Distribution of Deoxynivalenol in Soft Wheat Mill Streams[1]

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ABSTRACT

A study was made of the distribution of deoxynivalenol (DON) in mill streams of soft wheat infected to varying degrees with scab (*Fusarium graminearum*). Eleven lots of soft wheat ranging in DON contents from 0.03 to 2.89 ppm were each cleaned by screening, conditioned to 14% moisture, and milled on a Miag Multomat mill. Cleaning reduced DON content of wheat by an average of 16%, and screenings had 4.7-fold higher DON contents than cleaned wheat. DON was found in all mill fractions, which included straight-grade flour, four break flours, six reduction flours, break and reduction shorts, red dog, and bran. Mean DON concentration in straight-grade flour was about 90% of that in cleaned wheat. The different lots of wheat, regardless of DON concentration, generally gave similar fractional distributions of DON as indicated by correlation coefficients. Mean DON concentrations were lower in flours (except for first reduction) and higher in offals than in whole wheat. Among break flours, mean DON concentrations increased slightly from the first to the third break. However, the first reduction flour exhibited a higher mean DON concentration than subsequent reduction flours, with the lowest concentration found in the fifth reduction flour.

Wet weather during maturation of the 1982 wheat crop in parts of the midwestern United States caused an unusually high occurrence of scab, a disease caused by the fungus *Fusarium graminearum* (Schwabe) (Weise 1977). Occurrence of 4-deoxynivalenol (DON), a metabolite of *F. graminearum*, was also common in the scabby wheat (Bertelsen 1982). Because DON had been reported to exhibit various toxicological effects in animals (Ueno 1983), the possibility of DON contamination of wheat-based foods was a matter of concern (Anonymous 1982). In response to these concerns, this study was initiated to determine how DON would be distributed among various products from the milling of scabby soft wheat. Results from milling tests with various wheats have been reported (Hart and Braselton 1983, Scott et al 1983, Young et al 1984), but most of those wheats were from eastern Canada, and only one sample was from the United States (Hart and Braselton 1983).

MATERIALS AND METHODS

Ten commercial lots of soft wheat from the 1982 crop were obtained from Missouri and one from Ohio. All lots were cleaned and milled at the Soft Wheat Quality Laboratory (USDA-ARS-NCR), Ohio Agricultural Research and Development Center, Wooster. The wheat (102-127 kg) was cleaned by using a combination of screening and air flow similar to most commercial cleaners. Because test weights were low for some lots, air flow was set slightly lower than normal to avoid removing too much low-density wheat. Cleaned wheat, tempered to 14% moisture, was milled with a slightly modified Miag Multomat mill as shown in Figure 1. Mean flour yield was 67.2% based on cleaned, tempered wheat. Immediately after the milling tests were completed, all samples were sent to the U.S. Grain Marketing Research Laboratory, Manhattan, KS, for chemical analyses. Samples were stored in plastic bags or metal cans at 4°C.

For determining DON in whole wheats and mill fractions the method of Scott et al (1981), with minor modifications, was used, except that high-pressure liquid chromatography replaced gas chromatography for detecting DON in final extracts. The

