Two Physical Methods for the Decontamination of Four Cereals Contaminated with Deoxynivalenol and Zearalenone†

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Experiments were conducted to examine the efficacy of two physical methods (sieving and dehulling) in reducing the concentrations of deoxynivalenol (DON) and zearalenone (ZEN) in contaminated barley, wheat, corn, and rye. Coarsely ground barley, wheat, and corn containing 5–23 and 0.5–1.21 mg/kg DON and ZEN, respectively, were segregated into fractions of differing particle sizes by sieving through a series of screens. The retained fractions containing the larger particles (+9 mesh barley; + 9 mesh wheat; +16 mesh corn) contained 67–83% less toxin than was present in the whole kernel. Removing the hull material from barley prior to sieving resulted in a further 16% reduction in the DON content of the +9 mesh fraction (from a 73% reduction in intact barley to an 89% reduction in dehulled barley). The amount of material lost during the sieving procedure was 34, 55, and 69% of the total material for intact ground barley, wheat, and corn, respectively, and 39% for dehulled and ground barley. When barley, wheat, and rye were treated in a Scott-Strong dehuller (to remove the outer portion of the kernels), 40–100% of the DON and ZEN, 13–19% of the grain material, and 22–32% of the protein was removed from the grain. It was concluded that both sieving and dehulling can represent useful procedures for reducing mycotoxin levels in contaminated grain, under certain circumstances.

INTRODUCTION

Deoxynivalenol (DON, vomitoxin, 3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one) and zearalenone [ZEN, (*S*)-2,4-dihydroxy-6-(10′-hydroxy-6′-oxo-*trans*-1-undecenyl)benzoic acid μ-lactone] are naturally occurring secondary metabolites produced by several species of *Fusarium* fungi on a variety of cereal grains.

Feed contaminated with these mycotoxins has been associated with poor growth, reproductive problems, or illness in farm animals. Pigs are particularly susceptible to the adverse effects of DON and ZEN. DON, a trichothecene mycotoxin, has been reported to cause decreased feed intake and weight gain in pigs when fed at concentrations greater than 2 mg/kg feed, and feed refusal and vomiting when fed at concentrations greater than 20 mg/kg (Trenholm et al., 1988). ZEN, a resorcylic acid lactone with estrogenic activity, causes reproductive problems, swollen vulva, uterine enlargement, vaginal prolapse, and rectal prolapse in swine (Trenholm et al., 1988; Young et al., 1981; Chang et al., 1979).

Many physical and chemical methods for decontaminating *Fusarium* mycotoxin contaminated grain have been tried with varying degrees of success. Physical methods have included density segregation of contaminated kernels from noncontaminated kernels, in water and saturated sodium chloride (Babadoost et al., 1987) or sucrose solution (Huff and Hagler, 1985), food-processing practices such as milling (Lee et al., 1987; Seitz et al., 1985, 1986; Scott et al., 1984; Young et al., 1984), cleaning or washing (Seitz et al., 1985, 1986; Scott et al., 1983), and baking (Seitz et al., 1986; Young et al., 1984; Abbas et al., 1988; Tanaka et al., 1986; El-Banna et al., 1988), and, although not a decontamination method per se, dilution of contaminated grain with clean grain has also been used to reduce the toxicity of contaminated feedstuffs. Density segregation, milling, cleaning, and baking have been largely unsuccessful in completely removing the DON and ZEN from flour fractions or from whole wheat, except in wheat containing a very low concentration of ZEN (8 μg/kg), where the mycotoxin concentration in the flour produced by milling was below the detection limit of the assay and considered to be zero (Tanaka et al., 1986).

For a decontamination method to be commercially feasible it needs to be effective without creating new toxins, introducing new toxic compounds, or altering the nutritional value or other desirable parameters of food or feed; it should be simple and inexpensive and use existing technology (Young, 1985).

Two such methods (sieving and dehulling) were tested in the study reported here. The distribution of DON and ZEN in fractions of differing particle size from coarsely ground barley, wheat, and corn was investigated. In addition, the effect of dehulling barley, by floating off the hulls, on the concentration and hence distribution of DON and ZEN in these fractions was also investigated. Finally, the effect of dehulling barley, wheat, and rye by using a commercial Scott-Strong dehuller on the concentration of DON and ZEN in the outer and inner portions of the kernel was also investigated.

