which includes the strong DON NIR

absorption band at around 1900 nm. Therefore, extending the scanning range of the SKNIR spectrometer up to about 2000 nm may improve the ability of the instrument to detect and sort FDks

## Conclusions

The results obtained in these studies from the NIR absorption spectra of sound kernels and FDks and DON showed that DON has distinct peaks in the NIR region. The NIR absorption differences between the sound kernels and FDks may arise, in part, due to differences in DON levels of the grains. Thus, NIR spectrometry could be used as a rapid, non-destructive, single-kernel technique to evaluate grain

Table 1. Summary of wavelengths associated with near infrared absorption by FDks and DON.

Instrument	Sample spectra	Wavelength (nm)
ASD Spectrometer	DON, OD	1414, 1906
ASD Spectrometer	DON, 2 <sup>nd</sup> derivative	1408, 1904, 1919
SKNIR	FDks, OD	1205, 1400(broad peaks)
SKNIR	FDks, 2 <sup>nd</sup> derivative	1195, 1425
ASD Spectrometer	FDks, OD	1208, 1440, 1905, 2001
ASD Spectrometer	FDks, 2 <sup>nd</sup> derivative	1204, 1365, 1700

samples of wheat germplasm for *Fusarium* damage based on their DON levels. Our future studies will use this technique to estimate the DON levels of FDKs. Our preliminary results indicated that NIR absorption spectra could estimate DON levels in kernels having more than 60 ppm DON. Further studies are necessary to lower the DON detection limits of this technique, especially to detect DON levels in *Fusarium*-infected asymptomatic kernels having significant DON levels.

