Predicting Scab, Vomitoxin, and Ergosterol in Single Wheat Kernels Using Near-Infrared Spectroscopy

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ABSTRACT

Near-infrared spectroscopy (NIRS) was used to detect scab damage and estimate deoxynivalenol (DON) and ergosterol levels in single wheat kernels. Results showed that all scab-damaged kernels identified by official inspectors were correctly identified by NIRS. In addition, this system identified more kernels with DON than did a visual inspection. DON and ergosterol were predicted with standard errors of ≈40 and 100 ppm, respectively. All samples with visible scab had single kernels with DON levels >120 ppm, and some kernels contained >700 ppm of DON. This technology may provide a means of rapidly screening samples for potential food safety and quality problems related to scab damage.

Scab damage in wheat (*Triticum aestivum* L.) can occur when damp and cool conditions during the maturing and harvesting seasons create a favorable environment for the growth of the mold *Fusarium graminearum*. The mold can cause kernels to appear dull, lifeless, or chalky and may produce the toxin deoxynivalenol (DON). Scab does not uniformly affect all heads in a field or all kernels in a head. Often, only a portion of all heads or kernels is infected (Klenda 1995), with infection rates of heads reaching 50% (Liu 1985). The presence of scab adversely affects flour ash, flour color, glutenin levels, dough properties, and loaf volume (Dexter et al 1996). In addition, the toxin can cause digestive disorders, diarrhea, refusal to eat, and death in animals. DON is also a suspected human carcinogen (Luo et al 1990).

Besides adversely affecting grain quality and food safety, scab can reduce yields by 50%. Crop losses in the United States in some years have exceeded $1 billion (Liu 1985, Meronuck 1997). Also, in years when DON levels are excessive, individual millers may spend more than $1 million as they attempt to blend wheat to meet Food and Drug Administration (FDA) guidelines (Anonymous 1998). FDA guidelines are 1 ppm for finished wheat products for human consumption, 10 ppm for cattle or poultry feed, and 5 ppm for other animals (Herrman et al 1995). Cleaning, milling, and baking can reduce DON levels. However, concentrations in finished products may not be significantly less than levels measured before milling (Scott et al 1984, Abbas et al 1985, Seitz et al 1986, Nowicki et al 1988, Trigo-Stockli et al 1996).

Several methods exist to detect scab-damaged kernels, DON, or ergosterol levels. Ergosterol indicates the presence of fungal invasion (Seitz et al 1977). The Grain Inspection, Packers and Stockyards Administration (GIPSA) includes visual scab detection as part of their routine grain inspection procedures (USDA 1991). GIPSA inspection procedures consider kernels as scab-damaged if they have a significant amount of discoloration attributable to the fungus. However, kernels with little or no visible scab can have significant levels of ergosterol and DON (Seitz and Bechtel 1985) but not meet GIPSA criteria for damage. Chemical tests such as thin-layer chromatography, gas chromatography, or HPLC can measure DON or ergosterol levels (Miller et al 1983, Seitz and Bechtel 1985, Nowicki et al 1988, Trigo-Stockli et al 1996). However, these tests can take several hours to complete and typically measure DON or ergosterol in bulk samples. Bulk-sample results do not indicate whether the DON or ergosterol average values resulted from a few highly infected kernels or from many kernels infected at a lower level. Information about the distribution of DON in single kernels may assist millers in reducing DON to acceptable levels. Thus, a rapid, objective means of detecting scab-damaged kernels and indicating levels of DON and ergosterol is needed.

Ruan et al (1998) showed that machine vision could estimate scab damage more accurately than a human expert panel and can be fast. They did not attempt to use machine vision to estimate DON or ergosterol levels. Near-infrared spectroscopy (NIRS) can estimate wheat quality characteristics such as internal defects, color class, protein, and hardness (Delwiche and Norris 1993, Delwiche and Massie 1996, Dowell 1998, Dowell et al 1998). Thus, applying NIRS to the detection of scab-damaged kernels and the estimation of DON and ergosterol in wheat may be possible. The objective of this research was to assess the feasibility of single-kernel spectral analysis as an objective method for measuring scab-damaged wheat kernels and for measuring DON and ergosterol levels.

