APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1985, p. 482–486
0099-2240/85/080482-05$02.00/0

Vol. 50, No. 2

Effect of Cleaning, Milling, and Baking on Deoxynivalenol in Wheat†

H. K. ABBAS,[1] C. J. MIROCHA,[1]* R. J. PAWLOSKY,[1] AND D. J. PUSCH[2]

Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota 55108,[1] and Research and Development Laboratories, The Pillsbury Company, Minneapolis, Minnesota 55414[2]

Received 28 February 1985/Accepted 29 May 1985

Samples of wheat naturally infected by *Fusarium graminearum* Schwabe were obtained from mills in Oklahoma, Missouri, Kansas, and Minnesota and fields in Nebraska and Kansas in 1982; they were analyzed for deoxynivalenol (DON). The wheat was milled, and DON was found throughout all the milling fractions (bran, shorts, reduction flour, and break flour). The DON recoveries for each mill run ranged from 90 to 98%. These samples, regardless of DON concentration, also gave similar fractional distributions of DON. The greatest (21 ppm [21 μg/g]) concentration of DON was found in the bran, and the smallest (1 ppm) was found in the break flour. Cleaning and milling were not effective in removing DON; DON was not destroyed in the bread baked from the naturally contaminated whole wheat flour, but the effect on its concentration in the samples analyzed varied, the reduction ranging from 19 to 69%. The percent reduction found in the cleaned wheat ranged from 6 to 19%. DON concentrations in the following commercially made breads, caraway rye, seedless rye, and pumpernickel, were 45 ppb (ng/g), 39 ppb, and 0 ppb, respectively. The limits of detection by gas chromatography-mass spectrometry and high-pressure liquid chromatography for DON were 0.5 and 10 ng, respectively.

Deoxynivalenol (DON; vomitoxin, 12,13-epoxy-3,7,15-trihydroxy-trichothec-9-en-8-one) is a natural metabolite produced on a variety of cereal grains by *Fusarium graminearum* Schwabe (12). It was first found in infected grain and peptone-supplemented Czapek Dox medium by Morooka et al. (11) and later characterized by Yoshizawa and Morooka (20) in Japan. It is found in grains (corn, barley, feedstuff, and wheat) in many parts of the world, including Canada, Japan, and the United States (8, 9, 15–19) and is cause for concern from the viewpoint of animal and human health.

Recently, researchers have focused on the fate of trichothecenes during grain processing. In one study, Kamimura et al. (7) reported the effects of baking bread and preparing Chinese and Japanese noodles on levels of DON and five other trichothecenes. They also observed that soaking naturally contaminated ground wheat in water removed about 30% of the DON (and nivalenol). Scott et al. (14) reported the effects of experimental flour milling and bread baking on retention of DON in hard red spring wheat. They also found that DON is distributed throughout the milled products and is not destroyed upon making bread. Greenhalgh et al. (4) reported occurrence of DON, iso-DON, and other derivatives in bread and cereal products. Young et al. (21) reported the effect of milling and baking on the DON content of eastern Canadian wheat; they also reported that milling led to a fractionation of both DON and ergosterol, with higher levels in the outer parts of the kernel (e.g., bran) and lower levels in the endosperm. The effect of baking on the DON level in products is variable, ranging from no effect to 66% reduction in DON. L. M. Seitz et al. (in press, Cereal Chem.) reported the distribution of DON in soft wheat mill streams, and they found that the first reduction flour exhibited a higher mean DON concentration than subsequent reduction flours, with the lowest concentration found in the fifth reduction flour; they concluded that commercial milling is not particularly effective for removing DON.

Because levels of DON are affected by methods of food preparation, further study is necessary to determine what levels are found in finished foods. We have studied the fate of DON during the process of cleaning, milling, and bread making in naturally *Fusarium*-infected hard red winter wheat.

