Effects of Experimental Flour Milling and Breadbaking on Retention of Deoxynivalenol (Vomitoxin) in Hard Red Spring Wheat

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ABSTRACT

Hard spring wheat from the 1981 eastern Canadian crop naturally contaminated with deoxynivalenol (DON, vomitoxin) was cleaned and milled in an Allis-Chalmers experimental mill, and the straight grade flour was baked into bread. The cleaned wheat, dockage, bran, shorts, feed flour (red dog), straight grade flour, and bread were analyzed for DON by gas liquid chromatography using electron capture and mass spectrometric single-ion monitoring detection; two methods of cleanup gave comparable results. DON was distributed throughout the milled products and was not destroyed on making bread. The highest concentration of DON was found in dockage (16.7 μg/g); the cleaned wheat contained 4.6 μg/g, and the flour and bread (flour weight basis) contained an average of 4.1 and 4.2 μg/g in two milling and baking experiments.

The mycotoxin deoxynivalenol (DON) was first obtained from moldy Japanese barley primarily infected with *Fusarium* species and from a strain of *Fusarium roseum* isolated from the barley (Morooka et al 1972). It was initially given the trival name Rd-toxin until establishment of the chemical structure as the trichothecene 3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one (deoxynivalenol) (Yoshizawa and Morooka 1973). The same toxin was isolated in the United States from corn contaminated with *Fusarium* that caused emesis (vomiting) in swine and was descriptively named vomitoxin (Vesonder et al 1973). Grains infected with *Gibberella zeae* (perfect stage of *F. graminearum*) frequently bring about feed refusal as well as vomiting in swine (Vesonder and Hesseltine 1980). DON has been shown to be a powerful emetic agent in swine, dogs, and ducklings (Ueno 1980) and to cause feed refusal by swine and rats (Forsyth et al 1977; Vesonder et al 1973, 1979; Yoshizawa et al 1978). In a recent chronic study, the level in swine that did not affect feed consumption, feed conversion, weight gain, and certain blood properties was reported to be less than 1 μg/g of feed (Schuh et al 1982). DON was also fetotoxic in mice at maternal doses of 2.5 mg/kg of body weight per day (Khera et al 1982). Thus, the widespread occurrence of DON in grains (corn, barley, and wheat) in many parts of the world, including Canada and the United States (Bottalico et al 1981, Kamimura et al 1981, Kuroda et al 1979, Scott et al 1981, Trenholm et al 1981, Ueno 1980, Vesonder and Hesseltine 1980), is cause for concern from the viewpoint of animal and human health.

Little information is available on the fate of trichothecenes during grain processing. In one study, (Collins and Rosen 1981), wet-milling of corn removed about two thirds of the T-2 toxin initially present. Kamimura et al (1979) reported on 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) present. To assess human consumption of DON in finished foods, it is essential to extend available data on the stability of DON during food processing. We present some preliminary results on the retention of DON in a sample of naturally contaminated hard red spring wheat during milling and breadmaking.

MATERIALS AND METHODS

Wheat

A sample of Concord, a hard red spring feed wheat, from the 1981 Quebec crop was obtained for the study. Previously, Laboratory Services Division, Agriculture Canada had estimated that this sample contained 7.1 μg/g of DON.

Some sprouted kernels were present in the wheat, resulting in a falling number (ICC 1967) of only 115 sec. Other wheat characteristics included a test weight (using the 1-L Schopper Chondrometer) of 73.5 kg/hl, protein content (percent N × 5.7 by the standard Kjeldahl procedure) of 13.4%, and ash of 1.70% (AACC 1983).

Before it was prepared for milling, the wheat was cleaned on a Carter dockage tester. All of the dockage (0.90% by weight) and a representative portion of the cleaned wheat were retained for DON analysis.

Milling

The cleaned wheat was separated into two 2-kg lots for duplicate millings in an Allis-Chalmers laboratory mill using the GRL sifter flow of Black et al (1980). Wheat moisture was 16.3%, so the wheat was milled without further conditioning. The mill room was controlled for temperature (22° C) and relative humidity (60%).