MATERIALS AND METHODS

Sieving. Naturally contaminated intact barley (from Nova Scotia) containing 14.2–18.6 mg of DON/kg of 0.6–1.0 mg of ZEN/kg, Soft White Winter wheat (from Quebec) containing 5.4 mg of DON/kg, and corn (from Ontario) containing 23.3 mg of DON/kg of 1.2 mg of ZEN/kg kernels were coarsely ground (approximately 1–2 kg) in a bench-sized plate mill (Sprout-Waldren, Model 105-A, Koppers Co. Inc., Muncy, PA), adjusted to produce, predominantly, particles of greater than 2.0-mm size. The ground samples were then sieved through a series of four stacked 20-cm screens, Tyler equipment mesh sizes 9, 16, 20, and 24 (2.0-, 0.991-, 0.841-, and 0.707-mm opening size, respectively). The resulting five samples, one taken from each of the four screens (+9, +16, +20, and +24, consisting of particles of larger size than the holes in the corresponding screen) and one that passed through all four screens (−24, consisting of particles smaller than the holes

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0021-8561/91/1439-0356$02.50/0

Table I. Deoxynivalenol (DON) and Zearalenone (ZEN) Concentrations and Percentage Distribution (Percent Total) in the Various Sieved Fractions of Coarsely Ground Barley, Wheat, and Corn

	grain[a]								
	barley			wheat		corn		
	fraction wt, %	mycotoxin concn, mg/kg (% total)		fraction wt, %	mycotoxin concn,[b] mg/kg (% total)	fraction wt, %	mycotoxin concn, mg/kg (% total)	
		DON	ZEN		DON		DON	ZEN
whole kernel	100	14.2	0.50	100	5.44	100	23.1	1.21
+9	65.7	5.87 (27.2)	0.25 (32.9)	45.1	2.05 (17.0)	10.0	18.7 (8.1)	0.88 (7.3)
+16	16.5	26.2 (30.4)	0.58 (19.1)	0.0	0.0	21.2	20.3 (18.6)	0.77 (13.5)
+20	3.0			30.5	8.31 (46.6)	0.0		
+24	1.5			4.4	8.31 (6.7)	15.3	23.3 (15.4)	1.42 (18.0)
-24	13.3	35.0 (32.8)	1.46 (38.8)	20.0	8.06 (29.6)	53.6	25.0 (58.0)	1.58 (70.0)

[a] Fractions with insufficient amounts of material could not be analyzed. [b] The ZEN concentration of Quebec Winter wheat was below the detection level of the assay and assumed to be zero.

Table II. Deoxynivalenol (DON) Concentration and Percentage Distribution (Percent Total) in the Various Sieved Fractions of Intact Barley and Barley That Had Been Dehulled by Floatation in Dichloromethane

	intact barley[a]		dehulled barly[b]		hulls[c]
mesh size	fraction wt, %	total concn,[d] mg/kg (% total)	fraction wt, %	DON concn,[d] mg/kg (% total)	calcd fraction wt, %
+9	65.7	5.87 (27.2)	61.2	2.45 (10.6)	4.5
+16	16.5	26.2 (30.4)	13.8	14.7 (14.3)	2.7
+20	3.0		1.9		1.1
+24	1.5		1.2		0.3
-24	13.3	35.0 (32.8)	12.3	31.8 (27.5)	1.0

[a] Whole kernel DON concentration, 14.2 mg/kg; fraction weight, 100%. [b] Fraction weight and total DON percentages calculated by using values for intact barley given in footnote a. [c] Hull fraction weights calculated as the differences between those in intact barley and those in dehulled barley. Total weight of hull material in intact barley was 9.5%. DON concentration of hull material was 79.1 mg/kg or 52.9% of the total DON content of the intact barley. [d] +20 and +24 fractions could not be analyzed for DON due to the small quantity of material obtained.

in the 24-mesh screen), were each ground to a fine powder in a Retsch grinder (Sybron/Brinkham, Westbury, NY), containing a 0.5-mm screen, before being weighed and analyzed for their DON and ZEN concentrations. A similar procedure was carried out for coarsely ground barley that had been dehulled by floatation in dichloromethane prior to sieving.