MATERIALS AND METHODS

Ten samples of hard red spring wheat were collected from lots with high levels of scab damage. Lots originated from commercial sources and from scab nurseries. The GIPSA Board of Appeal and Review separated samples into scab-damaged and sound kernels. Kernels determined as sound kernels by GIPSA criteria were further separated into kernels that appeared healthy and kernels with any visible scab damage. Table I shows the number of kernels used for subsequent NIR, DON, and ergosterol measurements. About 45 kernels per sample were used for subsequent tests.

Spectra Collection and Data Analysis

A diode-array, near-infrared spectrometer integrated with a single-kernel characterization system (Perten Instruments, Reno, NV) was used to collect spectra from single wheat kernels. The spectrometer measures absorbance at 400–1,700 nm using an array of silicon and indium-gallium-arsenide sensors and collects data at 30 spectra/sec. The system can automatically deliver single kernels in the spectrometer viewing area at a rate of two kernels/sec. However, kernels were hand-placed in this research to minimize placement errors. Six spectra were collected from each kernel and averaged to reduce noise. Data were recorded in 5-nm increments. The spectra were stored on a hard disk for subsequent analysis using GRAMS/32 software (Galactic Industries Corp., Salem, NH).

Spectra were analyzed by using partial least squares (PLS) regression, a spectral decomposition technique similar to principal component regression (Martens and Naes 1989). The PLS regression uses

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concentration data during the decomposition process and includes as much information as possible into the first few loading vectors. It also takes advantage of the correlation between the spectral data and the constituent concentrations. All data were mean centered before analysis.

Chemical Analyses

Single kernel ergosterol levels were determined using methodology originally developed for bulk samples by Seitz et al (1977) and modified to include hydrolysis during extraction for improved ergosterol recovery (Schwadorf and Muller 1989). Each kernel was crushed in a mortar. The crushed material was weighed and transferred to a 10-mL test tube with a cap lined with Teflon. Methanol (2 mL) followed by 20% aqueous KOH (0.5 mL) was added, and the contents were heated at 65–70°C in a water bath for 30 min. The mixture, to which 1 mL of water was added, was extracted twice with 5 mL of hexane. The hexane extract was separated, evaporated to dryness, and redissolved in 200 µL of methanol. It was filtered and 70 µL was injected into the HPLC (UV detector set at 282 nm). The column was Zorbax 3-µm octadecylsilane (C18) (ODS), two 6.0 × 40 mm cartridges (MAC-MOD Analytical, Inc., Chadds Ford, PA) coupled together and held at 50°C. The mobile phase was methanol and water (98:2). The flow rate was 1.5 mL/min. The detection limit was 31 ng/injection (89 ng/kernel).

TABLE I
Deoxynivalenol (DON) and Ergosterol Levels (ppm) in Single Wheat Kernels Determined as Sound or Scab-Damaged by the Grain Inspection Packers and Stockyards Administration (GIPSA)

Scab Category	*n*	Avg	Min	Max	SD[a]
DON					
GIPSA sound					
No visible scab	60	0.2a[b]	nd[c]	2.8	0.5
Visible scab[d]	180	50.8b	nd	558.1	88.3
GIPSA scab[e]	31	236.0c	32.7	788.6	156.9
Ergosterol					
GIPSA sound					
No visible scab	40	4.6a	nd	19.0	5.1
Visible scab[d]	115	157.8b	nd	1,497.0	273.8
GIPSA scab[e]	15	568.4c	262.1	1,232.0	285.3

[a] Standard deviation.
[b] Means followed by the same letter are not significantly different ($P = 0.05$).
[c] None detected.
[d] Kernels with visible scab, but not meeting GIPSA criteria for scab.
[e] Scab damage as determined by GIPSA standards.