MATERIALS AND METHODS

Apparatus. High-pressure liquid chromatography (HPLC) with a Model 440 absorbance detector (Waters Associates, Inc., Milford, Mass.), a liquid chromatographic column-Zorbax reverse phase C18 (4.6 mm [inner diameter] by 25 cm) (Du Pont Co., Wilmington, Del.), an activated charcoal-alumina column (Myco Lab Co., Chesterfield, Mo., and gas chromatography-mass spectrometry (Hewlett-Packard gas chromatograph-5840 and mass spectrometer-5987) were used.

Wheat, flour, and bread. In 1982, samples of naturally *F. graminearum*-infected wheat were obtained from R. A. Wilcox, Kansas State University; B. Doupnik, University of Nebraska; and the Cargill Company, Minneapolis, Minn. Wheat, flour, and bread samples were obtained from Carl Smith, Jr., The Pillsbury Company, Minneapolis, Minn. Commercial bread samples were obtained from New York state. The samples were cleaned on a Carter dockage tester before they were prepared for milling. All of the wheat samples were shaken for 1/2 h on a shaker at high speed before representative samples were taken for DON analysis.

Isolations. Samples of wheat were examined mycologically for contamination by toxic fungi. Wheat kernels were surface sterilized for 1 to 2 min with a 5% solution of a commercial preparation of sodium hypochlorite. After being thoroughly rinsed in sterile water, the kernels were transferred directly to plates containing homemade potato glu-

* Corresponding author.

† Paper no. 14,289, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.

TABLE 1. Milling fractions of naturally *F. graminearum*-infected hard red winter wheat from 1982 crop

Source	Sample wt (g)	Fractions			
		Total wt (g)[a]	Type	Wt (g)	Proportion of milled, clean Wheat (%)
University of Nebraska					
Lot 1	1,179	1,009.8	Bran	180.5	17.9
			Shorts	81.5	8.1
			Reduction flour	612.7	60.7
			Break flour	135.1	13.4
Lot 2	1,267	1,148.9	Bran	199.5	17.4
			Shorts	104.0	9.0
			Reduction flour	692.4	60.0
			Break flour	153.0	13.3
Missouri					
Lot 1	1,123	989.5	Bran	196.5	19.9
			Shorts	97.4	9.8
			Reduction flour	555.1	56.1
			Break flour	140.5	14.2
Lot 2[b]	1,154	476.3[c]	Bran	267.2	53.8
			Shorts	20.1	4.1
			Reduction flour	114.8	23.1
			Break flour	94.2	19.0
Kansas State University	1,191	1,002.0	Bran	176.0	17.6
			Shorts	111.1	11.1
			Reduction flour	578.1	57.8
			Break flour	136.2	13.6

[a] Weight remaining after cleaning
[b] Soft red winter wheat.
[c] Total weight of bran, reduction flour, and break flour.

cose agar. Plates were incubated in the light at 25°C and examined at regular intervals, and the dominant fungi were isolated in pure culture and examined for *Fusarium* species.

DON standard. DON was purchased in analytically pure form from the Myco-Lab Co.

Milling. Untempered, infected clean wheat was milled with a Buhler automatic laboratory mill (model NLU-202; manufactured by Buhler Brothers, Inc., Uzwill, Switzerland and distributed in the United States by Buhler-Miag, Inc., Minneapolis, Minn.). Wheat fractions were collected from the first three break rolls (break flour), the first three reduction rolls (reduction flour), the shorts, and the bran (see Table 1).

Whole-wheat flour. The wheat was ground with a coffee grinder, (model 490-SP; Grindmaster of Kentucky, Inc., Louisville, Ky.). The grinder was set at no. 1 (the finest grind).

Bread. The whole-wheat bread was baked as outlined previously (1).