All bran, shorts, and feed flour (red dog) from each milling and a portion of each straight-grade flour were retained. These were rotated for 2 hr before representative samples were taken for DON analysis.

Flour Properties

Straight grade flours were analyzed separately for Kjeldahl protein (percent N × 5.7), ash (AACC 1983), and Kent-Jones color (Holas and Tipples 1978). Farinograph mixing properties were determined by standard AACC (1983) methods.

Baking

The straight grade flours were each baked into three loaves by the Canadian short process described by Preston et al (1982), which features a 30-min bake at 205° C. Loaf volume was measured by rapeseed displacement.

Each loaf was weighed before and after air-drying, then ground, blended, and a representative sample taken for DON analysis.

Methods of Analysis for DON

Two methods were employed. The same methanol-water extract was used for both. Method 1 was the published method for determination of DON in wheat (Scott et al 1981) with change in concentration of ammonium sulfate from 30% to 10%. In Method 2 (Scott et al 1982), 20 ml of filtered sample extract in methanol-water (1 + 1) after treatment with 10% ammonium sulfate was added to a Clin Elut extraction column (Analytichem International, no. 1020, Harbor City, CA). Eight 20-ml portions of ethyl acetate were used to elute the column, the ethyl acetate was evaporated without drying over anhydrous sodium sulfate, and the residue (≡0.8 g sample) was dissolved in 0.8 ml of toluene-acetonitrile (95 + 5). Gas liquid chromatography (GLC) was performed after derivatization with heptafluorobutyrylimidazole (Scott et al 1981). The final concentration of sample in the diluted

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derivatized extract was equivalent to 20 mg/ml. Concentration of DON in the diluted derivatized standard was 0.020 μg/ml. Estimations were made by comparison of peak heights, measured from the tangential (sloping) baseline (Fig. 1), in the injected sample with standard injected before and after the sample. A Varian 3700 gas chromatograph, equipped with a ^{63}Ni electron capture (EC) detector and 183 cm × 2 mm i.d. glass column packed with 3% OV-3 on Chromosorb W-HP (80/100 mesh), was used. The column temperature was 160° C, injector temperature 250° C, and detector temperature 300° C; nitrogen flow rate was 46 ml/min. The attenuation was 16×10^{-12} amp/mV.

Confirmation of GLC-EC results were by GLC-mass spectrometric single-ion monitoring (MS[SIM]) at m/z 884 at a resolution of 1,500 (10% valley) with operating conditions slightly different from those described previously (Scott et al 1981). The GLC column temperature was 200° C. One sample extract each of flour and bread was subjected to capillary GLC-MS(SIM) after both heptafluorobutyrate (HFB) and trimethylsilyl (TMS) ether derivatization. The latter procedure was done with TRI-SIL TBT (Pierce Chemical Co., Rockford, IL). Instrumentation consisted of a Varian 3700 gas chromatograph equipped with a 29-m DB-5 fused silica capillary column and splitless injection and coupled directly to a VG Micromass ZAB-2F mass spectrometer operated at 1,500 resolution, 70 eV electron energy, and ion source temperature 180° C. For the HFB derivative, the column temperature was programmed from 60° C (after 1 min) to 160° C in 2 min, then to 240° C at 5° C/min, whereas for the TMS derivative the initial temperature was 80° C (1 min); injector and transfer line temperatures were 200° C; and head pressure was 26 psi. Retention times were 12.4 and 15.1 min, respectively. Ions monitored were m/z 884 (HFB derivative) and 512 and 497 (TMS derivative).