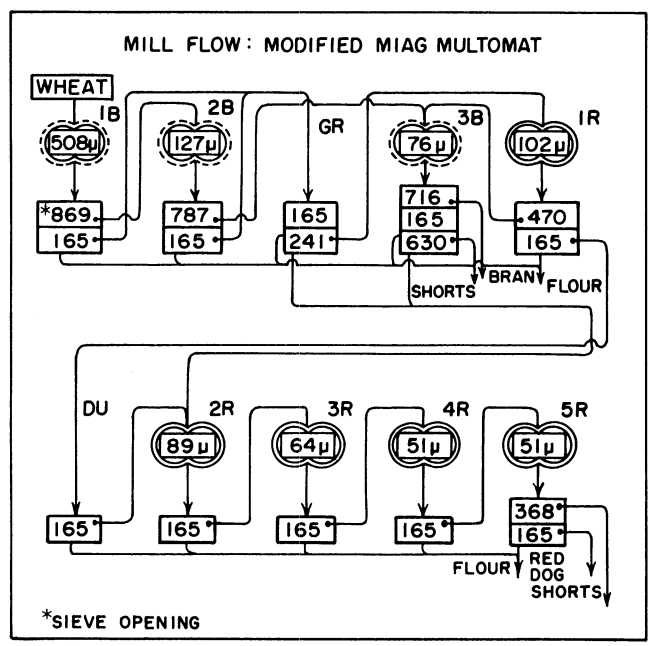

Fig. 1. Flow diagram for Miag Multomat mill. Numbers on the rolls are roll spacings in micrometers. Numbers in boxes are sieve openings in micrometers.

[1] Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others not mentioned.

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TABLE I
Composition and Test Weights of 11 Lots of Soft Wheat Used for the Milling Tests, and Deoxynivalenol (DON) Contents of Straight-Grade Flours

Lot	Variety	DON Contents (ppm) Uncleaned Wheat	Cleaned Wheat	Screening	Straight-grade Flour	Test Weight Cleaned Wheat (lb/bu)	Increase After Cleaning (%)	Screenings (% of Uncleaned Wheat)	Protein Contents of Cleaned Wheat[a] (%)	Ash Contents of Cleaned Wheat[a] (%)
1	Mix	1.98	1.16	11.40	1.37	60.3	1.9	4.9	10.8	1.58
2	Mix	0.24	0.32	NS[b]	0.15	62.4	1.0	2.5	10.4	1.65
3	Mix	2.40	1.91	NS	1.71	58.5	3.0	4.5	10.7	1.56
4	Pike	2.89	3.35	10.50	2.60	57.1	5.9	10.9	9.7	1.61
5	Hart	1.75	1.69	10.50	1.05	57.9	2.1	3.6	10.6	1.63
6	Stadler	0.18	0.13	0.20	0.14	58.8	1.6	2.0	8.9	1.45
7	Oasis	0.79	0.66	8.84	0.79	59.8	1.1	2.3	10.3	1.62
8	Pioneer	0.99	0.59	2.85	0.62	56.7	2.5	6.0	10.4	1.63
9	McNair	0.92	0.64	5.28	0.47	57.4	3.6	2.8	10.4	1.52
10	Arthur	1.36	0.81	1.31	1.00	56.5	3.7	15.1	12.9	1.73
11	Arthur-71	0.03	0.03	NS	0.01	60.4	0.7	1.2	9.5	1.53
	Mean	1.35[c]	1.13[c]	6.36	0.99	58.7	2.5	5.1	10.4	1.59

[a] 14% Moisture basis.
[b] NS = sample not available.
[c] Excluding lot 11.