## References

1. D.W. Parry, P. Jenkinson and L. McLeod, "Fusarium ear blight (scab) in small cereals—a review", *Plant Pathol.* **44**, 207 (1995). doi: [10.1111/j.1365-3059.1995.tb02773.x](https://doi.org/10.1111/j.1365-3059.1995.tb02773.x)
2. Anon, *Discussion Paper on Deoxynivalenol (DON). Joint FAO/WHO Food Standards Program. Codex Committee on Food Additives and Contaminants*. Thirty-eighth session. The Hague, Netherlands, 24–28 April (2006).
3. H.J. Dubin, L. Gilchrist, J. Reeves and A. McNab, Eds, *Fusarium head scab: Global status and prospects*. CIMMYT, Mexico, DF, Mexico. 130 pp. (1997).
4. M.P. McMullen, R. Jones and D. Gallenberg, "Scab of wheat and barley: A reemerging disease of devastating impact", *Plant Dis.* **81**, 1340 (1997). doi: [10.1094/PDIS.1997.81.12.1340](https://doi.org/10.1094/PDIS.1997.81.12.1340)
5. W.E. Nganje, S. Kaitibie, W.W. Wilson, F.L. Leistriz and D.A. Bangsund, "Economic impacts of *Fusarium* head blight in wheat and barley: 1993–2001", *Agribusiness and Applied Economics Report No. 538*. Department of Agribusiness and Applied Economics, North Dakota State University, Fargo, ND, USA (2004).
6. S.R. Delwiche, T.C. Pearson and D.L. Brabec, "High-speed optical sorting of soft wheat for reduction of deoxynivalenol". *Plant Dis.* **89**, 1214 (2005). doi: [10.1094/PD-89-1214](https://doi.org/10.1094/PD-89-1214)
7. R. Ruan, S. Ning, A. Song, A. Ning, R. Jones and P. Chen, "Estimation of *fusarium* scab in wheat using machine vision and a neural network", *Cereal Chem.* **75**, 455 (1998). doi: [10.1094/CCHEM.1998.75.4.455](https://doi.org/10.1094/CCHEM.1998.75.4.455)
8. A. Agostinelli, N. Mundell and D.V. Sanford, "Percentage of *fusarium* damaged kernels measured by air separation", in *Proc. of the National Fusarium Head Blight Forum*, 2–4 Dec 2008, Indianapolis, IN, USA, Ed by S.M. Canty, E. Walton, A. Clark, D. Ellis, J. Mundell and D.A. Van Sanford. University of Kentucky, Lexington, KY, USA, p.133 (2008).
9. G.H. Bai, R. Plattner, A. Desjardins and F. Kolb, "Resistance to *Fusarium* head blight and deoxynivalenol accumulation in wheat", *Plant Breeding* **120**, 1 (2001). doi: [10.1046/j.1439-0523.2001.00562.x](https://doi.org/10.1046/j.1439-0523.2001.00562.x)
10. A. Ludewig, U. Kabsch, J.A. Verreet, "Comparative deoxynivalenol accumulation and aggressiveness of isolates of *Fusarium graminearum* on wheat and the influence on yield as affected by fungal isolate and wheat cultivar", *J. Plant Dis. Protect.* **112**, 329 (2005).
11. A. Mesterhazy, T. Bartok, C.G. Mirocha and R. Komoroczy, "Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding", *Plant Breeding* **118**, 97 (1999). doi: [10.1046/j.1439-0523.1999.118002097.x](https://doi.org/10.1046/j.1439-0523.1999.118002097.x)
12. T. Miedaner, B. Schneider and H.H. Geiger, "Deoxynivalenol (DON) content and *Fusarium* head blight resistance in segregating populations of winter rye and winter wheat", *Crop Sci.* **43**, 519 (2003).
13. K.J. Odenbach, J.D. Salgado, L.V. Madden and P.A. Paul, "Influence of cultivar resistance, infection timing, and inoculum density on FHB development and DON accumulation in asymptomatic wheat spikes", in *Proc. of the National Fusarium Head Blight Forum*, 2–4 Dec 2008, Indianapolis, IN, USA, Ed by S.M. Canty, E. Walton, A. Clark, D. Ellis, J. Mundell and D.A. Van Sanford. University of Kentucky, Lexington, KY, USA, p.50 (2008).
14. A. Champeil, T. Dore and J.F. Fourbet, "*Fusarium* head blight: Epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains", *Plant Sci.* **166**, 1389 (2004). doi: [10.1016/j.plantsci.2004.02.004](https://doi.org/10.1016/j.plantsci.2004.02.004)
15. M. Nita, K. Tilley, E. De Wolf and G. Kuldau, "Effects of moisture during and after anthesis on the development of *Fusarium* head blight of wheat and mycotoxin production", in *Proc. of the National Fusarium Head Blight Forum*, Milwaukee, WI. 11–13 Dec. 2005, Ed by S.M. Canty, T. Boring, J. Wardwell, L. Siler and R.W. Ward. Michigan State University, East Lansing, Michigan, USA, pp. 125–128. (2005).
16. K.T. Willyerd, D.D. Archibald, K. Boroczky, E.D. DeWolf and G.A. Kuldau, "Effects of temperature on deoxynivalenol translocation and *F. Graminearum* infection of wheat heads", in *Proc. of the National Fusarium Head Blight Forum*, 2–4 Dec 2008, Indianapolis, IN, USA, Ed by S.M. Canty, E. Walton, A. Clark, D. Ellis, J. Mundell and D.A. Van Sanford. University of Kentucky, Lexington, KY, USA, p. 74 (2008).
17. F.E. Dowell, M.S. Ram and L.M. Seitz, "Predicting scab, vomitoxin, and ergosterol in single wheat kernels using near-infrared spectroscopy", *Cereal Chem.* **76**, 573 (1999). doi: [10.1094/CCHEM.1999.76.4.573](https://doi.org/10.1094/CCHEM.1999.76.4.573)
18. R. Ruan, Y. Li, X. Lin and P. Chen, "Non destructive determination of deoxynivalenol levels in barley using near-infrared spectroscopy", *Appl. Eng. Agric.* **18**, 549 (2002).
19. J.R. Morrey, "On determining spectral peak positions from composite spectra with a digital computer", *Anal. Chem.* **40**, 905 (1968). doi: [10.1021/ac60262a006](https://doi.org/10.1021/ac60262a006)
20. J. Workman and L. Weyer, *Practical Guide to Interpretive Near-Infrared Spectroscopy*. CRC Press, Boca Raton, FL, USA (2008).

- 21.** D.B. Bechtel, L.A. Kaleikau, R.L. Gaines and L.M. Seitz, "The effects of *Fusarium graminearum* infection on wheat kernels", *Cereal Chem.* **62**,191 (1985).
- 22.** D. Boyacioglu and N.S. Hettiarachchy, "Changes in some biochemical components of wheat grain that was infected with *Fusarium graminearum*", *J. Cereal Sci.* **21**, 57 (1995). doi: [10.1016/S0733-5210\(95\)80008-5](https://doi.org/10.1016/S0733-5210(95)80008-5)