TABLE II
Deoxynivalenol (DON) Levels (ppm) in Samples Predicted as Sound or Scab-Damaged by a Partial Least Squares (PLS) Calibration Developed from Near-Infrared Spectroscopy (NIRS) of Single Wheat Kernels

	No. Kernels	% Correct[a]	Avg DON	SD[b]
Calibration set				
Scab[c]	21	100%	218.8a[d]	134.3
Sound[e]	77	100%	nd[f]	···
Test set				
Scab[c]	10	100%	272.1a	191.0
Sound				
PLS				
Sound[g]	77	100%	3.2b	11.1
Scab[h]	86	0%[g]	103.6c	104.3

[a] According to standards of the Grain Inspection, Packers and Stockyards Administration (GIPSA).
[b] Standard deviation.
[c] Scab as determined by GIPSA personnel
[d] Means followed by the same letter are not significantly different ($P = 0.05$).
[e] Kernels with no visible scab.
[f] None detected.
[g] Classed as sound by visual inspection and by PLS.
[h] Kernels visibly sound by GIPSA criteria but classed as scab-damaged by PLS.

Single kernel DON levels were measured by Romer Labs, Inc. (Union, MO). Each kernel was crushed and weighed into a glass culture tube. The samples were extracted by adding 2 mL of acetonitrile and water (84:16) and vortexing for 5 min. The entire contents of the culture tube were transferred to a 216 column (Romer Labs). The column was rinsed with 9 mL of acetonitrile and water (90:10) to elute the DON. All of the rinse solution was then taken to dryness, and the contents were reconstituted with mobile phase (250 µL); 150 µL was injected into the HPLC (UV detector set at 220 nm). The column was a SPHER15, RP18, 5-µm, 0.4 × 10 cm (Perkin Elmer Corp., Norwalk, CT). The mobile phase was water-methanol-acetonitrile (92:4:4). The flow rate was 1.0 mL/min and retention time was 8.5 min. Detection limit was 4 ng of DON. DON and ergosterol were not measured on the same kernel.

RESULTS AND DISCUSSION

Relationship of DON and Ergosterol to Scab

Table I shows DON and ergosterol levels from single wheat kernels. Kernels determined by GIPSA as scab-damaged averaged 236.0 ppm of DON and 568.4 ppm of ergosterol. Kernels with visible scab but not meeting GIPSA criteria for scab damage contained an average of 50.8 and 157.8 ppm of DON and ergosterol, respectively. Kernels with no visible scab had DON and ergosterol levels as high as 2.8 and 19.0 ppm, respectively. Ergosterol and DON levels in sound kernels are higher than expected for truly sound grain. Levels are probably inflated because all samples came from wheat subject to conditions conducive to scab infection.

Figures 1 and 2 show the distribution of DON and ergosterol levels in single kernels identified by GIPSA as sound or scab-damaged. All kernels identified by GIPSA as scab-damaged had detectable amounts of DON and ergosterol. In addition, detectable amounts of DON or ergosterol were found in 60 and 84%, respectively, of kernels identified by GIPSA as sound. Thus, current official grading procedures identify kernels with high levels of DON and ergosterol. However, many kernels not identified by current inspection procedures still contain significant levels of DON and ergosterol.

Figures 1 and 2 also show that more kernels had detectable ergosterol than DON. All fungi normally found on grains produce ergo-

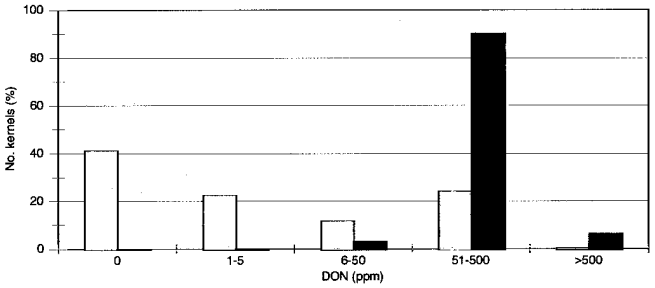

Fig. 1. Distribution of deoxynivalenol (DON) in single wheat kernels determined as sound (white bars) or scab-damaged (black bars) by official grading procedures.