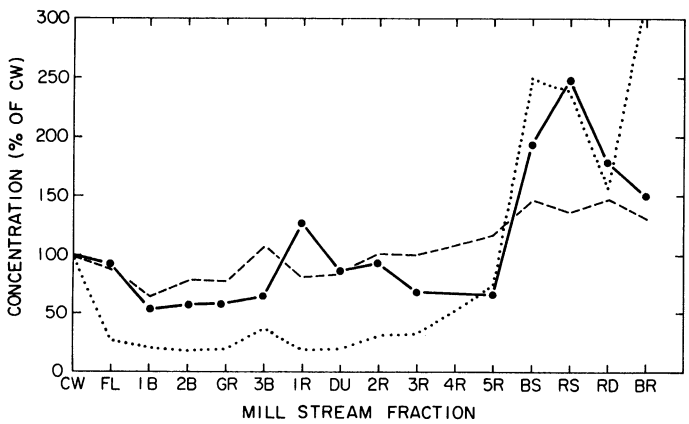

Fig. 2. Deoxynivalenol (DON) (——), ash (· · · ·), and protein (- - - -) contents of soft wheat mill fractions expressed as percentages of the respective component concentrations in cleaned wheat (see Materials and Methods). Fractions include cleaned wheat (**CW**), straight-grade flour (**FL**), break flours (**1B, 2B, 3B**), grader (**GR**), reduction flours (**1R, 2R, 3R, 4R, 5R**), duster (**DU**), break shorts (**BS**), reduction shorts (**RS**), red dog (**RD**), and bran (**BR**). DON values are means of 10 lots (lot 11 excluded), and ash and protein values are means of 11 lots. Means and ranges of coefficients of variation (%) were: 31.0, 12–47 for DON; 9.7, 4.1–20.8 for ash; and 3.6, 2.0–5.3 for protein.

methodology has been described previously (Seitz and Bechtel 1985). Each sample was analyzed once, except when an obviously incorrect result necessitated a reanalysis.

Protein, ash, and moisture were determined by AACC methods 08-01, 46-10, and 44-15A (1976), respectively. The ash method was modified such that samples were placed in the muffle furnace at 427°C (800°F), ignited, then heated at 496°C (925°F) for 16 hr, and finally at 538°C (1,000°F) for 4 hr.

To obtain an overall picture of the distribution of DON among mill fractions, the data were processed as follows: First, for each lot, DON concentrations in fractions were expressed as a percentage of the DON concentration in the cleaned wheat. Then, for each fraction, mean DON concentrations were calculated and plotted (Fig. 2). Protein and ash data were processed in the same way.

RESULTS AND DISCUSSION

Cleaning the scab-infected wheat by using a method similar to that used by commercial mills was not particularly effective for removing DON (Table I). Average DON content of cleaned wheat was only 16% lower than that in uncleaned wheat. Screenings

TABLE II
Correlation Matrix for Deoxynivalenol Contents of All Mill Fractions

Lot	2	3	4	5	6	7	8	9	10
1	0.90	0.95	0.92	0.94	0.90	0.90	0.92	0.91	0.70
2		0.94	0.88	0.98	0.87	0.93	0.93	0.79	0.49
3			0.90	0.97	0.93	0.96	0.93	0.87	0.62
4				0.89	0.88	0.85	0.94	0.94	0.54
5					0.93	0.94	0.94	0.83	0.51
6						0.85	0.86	0.89	0.40
7							0.95	0.79	0.66
8								0.86	0.59
9									0.54

contained about 5.6- and 4.7-fold higher DON concentrations than in cleaned and uncleaned wheats, respectively. High DON contents of the screenings resulted from the presence of lightweight, severely scab-infected kernels or pieces of kernels. However, the screenings sometimes consisted of enough other lightweight kernels and other materials to cause only moderate correlation between DON content of uncleaned wheat and percentage of screenings ($r = 0.54$, but 0.47 with lot 11 excluded). Test weights of all lots were increased by cleaning (Table I) and amount of increase correlated with DON content of uncleaned wheat ($r = 0.74$). Test weight of screenings averaged about 45 lb/bu.

The inefficient removal of DON by the cleaner is explained by considering recent results from chemical, physical, and microscopical studies of scab-infected hard red winter wheat, which showed that degree of *Fusarium* infection varied considerably among kernels, and that kernels with nearly normal size and weight had significant fungal invasion and DON contents (Seitz and Bechtel 1985). Infected kernels with near-normal size and weight cannot be selectively removed by the cleaner. Tests with eastern Canadian wheats (Scott et al 1983, Young et al 1984) and with several hard red winter wheats from Kansas (Seitz et al 1984) also showed that the DON content was reduced only slightly by cleaning.