Dehulling Using Dichloromethane. All work with dichloromethane was performed in a fume hood equipped with an extractor fan and an exhaust system. Ground barley (103.5 g) was immersed in dichloromethane (600 mL) for 5–10 min. The hull material, which floated to the surface of the liquid, was removed. The dichloromethane was decanted from the remainder of the barley, and excess solvent was removed by evaporation at room temperature in a fume hood overnight.

Dehulling Using a Scott-Strong Dehuller. Intact barley (415 g), Opel wheat (257 g, containing 2.4 mg of DON/kg), and Keenan rye (201 g, containing 0.08 mg of DON/kg) kernels were treated in a Scott-Strong dehuller for 25 (barley and wheat) or 35 (rye) to shave off the outer coat and hence divide the kernels into outer and inner fractions. The outer fraction was collected in a tray as a fine powder. The inner fraction was then ground to a fine powder, and both the inner and outer fractions were weighed before being analyzed for their DON, ZEN, and protein concentrations. To discover the optimum contact time for maximum DON removal from the inner fraction, barley was dehulled for 15, 25, or 35 s and the resulting inner and outer fractions were analyzed for their DON and ZEN concentrations.

Analysis. High-pressure liquid chromatography was performed on equipment obtained from Spectra-Physics (Spectra-Physics, San Jose, CA): Model SP-8810 isocratic pump, SP-4290 integrator, SP-8780 autosampler; Kratos Spectroflow 773 variable-wavelength detector (Kratos Analytical Inc., Ramsey, NJ) set at 220 nm for DON analysis, Kratos Spectroflow 980 programmable fluorescence detector (236-nm excitation wavelength, 418-nm cutoff emission filter for ZEN analysis).

LC columns used were stainless steel, 250 × 4.6 mm i.d., packed with reverse-phase RP-18, 5 μm OD-5A Spheri-5 (Brownlee Labs, Santa Clara, CA). A guard column (Waters Scientific Ltd., Mississauga, ON), filled with Spherisorb S10-0DS1, 10 μm (Phase Separations, Norwalk, CT), was inserted between injector and LC columns. All analyses were done in duplicate and compared by the method of external standards using three different concentrations of freshly prepared DON and ZEN standards.

DON was analyzed by the method of Trenholm et al. (1985) and ZEN by a modification of the method of Trenholm et al. (1984). For ZEN analysis a 50-g sample was extracted in 250 mL of acetonitrile/water (21:4) for 2.5 h. Two milliliters of phosphate buffer (pH 7.8) was added to 10 mL of the supernatant. The sample was mixed and evaporated at 45 °C to 1–2 mL under a gentle stream of nitrogen. The remaining residue was extracted with 10 mL of 2-propanol (10%) in ether. The upper ether extract was separated and chilled on ice. Two milliliters of chilled NaOH (0.2 M) was added and the mixture mixed thoroughly for 2 min. The upper ether layer was discarded and the remaining solution neutralized with 0.5 M acetic acid to pH 7.0; phenol red (3 drops) was used as the pH indicator. The mixture was extracted twice with 3 mL of 2-propanol (10%) in ether. The ether extracts were pooled and evaporated to dryness under nitrogen at room temperature. Methanol (3.64 mL), acetonitrile (1.82 mL), and water (4.55 mL) were added sequentially to the dried residue, and the solution was mixed thoroughly. Strict safety precautions were observed when working with ether. An aliquot of the cloudy extract, equivalent to 20 mg of sample, was then injected from a 100-μL loop onto the HPLC column. ZEN peaks were monitored with a fluorescence detector.

Protein was analyzed in duplicate by the method of Hambleton and Noel (1975). Acid detergent fiber (ADF) analysis was performed according to the method of Goering and Van Soest (1970).