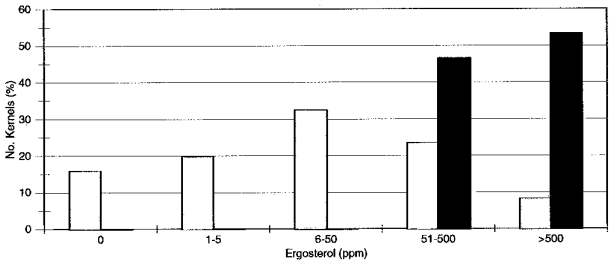

Fig. 2. Distribution of ergosterol in single wheat kernels determined as sound (white bars) or scab-damaged (black bars) by official grading procedures.

sterol. Thus, the presence of ergosterol may either indicate the presence of other fungi, or that ergosterol was present before DON was produced. O'Neill et al (1993) reported that 14 days or more was required for DON to be detected after grain was inoculated with *F. graminearum*.

Identification of Scab-Damaged Kernels Using Spectral Characteristics

A calibration was developed from PLS regressions to predict scab damage using single-kernel spectral characteristics. The calibration equation (six factors) included only kernels determined by GIPSA as scab-damaged and kernels with no measurable DON. Results from the calibration and test sets are shown in Table II.

Results showed that all kernels identified as scab-damaged by GIPSA and all kernels with 0 ppm of DON were correctly classified. There were many kernels predicted by the calibration as scab-damaged that had been classified as sound by GIPSA. However, all of these kernels had >0 ppm of DON and averaged 103.6 ppm (Table II). Thus, the NIR system identified more kernels as scab-damaged than did the visual inspection, and all of these tested positive for DON. Therefore, this system should more accurately identify scab-damaged kernels with significant levels of DON. This is illustrated in Fig. 1, which shows the distribution of DON in kernels graded by GIPSA. Similar results were shown in the ergosterol analysis.

Prediction of DON and Ergosterol Levels

A DON prediction equation (nine factors, n = 114) was developed using only kernels with >5 ppm of DON. Including kernels in the calibration with <5 ppm of DON resulted in poorer predictions. The prediction equation was applied only to those kernels predicted previously as scab-damaged. Figure 3 shows predicted DON levels (r^2 = 0.64, SE = 44 ppm, n = 88). Only one kernel was erroneously predicted as having 0 ppm of DON. Although a standard error (SE) of 44 ppm is probably not acceptable for predicting bulk DON levels, it may be acceptable for detecting samples that require further chemical analysis. All samples had some kernels with >120 ppm of DON. Thus, even with a SE of 44 ppm, there would be a >99% probability that at least one kernel in each of the samples in this study would be identified as having a significant amount of DON. This information could prompt the seller or buyer that additional chemical testing of the bulk sample may be warranted.

Additional data supporting the use of this system as a screening tool comes from *F. graminearum* and DON levels reported by Love and Seitz (1987). They showed that the presence of *F. Graminearum* was not uniform but occurred in ≈20–70% of individual kernels from samples with detectable levels of DON. Related research on aflatoxin in corn and peanuts also shows infection is not uniform and that a small percentage of kernels are usually highly contaminated at levels several times the average (Fennell et al 1973, Whitaker 1977). For kernels with detectable DON in the present study, 34% of single kernels had >100 ppm and 16% had >200 ppm of DON. Thus, if infection is not uniform, some of those infected kernels should have levels exceeding the SE of this system and will have a high probability of being detected.