Preparation, extraction, clean up, and determination. The samples were prepared, extracted, and analyzed by a slight modification of the method described in detail by Chang et al. (2). Briefly, 25 or 50 g of ground sample was extracted with 200 ml of extracting solvent (acetonitrile-water [84:16, vol/vol]) in a Waring blender for 3 min at high speed. The extract was filtered through Whatman no. 4 filter paper, and 10-ml portions of the filtrate were placed on top of an activated charcoal-alumina column. When the filtrate reached the top of the packing, the column was eluted with 10 ml of extracting solvent. The combined eluate was collected in a 200-ml round-bottom flask and evaporated to dryness on a rotary evaporator. The residue was dissolved in acetone and transferred to a 2-dram vial. The solution of acetone was evaporated to dryness under a gentle flow of nitrogen gas with gentle heating. The residue was dissolved in 2 ml of methanol-water (23:77 or 10:90, vol/vol) and immediately mixed on a vortex mixer for 1 min before the DON analysis by HPLC. The chemical identity of DON was confirmed by derivatizing with TBT (Pierce Chemical Co., Rockford, Ill.) and analyzing by gas chromatography-mass spectrometry and selected ion monitoring (10) in the negative chemical ionization mode with methane at 0.5 torr (66.7 Pa) source pressure. The mass spectrometer was tuned with perfluorotributylamine in the negative ion mode, and the ion 634 was optimized for maximum sensitivity.

DON quantitation. The flow rate of the HPLC was set to 1.5 ml/min on a preconditioned column with several column volumes of the mobile phase (methanol-water [23:77 or 10:90, vol/vol]). The UV monitor was set to 254 nm, with a sensitivity of 0.02 or 0.005 absorbance units, full scale. The recorder was set to 10 in. (25 cm)/h. Injection of the standard solution (2.5 ng/μl) was repeated until peak heights and retention time were constant. Calculation of DON concentrations was performed by the peak height method of quantification.

RESULTS

F. graminearum was the only fungus isolated from wheat samples obtained from Nebraska, Missouri, and Kansas. Bread was made only from the naturally *F. graminearum*-infected wheat obtained from Kansas. The wheat was cleaned and milled, and various milling fractions (bran, shorts, reduction flour, and break flour) (Table 1) were collected and analyzed for DON by HPLC. The percent recovery (spiked sample, 1 ppm [1 μg/g]) was 80.3 ± 0.9% by the above method for DON analysis. DON was detected in all samples. Detection of the DON concentration in these milling fractions was variable, ranging from 21.3 ppm (Nebraska lot 1, bran) to 1.1 ppm (Missouri lot 2, break flour). The highest concentration of DON was found in the bran

TABLE 2. Concentrations of DON in naturally *F. graminearum* infected hard red winter wheat and milling fractions

Source	DON concn (μg/g) in[a]:					
	Uncleaned wheat	Clean wheat	Bran	Shorts	Reduction flour	Break flour
University of Nebraska						
Lot 1	8.8 ± 0.20	7.9 ± 0.04	21.3 ± 0.34	17.5 ± 0.21	3.5 ± 0.17	3.1 ± 0.06
Lot 2	7.9 ± 0.23	6.4 ± 0.11	13.7 ± 0.09	9.1 ± 0.08	3.8 ± 0.06	3.1 ± 0.03
Missouri						
Lot 1	9.6 ± 0.25	7.8 ± 0.08	18.6 ± 0.32	15.4 ± 0.35	3.7 ± 0.03	1.2 ± 0.12
Lot 2	8.1 ± 0.15	7.1 ± 0.05	11.3 ± 0.23	NA[b]	1.8 ± 0.08	1.1 ± 0.40
Kansas State University	9.1 ± 0.25	8.6 ± 0.05	16.8 ± 0.56	9.1 ± 0.07	5.9 ± 0.31	2.8 ± 0.04

[a] Each value is the mean of four determinations of each sample ± standard deviation. DON was quantitated by HPLC.
[b] NA, Not applicable. Shorts were not obtained in sufficient weight for analysis of DON.

fraction in all samples. Milling of the wheat brought about a 1.6 to 9.9% reduction of DON concentrations. The highest concentration was found in the bran, followed by that in shorts, reduction flour, and break flour in all samples analyzed. Average concentrations of DON and standard deviations in the wheat, various milling fractions, and bread (made from the infected wheat) are shown in Table 2.

It is evident that results obtained by gas chromatography-mass spectrometry and selected ion monitoring refer to DON and that no interference was present (Tables 2 and 3). DON in all samples was observed at 11.14 min, as shown in Fig. 1.