RESULTS AND DISCUSSION

From Table I it is evident that DON and ZEN were not homogeneously distributed throughout the fractions of differing particle size in coarsely ground barley, wheat, and corn. In general, lower concentrations (and smaller

Table III. Deoxynivalenol (DON) and Zearalenone (ZEN) Concentrations and Percentage Distribution (Percent Total), Acid Detergent Fiber (ADF), and Dry Matter (DM) Content in the Inner and Outer Fractions of Barley Dehulled in a Scott-Strong Dehuller for Various Contact Times

	15-s contact time					25-s contact time					35-s contact time				
		mycotoxin concn, mg/kg (% total)					mycotoxin concn, mg/kg (% total)					mycotoxin concn, mg/kg (% total)			
fraction	fraction wt, %	DON	ZEN	ADF, %	DM, %	fraction wt, %	DON	ZEN	ADF, %	DM, %	fraction wt, %	DON	ZEN	ADF, %	DM, %
whole kernel	100	16.40	0.60	6.92[a]	96.3	100	15.40	0.95	6.93[a]	95.6	100	18.60	0.73	6.60[a]	96.0
inner	79.0	5.11 (24.6)	0.22 (29.0)	2.39	96.1	76.4	2.71 (13.4)	0.21 (16.9)	1.57	95.8	75.9	2.91 (11.9)	0.15 (15.6)	1.51	96.0
outer	21.0	58.97 (75.5)	2.02 (70.7)	23.95	97.0	23.6	56.40 (86.4)	3.36 (83.5)	24.26	95.0	24.1	67.90 (88.0)	2.57 (84.8)	22.62	96.0

[a] Values calculated.

Table IV. Deoxynivalenol (DON) and Zearalenone (ZEN) Concentrations and Percentage Distribution (Percent Total) in Barley, Wheat, and Rye Dehulled in a Scott-Strong Dehuller

	grain[a]								
	barley			wheat			rye		
		mycotoxin concn, mg/kg (% total)			mycotoxin concn, mg/kg (% total)			mycotoxin concn, mg/kg (% total)	
fraction	fraction wt, %	DON	ZEN	fraction wt, %	DON	ZEN	fraction wt, %	DON	
whole kernel	100	0.53	0.02	100	2.45	0.08	100	0.08	
inner	86.3	0.37 (60.3)	0.00[b] (0.0)	81.4	1.43 (47.5)	0.00[b] (0.0)	81.7	0.00[b] (0.0)	
outer	13.7	1.52 (39.3)	0.15 (103)	18.6	6.89 (52.3)	0.42 (97.7)	18.3	0.48 (110)	

[a] Contact time in the dehuller was 25 s for barley and wheat and 35 s for rye. [b] Below the detection limit of the assay and therefore assumed to be zero.

total amounts) of toxins were found in the fractions containing the largest particles (+9 mesh for barley and wheat and +16 mesh for corn); conversely, higher concentrations (and greater total amounts) were found in the fractions containing the smaller particles. By removing these smaller particles, approximately 73 and 67% of the total DON and ZEN, respectively, were removed from the barley, 83% of the total DON was removed from the wheat, and 73 and 79% of the total DON and ZEN, respectively, were removed from the corn. However, under these conditions 34, 55, and 69% of the total weights of the barley, wheat, and corn, respectively, were removed also. Since such large losses of grain material would be uneconomical in a commercial situation (A. Kelly, personal communication), this decontamination procedure would be economically feasible only during conditions of extensive mycotoxin contamination of the grain crop.

Earlier studies have suggested that in cases of relatively moderate *Fusarium* infestation in wheat the degree of DON and ZEN contamination is usually greatest at the exterior of the kernel (Lee et al., 1987; Seitz et al., 1985, 1986; Tanaka et al., 1986). A similar distribution is suspected for other grains as well.

In the present experiment the hulls of barley were found to contain a high concentration of DON (79.1 mg/kg), and although it represented only 9.5% of the total kernel weight, the hull material contained 52.9% of the total toxin (Table II). This agrees with the findings of other workers, who showed that the chaff of various cereals, contaminated with DON, had a consistently higher DON concentration than the inner portion of the kernel (Miller et al., 1985). In the present experiment, during coarse grinding the hull material was fractured into various sized slivers that were distributed throughout the fractions but predominantly into those containing the largest particles (+9 and +16 mesh). Consequently, when the hull material was removed prior to sieving coarsely ground barley, the largest reductions in DON concentration and content occurred in the +9- and +16-mesh fractions. A considerable reduction in DON contamination was achieved when ground barley was first dehulled and then sieved to eliminate the particles smaller than +16 mesh; approximately 75% of the DON was removed, while only 25% of the barley material was lost.