The above calibration was validated by randomly removing ≈20% of the kernels from the calibration set, developing a calibration with the remaining 80%, then predicting that 20% not in the calibration set. Results from this validation set (r^2 = 0.66, SE = 52) were similar to those reported above.

Ergosterol was predicted using an equation (eight factors) developed using kernels with >50 ppm of ergosterol. As with the DON prediction, the ergosterol prediction equation was applied only to those kernels previously predicted as scab-damaged. Figure 4 shows predicted ergosterol values (r^2 = 0.64, SE = 108 ppm, n = 46) where only one kernel was erroneously predicted with 0 ppm of ergosterol.

Calibrations were attempted using the weight of DON or ergosterol (in ng) in each kernel instead of ppm. However, prediction results using ng resulted in lower r^2 values than those achieved when using ppm.

Relationship of Kernel Weight to DON and Ergosterol Levels

Figure 5 shows the relationship of kernel weight to DON and ergosterol levels. For both DON and ergosterol, levels decreased as kernel weight increased. This relationship between DON and kernel weight agrees with results reported by others (Bechtel et al 1985, Snijders and Perkowski 1990). This relationship may be due to smaller kernels having higher concentrations of DON and ergosterol, or to damaged kernels being less dense than sound kernels.

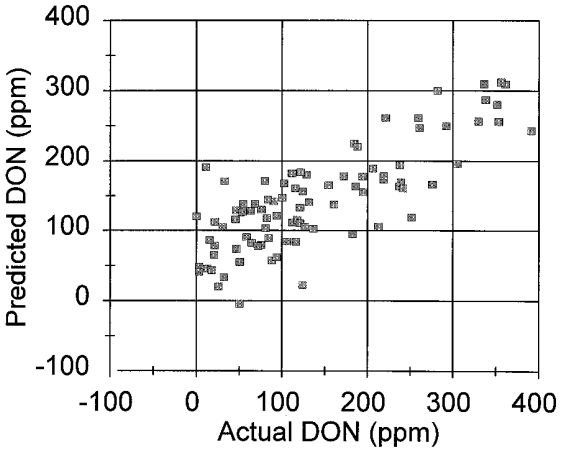

Fig. 3. Single wheat kernel deoxynivalenol (DON) levels predicted using near-infrared spectroscopy (NIRS).

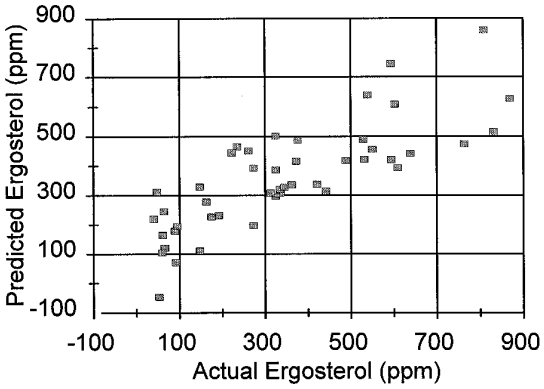

Fig. 4. Single wheat kernel ergosterol levels predicted using near-infrared spectroscopy (NIRS).

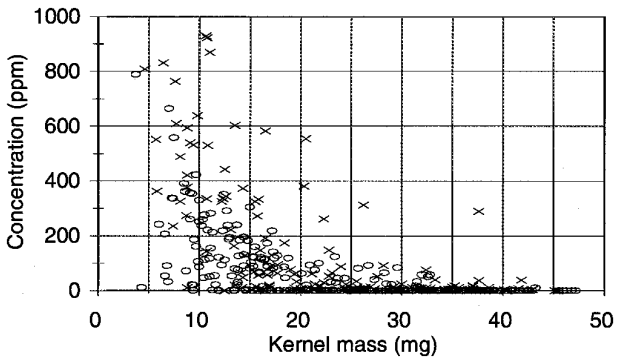

Fig. 5. Relationship between single kernel weight and levels of deoxynivalenol (DON) (○) and ergosterol (×).