Cleaning was not effective in removing DON (Table 2). The percent reduction in DON concentrations found in the cleaned wheat obtained from these wheat samples was 10.2, 19, 18.8, 12.4, and 5.5% for University of Nebraska lot 1, University of Nebraska lot 2, Missouri lot 1, Missouri lot 2, and Kansas State University samples, respectively.

Milling was not effective in removing DON (Table 2). The DON recoveries for the mill run of cleaned wheat were 98.2, 92.4, 95.6, 98.4, and 90.1% for University of Nebraska lot 1, University of Nebraska lot 2, Missouri lot 1, Missouri lot 2, and Kansas State University samples, respectively. These recoveries were determined by taking the mill fraction weights of Table 1 and multiplying with the DON concentrations of Table 2 to obtain the amount of DON in each fraction and in the total.

Other samples of wheat, flour, and bread were analyzed by the above method for DON. These results of triplicate determinations of corresponding samples are shown in Table 3.

Commercial bread samples obtained from New York were analyzed by gas chromatography-mass spectrometry and selected ion monitoring because the DON detection limit was 0.5 ng, whereas the DON limit with HPLC was 10 ng. DON was found in the commercial caraway rye and seedless rye breads at concentrations of 45 and 39 ppb (45 and 39 ng/g), respectively, and in bread made from infected wheat from Missouri (X-1) and Kansas (X-3) but not in the commercial pumpernickel bread or in whole-wheat bread made from wheat from Oklahoma (X-2) or Minnesota (X-4), as shown in Table 3.

DON was not destroyed by making bread from the naturally contaminated wheat (Table 3); however, the bread had a lower concentration of DON than that in the starting wheat samples. The starting wheat samples from Kansas State University, Missouri (X-1), and Kansas (X-3) had concentrations of DON of 9,100, 537.9, and 379.3 ppb, whereas the bread made from the same samples had DON concentrations of 6,100 ± 30, 435.3, and 116.2 ppb, respectively. These results correspond to a percent reduction of 33.0, 19.1, and 69.4%, respectively. Calculations were based on a flour weight basis of the baked bread samples. Others have also reported that baking reduced the concentration of DON in bread made from naturally contaminated wheat (3, 14).

DISCUSSION

Determinations of concentrations of DON in contaminated wheat at harvesting and during processing have been carried out recently in several countries with varied results. In Japan, concentrations of up to 40 ppm of DON and nivalenol have been reported in both wheat and barley (8). In Canada, DON has been measured at up to 8.5 ppm in Ontario white winter wheat (15, 16) and up to 5.5 ppm in 1982 scabby wheat from parts of the midwestern United States (Kansas, Nebraska, and Illinois) (5). In the United Kingdom, homegrown wheat was found to contain only up to 0.4 ppm of DON (13). *F. graminearum* is the major causative agent of DON contamination of grains (12). Thus, it may be that the differences in the degree of DON contamination of wheat grown in different areas are due to a geographical or seasonal variation in the occurrence of *F. graminearum*. This explanation is supported by the work of Osborne and Willis (13).

DON was not removed by cleaning wheat, but the concentrations of DON were less than those found in the uncleaned wheat samples (Table 2). Young et al. (21) reported that the percent reduction in the concentrations of DON during cleaning for industrially milled Ontario white winter wheat and pilot-milled Ontario soft white winter wheat are 12.5 and 23.45%, respectively. The reduction in

TABLE 3. Concentrations of DON in wheat, flour, and bread made from various samples of wheat[a]

Mill, location[b]	DON concn (ng/g) in:		
	Wheat	Whole-wheat flour	Whole-wheat bread
Missouri, X-1	537.9 ± 9.95	520.9 ± 13.97	435.3 ± 0.95
Oklahoma, X-2	ND[c]	ND	ND
Kansas, X-3	379.3 ± 19.90	305.9 ± 27.16	108.9 ± 7.05
Minnesota, X-4	ND	ND	ND

[a] Samples were obtained from C. Smith, The Pillsbury Co., Minneapolis, Minn., in 1982. Each value is a result of triplicate samples of each sample ± standard deviation. DON was quantitated by HPLC.
[b] Composition of wheat: Missouri, 100% Kansas wheat; mixture of Oklahoma, Texas, and Kansas wheat; Kansas, 100% Kansas wheat; Minnesota, mixture of North and South Dakota wheat.
[c] ND, Not detected.