The average DON concentration in straight-grade flour was 92% of that in cleaned wheat when the average was calculated as described in Materials and Methods. By using the means shown in Table I, average DON concentration in straight-grade flour was 88% of that in cleaned wheat and 73% of that in uncleaned wheat. DON concentrations in straight-grade flours and cleaned wheats were highly correlated ($r = 0.95$, Table I). The U.S. Food and Drug Administration has recommended that wheat containing DON at a level of 2 ppm or higher should not enter the milling process and that finished wheat products containing DON at a level of 1 ppm or higher should not be used for human consumption (Anonymous 1982). To meet the latter recommendation our results suggest that,

TABLE III
Correlation Matrix for Deoxynivalenol Contents of Flour Fractions

Lot	2	3	4	5	6	7	8	9	10
1	0.79	0.96	0.98	0.87	0.89	0.65	0.81	0.90	0.51
2		0.86	0.82	0.73	0.59	0.66	0.81	0.84	0.47
3			0.98	0.78	0.83	0.72	0.82	0.96	0.63
4				0.85	0.90	0.65	0.84	0.93	0.51
5					0.87	0.56	0.90	0.78	0.09
6						0.68	0.85	0.83	0.23
7							0.78	0.80	0.44
8								0.86	0.19
9									0.56

on the average, a lot of wheat should not be considered for milling if its DON content exceeds about 1.3 ppm before cleaning or about 1.1 ppm after cleaning.

All of the fractions from the milling of DON-contaminated lots contained DON (Fig. 2). Concentration levels were lower in flour fractions (except for first reduction) and higher in offal fractions than in cleaned wheat. This pattern of DON distribution was very similar among lots, regardless of the DON content of the whole wheat, as indicated by generally high correlation coefficients (Table II). The lowest correlations were associated with lot 10, which, as discussed later, differed from the other lots in the distribution of DON among flour fractions. Even with lot 11, which contained about 0.03 ppm DON in the cleaned wheat, DON contents of flours and bran were 0.01 to 0.02 ppm, red dog and break shorts about 0.07 ppm, and reduction shorts about 0.10 ppm.

Our results, as well as results from other tests (Scott et al 1983, Young et al 1984) indicating a fractionation of DON among all mill fractions, are consistent with recent findings concerning the distribution of the fungus in infected kernels. Results of microscopical analyses demonstrated that the fungus showed a preference to aleurone and pericarp tissues, but hyphae were found throughout the entire endosperm tissue (Bechtel et al 1985). Offal fractions are composed mostly of aleurone and pericarp tissues (high ash and protein), whereas flour fractions are composed of mostly endosperm tissue (low ash and protein). By using the data in Figure 2, we found that DON correlated nearly as well with protein ($r = 0.75$) and ash ($r = 0.80$) as protein and ash did with each other ($r = 0.82$). Young et al (1984) reported a positive correlation ($r = 0.80$) between ergosterol and DON in flour and offal fractions, which indicated that the DON was associated with fungal growth rather than transported from the outer to the inner part of the kernel.

The DON contents of some of the flour fractions were unexpected. High-ash flours, which contain more aleurone and subaleurone tissues than low-ash flours, could reasonably be expected to have higher DON concentrations. A very slight trend in this direction was observed with the break flours (Fig. 2). However, the observed trend for reduction flours was opposite that expected (Fig. 2). In all lots, the first reduction flour had the highest DON concentration, but it was low in ash and should have contained little aleurone or subaleurone tissue. Distribution among flours was generally similar among lots (Table III), except for lot 10, which showed a definite increase in DON content from the first to the third break flour and from the second to the fifth reduction flour (Fig. 3). Protein and ash contents of these flours followed the expected trends (Fig. 3). The DON distribution pattern for flours from lot 10 was similar to that observed for scab-infected hard red winter wheats milled with a Miag Multomat mill (Seitz et al 1984).