Regner et al (1994) reported that small kernels do tend to have higher ergosterol contents than larger kernels, but that small kernels have little influence on total ergosterol contents of the lot. Martin et al (1998) reported that scab-damaged kernels are less dense than sound kernels. The ratio of ergosterol to DON levels, which for GIPSA scab in Table I was ≈2.4, agrees well with the ratio reported by Love and Seitz (1987).

Wavelengths Used in Classifications

Beta coefficients calculated by PLS were examined to determine wavelengths used in scab, DON, and ergosterol predictions. Important wavelengths were noted throughout the 500–1,700 nm range, indicating that absorption in the visible region and absorption arising from O-H (≈750, 950, and 1,400 nm), C-H (≈1,200, 1,400, and 1,650 nm), and N-H (≈1,050 and 1,500 nm) overtones contributed to classifications. Bechtel et al (1985) reported that scab affects starch and protein, which absorb NIR radiation at the overtones noted above.

In summary, these results show that NIRS can detect scab-damaged kernels and estimate DON and ergosterol levels in single kernels. This technology could be used to rapidly screen incoming samples for potential wheat quality problems related to scab damage.

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LITERATURE CITED

Abbas, H. K., Mirocha, C. J., Pawlosky, R. J., and Pusch, D. J. 1985. Effect of cleaning, milling, and baking on deoxynivalenol in wheat. Appl. Environ. Microbiol. 50:482-486.

Anonymous. 1998. In the industry. GEAPS In-Grain. Grain Elevator and Processing Society 18:8.

Bechtel, D. B., Kaleikau, L. A., Gaines, R. L., and Seitz, L. M. 1985. The effects of *Fusarium graminearum* infection on wheat kernels. Cereal Chem. 62:191-197.

Delwiche, S. R., and Massie, D. R. 1996. Classification of wheat by visible and near-infrared reflectance from single kernels. Cereal Chem. 73:399-405.

Delwiche, S. R., and Norris, K. H. 1993. Classification of hard red wheat by near-infrared diffuse reflectance spectroscopy. Cereal Chem. 70:29-35.

Dexter, J. E., Clear, R. M., and Preston, K. R. 1996. Fusarium head blight: Effect on the milling and baking of some Canadian wheats. Cereal Chem. 73:695-701.

Dowell, F. E. 1998. Automated color classification of single wheat kernels using visible and near-infrared reflectance. Cereal Chem. 75:142-144.

Dowell, F. E., Throne, J. E., and Baker, J. E. 1998. Automated nondestructive detection of internal insect infestation of wheat kernels using near-infrared reflectance spectroscopy. J. Econ. Entomol. 91:899-904.

Fennell, D. I., Bothast, R. J., Lillehoj, E. B., and Peterson, R. E. 1973. Bright greenish-yellow fluorescence and associated fungi in white corn naturally contaminated with aflatoxin. Cereal Chem. 50:405-414.

Herrman, T., Bequette, R. K., McCluskey, P. J., Pedersen, J. R., Ponte, J. G., and Shroyer, J. P. 1995. Quality oriented marketing of hard red winter wheat. MF-2119. Cooperative Extension Service. Kansas State University: Manhattan, KS.

Klenda, D. K. 1995. Diversity of wheat scab fungus populations on a small spatial scale. J. Nat. Resour. Life Sci. Educ. 24:77-79.

Liu, Z. Z. 1985. Recent advances in research on wheat scab in China. Pages 174-181 in: Wheat for More Tropical Environments. CIMMYT: Mexico City.

Love, G. R., and Seitz, L. M. 1987. Effects of location and cultivar on Fusarium head blight (scab) in wheat from Kansas in 1982 and 1983. Cereal Chem. 64:124-128.