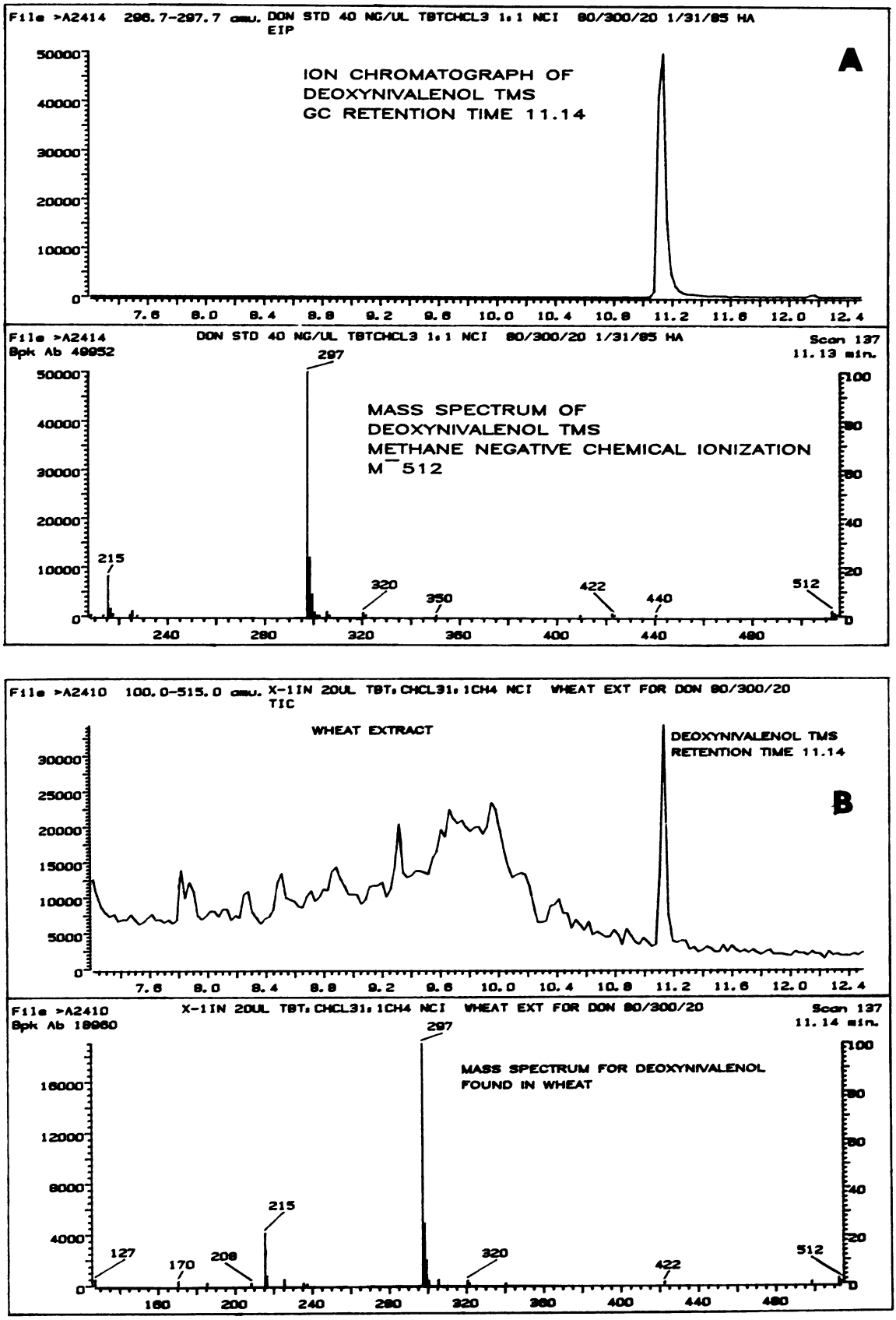

FIG. 1. (A) Total ion chromatograph and mass spectrum of DON-trimethylsilane derivative at retention time of 11.14 min. (B) Ion chromatograph of wheat extract DON-trimethylsilane eluted at 11.14 min. Mass spectra confirm its presence.

the DON concentrations during wheat cleaning may be due to removal of some of the infected kernels by the cleaning process.