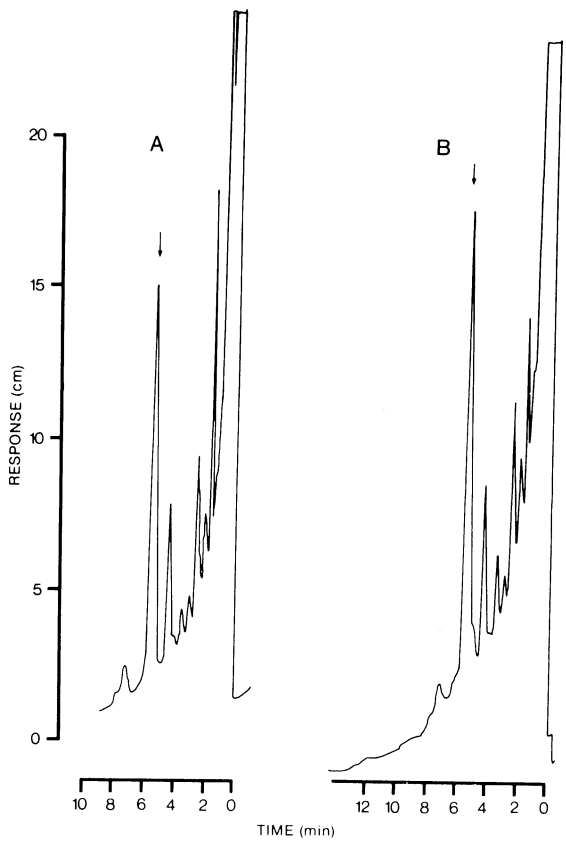

Fig. 1. GLC-EC of DON (indicated by arrow, retention time 5.3 min) in flour (sample B1) by **A** Method 1 and **B** Method 2; 10 μg flour equivalent injected. EC detector sensitivity to DON was 37% higher when chromatogram B was run.

TABLE I
Distribution of Milling Products and Average Concentrations[a] and Amounts of DON in Wheat, Milling Products, and Bread

Product	Product Distribution[b] (%) Milling A	Milling B	DON, μg/g Milling A	Milling B	Total Amount of DON, mg[c]
Cleaned wheat	100		4.62		18.48
Dockage	0.90		16.7		0.60
Bran	16.5	16.7	5.03	4.16	3.05
Shorts	4.6	4.6	6.30	7.42	1.26
Feed flour	2.8	2.9	10.3	5.61	0.90
Straight-grade flour	75.9	75.3	4.25	3.96 [3.06][d]	12.42
Bread[e]	···	···	4.32 [3.86][d]	4.00	···

[a] Determined by GLC-EC by two methods and corrected for recoveries.
[b] Straight grade flour as proportion of cleaned wheat to first break on a constant moisture basis; other products on as is moisture basis.
[c] Based on total of 4 kg wheat milled in both millings A and B.
[d] Includes one doubtful analysis.
[e] On equivalent flour weight basis.

RESULTS AND DISCUSSION

The distribution of milled products was very consistent between the two millings (Table I). Although Concord is considered to be of feed quality, milling properties were fairly adequate. Straight grade flour yield was in excess of 75% for both millings.

Flour color was much poorer than would be predicted from the flour ash (Table II). Farinograph development time was short and mixing tolerance index quite high, as is often the case for sprouted wheat flour. Nevertheless, dough properties were satisfactory for baking, and on a unit protein basis loaf volume approached that normally achieved for high-quality hard red spring wheat flour (Preston et al 1982).

Average concentrations of DON in the various milling fractions and in bread are presented in Table I, and individual determinations are shown in Table III. One extract of each type of product prepared by both Methods 1 and 2 was subjected to confirmation by GLC-MS (SIM) on a packed column, and it is evident that results obtained by GC-EC refer to DON and that no interferences were present (Table III). Furthermore, capillary GLC-MS (SIM) on flour and bread samples A1 extracted by Method 1 showed only one peak for the HFB derivative. Very minor extraneous TMS peaks, 0.9 and 2.6% of the DON peak when monitored at m/z 512, were observed in the bread sample only at retention times of 14.4 and 15.4 min, respectively (DON at 15.1 min). These could represent isomers of DON formed during the bread-making process, but amounts were negligible. Table III also shows that the two cleanup methods gave comparable results at the high levels of naturally occurring DON used in this study; chromatograms were similar, although a small shoulder was observed by GLC-EC for Method 2 just before the retention time