Luo, Y., Yoshizawa, T., and Katayama, T. 1990. Comparative study on the natural occurrence of *Fusarium* mycotoxins (Trichothecenes and Zearalenone) in corn and wheat from high- and low-risk areas for human esophageal cancer in China. Appl. Environ. Microbiol. 56:3723-3726.

Martens, H., and Naes, T. 1989. Multivariate Calibrations. John Wiley and Sons: Guildford, UK.

Martin, C., Herrman, T. J., Loughin, T., and Oentong, S. 1998. Mycropycnometer measurement of single kernel density of healthy, sprouted, and scab-damaged wheats. Cereal Chem. 75:177-180.

Meronuck, R. A. 1997. Effect of wheat scab and karnal bunt on quality. Pages 391-396 in: Proceedings International Wheat Quality Conference. J. L. Steele and O. K. Chung, eds. Grain Industry Alliance: Manhattan, KS.

Miller, J. D., Young, J. C., and Trenholm, H. L. 1983. *Fusarium* toxins in field corn. I. Time course of fungal growth and production of deoxynivalenol and other mycotoxins. Can. J. Bot. 61:3080-3087.

Nowicki, T. W., Gaba, D. G., Dexter, J. E., Matsuo, R. R.,and Clear, R. M. 1988. Retention of the Fusarium mycotoxin deoxynivalenol in wheat during processing and cooking of spaghetti and noodles. J. Cereal Sci. 8:189-202.

O'Neill, K. O., Damoglou, A. P., and Patterson, M. F. 1993. Toxin production by *Fusarium culmorum* IMI 309344 and *F. graminearum* NRRL 5883 on grain substrates. J. Appl. Bacteriol. 74:625-628.

Regner, S., Schnurer, J., and Jonsson, A. 1994. Ergosterol content in relation to grain kernel weight. Cereal Chem. 71:55-58.

Ruan, R., Ning, S., Song, A., Ning, A., Jones, R., and Chen, P. 1998. Estimation of *Fusarium* scab in wheat using machine vision and a neural network. Cereal Chem.75:455-459.

Schwadorf, K., and Muller, H.-M. 1989. Determination of ergosterol in cereals, mixed feed components and mixed feeds by liquid chromatography. J. Assoc. Off. Anal. Chem. 72:457-462.

Scott, P. M., Kanhere, S. R., Dexter, J. E., Brennan, P. W., and Trenholm, H. L. 1984. Distribution of the trichothecene mycotoxin deoxynivalenol (vomitoxin) during the milling of naturally contaminated hard red spring wheat and its fate in baked products. Food Add. Contam. 1:313-323.

Seitz, L. M., and Bechtel, D. B. 1985. Chemical, physical, and microscopical studies of scab-infected hard red winter wheat. J. Agric. Food Chem. 33:373-377.

Seitz, L. M., Eustace, W. D., Mohr, H. E., Shogren, M. D., and Yamazaki, W. T. 1986. Cleaning, milling, and baking tests with hard red winter wheat containing deoxynivalenol. Cereal Chem. 63:146-150.

Seitz, L. M., Mohr, H. E., Burroughs, R., and Sauer, D. B. 1977. Ergosterol production as an indicator of fungal invasion in grains. Cereal Chem. 54:1207-1217.

Snijders, C. H. A., and Perkowski, J. 1990. Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. Phytopathology 80:566-570.

Trigo-Stockli, D. M., Deyoe, C. W., Satumbaga, R. F., and Pedersen, J. R. 1996. Distribution of deoxynivalenol and zearalenone in milled fractions of wheat. Cereal Chem. 73:389-391.

Whitaker, T. B. 1977. Sampling granular foodstuffs for aflatoxin. Pure Appl. Chem. 49:1709-1717.

USDA. 1991. Inspecting grain. Practical procedures for grain handlers. Federal Grain Inspection Service: Washington, DC.

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