The distribution of the DON was not uniform in the milling fractions of the samples, showing the highest concentration of DON in the outer layers of the wheat (Table 2). Others have also reported various concentrations of DON in different milling fractions, for example, Hart and Braselton (6) have reported concentrations of DON of 2, 0.8, 0.8, 2.5, 0.9, 0.9, 0.7, 0.4, and 0.8 ppm in whole wheat, bran, shorts, red dogs, first milling, second milling, bread 1, bread 2, and bread 3, respectively. Scott et al. (14) have reported various concentrations of DON of 4.62, 4.60, 6.80, 7.96, 4.11, and 4.16 ppm in wheat, bran, shorts, feed flour, straight-grade flour, and naturally contaminated bread, respectively. It may be postulated that the invasion of fungus into the wheat is not uniform.

DON was not destroyed by making bread, but the concentrations of DON were lower in all bread samples than in the starting wheat samples (Table 3). Kamimura et al. (7) reported that only 51% of DON (range, 36 to 66%) survived baking at 210°C for 20 min. The reduction in the DON concentrations during bread making may be due to the bread-making procedure, e.g., thermal decomposition and isomerization of DON (4).

The results presented in this paper show that the incidence and concentrations of DON in a limited number of samples from Nebraska, Missouri, and Kansas (milling wheat fractions, whole-wheat breads) and other samples from different parts of the United States were significant. The Food and Drug Administration suggestions for DON concentrations in uncleaned wheat were recently set at 2 ppm, a level which was well exceeded by the naturally infected samples (Table 2). Cleaning, milling, and baking were not totally effective in removing DON, showing the range of reductions of 24.2 to 71.3% from uncleaned wheat to bread. This compares well with the assumption of the Canadian Government of an overall 40% reduction in DON concentrations upon manufacturing products from uncleaned wheat (21). The results showed that wheat, milling fractions, and bread intended for human consumption can contain DON and clearly indicate that DON was distributed through all fractions of the milled wheat.

ACKNOWLEDGMENT

We gratefully acknowledge Karl W. Gildemeister, The Pillsbury Co., Minneapolis, MN 55414, for cleaning, milling, and baking the bread.