TABLE II
Flour, Farinograph, and Bread Properties

Property	Milling A	Milling B
Flour		
Protein (% N × 5.7)	11.5	11.3
Color (Kent-Jones units)	5.5	5.5
Ash (%)	0.51	0.50
Farinograph		
Absorption (%)	59.1	59.1
Development time (min)	2.00	2.00
Mixing tolerance index (BU)[a]	120	130
Bread		
Baking absorption (%)	59.0	59.0
Loaf volume (cc)	1,580	1,520

[a] Brabender units.

of DON (Fig. 1). Recoveries of DON added to blank ground wheat were also essentially equivalent for the two methods (Table III). Concentrations of DON in bread were determined on a dry weight basis; dry loaf weights averaged 227.5 g (range 216.1–233.9 g) and before drying the average weight was 290.8 g (range 289.5–293.3 g). When the concentrations of DON in each loaf were converted to an equivalent flour weight basis, corrections for method recoveries applied, and determinations averaged for each type of product, the results shown in Table I for millings A and B were obtained.

Our studies clearly show that DON was distributed throughout the products of the flour milling process and that none was lost (Table I). A higher concentration (but only 3.2% of the weight of DON present) was found in the dockage, so cleaning of wheat brings about a slight reduction of DON levels. DON was not destroyed on making bread from the naturally contaminated straight grade flour. This result is in agreement with recent observations (El-Banna et al 1983) on making Egyptian bread from naturally contaminated and spiked whole wheat flour (350° C baking for 2 min). Kamimura et al (1979) reported that only 51% of DON (range 36–66%) survived baking at 210° C for 20 min. However, they did not report method recovery data.

The high retention of DON in flour and bread in the current study is of concern to food regulatory authorities. It would be premature to draw definite conclusions from just one set of experiments. Further investigations into the stability of DON during wheat processing, preferably using pilot scale and commercial equipment and at lower levels of DON contamination, are urgently required and are currently in progress on both hard and soft wheat.

TABLE III
Analyses of Wheat, Milling Products, and Bread for DON[a]

			DON, μg/g			
			GLC-EC		GLC-MS (SIM)	
Sample	Milling	Experiment	Method 1	Method 2	Method 1	Method 2
Cleaned wheat		1	4.27	5.32	4.72	4.65
		2	3.73	3.51	...	...
Dockage			9.08 11.7[b]	20.0	16.1	22.8
Bran	A	1	4.38	5.53	4.45	5.57
		2	4.01	4.40	...	...
	B	1	...	3.97	...	...
		2	3.72	3.85	...	...
Shorts	A		5.26	6.21	6.23	7.12
	B		6.87	6.63		
Feed flour	A		9.11	9.78	8.33	7.50
	B		4.89	5.32	...	...
Flour	A	1	3.93 4.15[b]	4.58	4.99	6.65
		2	3.40	3.42	...	...
	B	1	3.81	3.77	...	...
		2	3.03	[0.32][c]	...	...
Bread (dry weight basis)	A	1	3.43 3.32[b]	5.31	4.14	4.32
		2	[1.16][c]	3.41	...	...
		3	2.91	3.04	...	...
	B	1	3.36	4.53	3.73	4.48
		2	2.68	2.93	...	...
		3	2.49	2.86	...	...
			Method Recovery, %[d]			
		1	77	86	67	68
		2	91	114	...	...
		3	90	89	...	...
Average recovery		86	96			

[a] Uncorrected for recovery, single determinations.
[b] Repeated derivatization of extract.
[c] Analyses doubtful.
[d] DON (1 μg/g) added to ground wheat containing no detectable DON.

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