The relatively high DON concentration in the first reduction fraction may have resulted from changes in kernel endosperm fracturing characteristics caused by the fungus. Microscopical studies have shown that the fungus utilizes storage proteins, removes cell walls, and damages starch granules in heavily infected grain (Bechtel et al 1985). Apparently the fracturing of such kernels

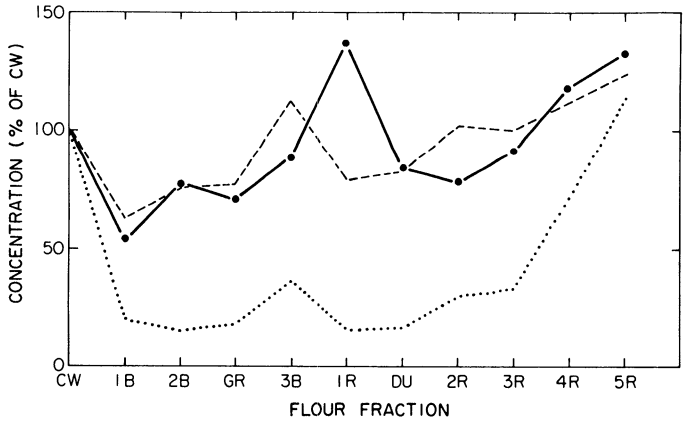

Fig. 3. Deoxynivalenol (——), ash (· · · ·), and protein (- - - -) contents, expressed as percentages of the respective component concentrations in cleaned wheat, of flour fractions from the milling of lot 10 wheat. Refer to Figure 2 for identification of fractions.

by the first- and second-break rolls tended to provide particles of a size range susceptible to reduction to flour by the first reduction rolls (Fig. 1). Infected particles of appropriate size range for diversion to the grader were reduced by the second reduction rolls, accounting for the higher-than-expected concentration of DON in the second reduction flour. Although gross mill-stream yields from infected grain did not appear to differ significantly from those milled from normal grain of the same cultivars, it is possible that certain highly infected particles fractured abnormally, thus altering their particle distribution.

LITERATURE CITED

ANONYMOUS. 1982. Food Chem. News, October 4, p. 3.

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1976. Methods 08-01 and 46-10, approved April 1961; Method 44-15A, approved October 1975. The Association: St. Paul, MN.

BECHTEL, D. B., KALEIKAU, L. A., GAINES, R. L., and SEITZ, L. M. 1985. Effects of *Fusarium graminearum* infection on kernels from scabby wheat. Cereal Chem. 62:191.

BERTELSEN, A. 1982. Toxins found in several states' wheat. Feedstuffs 54(29):1.

HART, L. P., and BRASELTON, W. E., Jr. 1983. Distribution of vomitoxin in dry milled fractions of wheat infected with *Gibberella zeae*. J. Agric. Food Chem. 31:657.

SCOTT, P. M., KANHERE, S. R., LAU, P.-Y., DEXTER, J. E., and GREENHALGH, R. 1983. Effects of experimental flour milling and breadbaking on retention of deoxynivalenol (vomitoxin) in hard red spring wheat. Cereal Chem. 60:421.

SCOTT, P. M., LAU, P.-Y., and KANHERE, S. R. 1981. Gas chromatography with electron capture and mass spectrometric detection of deoxynivalenol in wheat and other grains. J. Assoc. Off. Anal. Chem. 64:1364.

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.

SEITZ, L. M., EUSTACE, W. D., SHOGREN, M. D., BECHTEL, D. B., and YAMAZAKI, W. T. 1984. Cleaning, milling, and baking tests with scab-infected hard red winter wheat. (Abstr.) Cereal Foods World 29:499.

UENO, Y. 1983. General toxicology. Page 135 in: Developments in Food Science, Vol. 4, Trichothecenes-Chemical, Biological and Toxicological Aspects. Y. Ueno, ed. Elsevier: New York.

WEISE, M. V. 1977. Fungal diseases. Page 16 in: Compendium of Wheat Diseases. Am. Phytopathol. Soc.: St. Paul, MN.

YOUNG, J. C., FULCHER, G. R., HAYOE, J. H., SCOTT, P. M., and DEXTER, J. E. 1984. Effect of milling and baking on deoxynivalenol (vomitoxin) content of eastern Canadian wheats. J. Agric. Food Chem. 32:659.

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