LITERATURE CITED

1. **American Association of Cereal Chemists.** 1983. Approved methods of the American Association of Cereal Chemists, 8th ed. American Association of Cereal Chemists, St. Paul, Minn.
2. **Chang, H. L., J. W. Devries, P. A. Larson, and H. H. Patel.** 1984. Rapid determination of deoxynivalenol (vomitoxin) by liquid chromatography using modified Romer column clean up. J. Assoc. Off. Anal. Chem. **66**:52–54.
3. **El-Banna, A. A., P. Y. Lau, and P. M. Scott.** 1983. Fate of mycotoxins during processing of foodstuffs. II-Deoxynivalenol (vomitoxin) during making of Egyptian bread. J. Food Prot. **46**:484–486.
4. **Greenhalgh, R., J. Gilbert, R. R. King, B. A. Blackwell, J. R. Startin, and M. J. Shepherd.** 1984. Synthesis, characterization, and occurrence in bread and cereal products of an isomer of 4-deoxynivalenol (vomitoxin). J. Agric. Food Chem. **32**:1416–1420.
5. **Hagler, W. M., Jr., K. Tyczkowska, and P. B. Hamilton.** 1984. Simultaneous occurrence of deoxynivalenol, zearalenone, and aflatoxin in 1982 scabby wheat from the midwestern United States. Appl. Environ. Microbiol. **47**:151–154.
6. **Hart, L. P., and W. E. Braselton, Jr.** 1983. Distribution of vomitoxin in dry milled fractions of wheat infected with *Gibberella zeae*. J. Agric. Food Chem. **31**:657–659.
7. **Kamimura, H., M. Nishijima, K. Saito, K. Yasuda, A. Ibe, T. Nagayama, H. Ushiyama, and Y. Naoi.** 1979. The decomposition of trichothecene mycotoxins during food processing. Studies on mycotoxins in foods. XII. J. Food Hyg. Soc. Jpn. **20**:352–357.
8. **Kamimura, H., M. Nishijima, K. Yasuda, K. Saito, A. Ibe, T. Nagayama, H. Ushiyama, and Y. Naoi.** 1981. Simultaneous detection of several *Fusarium* mycotoxins in cereals, grains, and foodstuffs. J. Assoc. Off. Anal. Chem. **64**:1067–1073.
9. **Kuroda, H., T. Mori, C. Nichioka, H. Okasaki, and M. Takagi.** 1979. Studies on gas chromatographic determination of trichothecene mycotoxins in food. J. Food Hyg. Soc. Jpn. **20**:137–142.
10. **Mirocha, C. J., S. V. Pathre, B. Schauerhamer, and C. M. Christensen.** 1976. Natural occurrence of *Fusarium* toxins in feedstuff. Appl. Environ. Microbiol. **32**:553–556.
11. **Morooka, N., N. Uratsuji, T. Yoshizawa, and H. Yamamoto.** 1972. Studies on toxic substances in barley infected with *Fusarium* spp. J. Food Hyg. Soc. Jpn. **13**:368–375.
12. **Neish, G. A., and H. Cohen.** 1981. Vomitoxin and zearalenone production by *Fusarium graminearum* from winter wheat and barley in Ontario. Can. J. Plant Sci. **61**:811–815.
13. **Osborne, B. G., and K. H. Willis.** 1984. Studies into the occurrence of some trichothecene mycotoxins in UK home-grown wheat and in imported wheat. J. Sci. Food Agric. **35**:579–583.
14. **Scott, P. M., R. Kanhere, P. Y. Lau, J. E. Dexter, and R. Greenhalgh.** 1983. Effects of experimental flour milling and bread-baking on retention of deoxynivalenol (vomitoxin) in Hard Red Spring wheat. Cereal Chem. **60**:421–424.
15. **Scott, P. M., P. Y. Lau, and S. R. Kanhere.** 1981. Gas chromatography with electron capture and mass spectrometric detection of deoxynivalenol in wheat and other grains. J. Assoc. Off. Anal. Chem. **64**:1364–1371.
16. **Trenholm, H. L., W. P. Cochrane, H. Cohen, J. I. Elliot, E. R. Farnworth, D. W. Friend, R. M. G. Hamilton, G. A. Neish, and J. R. Standish.** 1981. Survey of vomitoxin contamination of the 1980 white winter wheat crop of Ontario. J. Am. Oil Chem. Soc. **58**:992A–994A.
17. **Ueno, Y.** 1980. Trichothecene mycotoxins. Mycology, chemistry and toxicology, p. 301–353. *In* H. H. Draper (ed.), Advances in nutritional research, vol. 3. Plenum Publishing Corp., New York.
18. **Vesonder, R. F., and C. W. Hesseltine.** 1980/1981. Vomitoxin: natural occurrence on cereal-grains and significance as a refusal and emetic factor to swine. Process Biochem. **16**:12–15.
19. **Yoshizawa, T., and H. Hosokawa.** 1983. Natural occurrence of deoxynivalenol and nivalenol, trichothecene mycotoxins, in commercial foods. J. Food Hyg. Soc. Jpn. **24**:413–415.
20. **Yoshizawa, T., and N. Morooka.** 1973. Deoxynivalenol and its monoacetate. New mycotoxins from *Fusarium roseum* and moldy barley. Agric. Biol. Chem. **37**:2933–2934.
21. **Young, J. C., R. G. Fulcher, J. H. Hayhoe, P. M. Scott, and J. E. Dexter.** 1984. Effect of milling and baking on deoxynivalenol (vomitoxin) content of eastern Canadian wheats. J. Agric. Food Chem. **32**:659–664.