A more practical method for removing the hulls from grain is by the use of a Scott-Strong dehuller. This commercial dehuller, designed specifically for barley, was used to grind off the outer surface of barley, wheat, and rye kernels to remove the surface contamination of DON and ZEN. Due to the shape and size of the corn kernel, the dehuller could not be used to remove the outer portion of the corn. Increasing the contact time in the dehuller resulted in the removal of more of the outer surface of the barley, wheat, or rye, as indicated by the ADF concentrations in the inner and outer portions of the kernel, the ADF of the kernels being found principally in the outer portion. For barley, containing 15–19 mg of DON/kg of 0.6–1.0 mg of ZEN/kg, dehulling resulted in a reduction in the DON and ZEN concentration of the grain. A greater reduction was observed as contact time in the dehuller was increased (Table III). The DON and ZEN remaining were approximately 25 and 29% of the total after 15 s, 13 and 17% after 25 s, and 12 and 15%, respectively, after 35 s of dehulling. The amount of barley material retained even after the 35-s dehulling period was 76% of that in the whole kernel (86% if the hull weight was disregarded). Only marginal increases in material loss occurred with increasing dehulling times.

At lower levels of contamination, dehulling barley, wheat, and rye in a Scott-Strong dehuller resulted in substantially lower mycotoxin concentrations in the remaining portion of the kernel than in the whole kernel (Table IV). A 25-s contact time resulted in the complete removal of ZEN from barley and wheat, contaminated at 0.02 and 0.08 mg/kg, respectively, and a 35-s contact time resulted in the complete removal of DON from rye

Table V. Concentration and Relative Distribution (Percent Total) of Protein in the Inner and Outer Portions of Barley, Wheat, and Rye Dehulled in a Scott-Strong Dehuller

	grain[a]					
	barley		wheat		rye	
fraction	fraction wt, %	protein concn,[b] g/100 g (% total)	fraction wt, %	protein concn,[b] g/100 g (% total)	fraction wt, %	protein concn,[b] g/100 g (% total)
whole kernel	100	14.8	100	13.9	100	9.1
inner	76.4	14.0 (72.3)	81.4	13.4 (78.4)	81.7	7.6 (68.1)
outer	23.6	17.3 (27.6)	18.6	16.1 (21.6)	18.3	16.1 (31.9)

[a] Contact time in the dehuller was 25 s for barley and wheat and 35 s for rye. [b] Protein concentration for rye and barley = nitrogen % × 6.25 and for wheat = nitrogen % × 5.7.

contaminated at 0.08 mg/kg. Thus, it would appear that for ZEN in barley and wheat and DON in rye all of the mycotoxin was located at the exterior of the kernel and could be eliminated by removing this area. Similarly, Tanaka et al. (1986) showed that after wheat containing 8 μg of ZEN/kg was milled, no ZEN could be detected in the flour fractions. They concluded that ZEN was heterogeneously located on wheat kernels with more present at the exterior. Young et al. (1984) also showed that *Fusarium* infestation and DON concentrations in Soft White Winter wheat contaminated with 0.45 mg of DON/kg were greatest at or near the surface of the kernel. However, if the overall concentration of toxin was increased, the proportion of DON in the inner portion of the kernel relative to the outer portion also was increased. Similarly, Seitz et al. (1985, 1986) reported that DON contamination was greatest at or near the surface of the kernels of Soft White and Hard Red Winter wheat contaminated with 0.03–5.10 mg of DON/kg.

In the present study, although all the ZEN was eliminated, only approximately 40 and 52% of the DON in barley and wheat, respectively, were eliminated by the dehulling process. This agrees with Tanaka et al. (1986), who showed that although the flour fracdtion of DON- and ZEN-contaminated wheat contained no ZEN, approximately two-thirds of the DON in the original wheat was carried over into the flour. The present results suggest that even at low levels of contamination either the *Fusarium* fungus is able to penetrate the inner area of the kernel or DON can leach into this area from the exterior surface. From the results of Young et al. (1984) it seems more probable that the fungus had penetrated and produced DON in the inner part of the kernel.

Dehulling resulted in a reduction in the protein content of 27.7, 21.6, and 31.9% in barley, wheat, and rye, respectively (Table V), compared to that in the whole kernel. In wheat, protein is not homogeneously distributed but appears to be more concentrated in the bran with 50% more protein being present here than in the flour derived from the inner kernel (Young et al., 1984). Thus, the losses in protein observed in the present study, although quite large, would not be as economically significant for grain destined to be milled for flour as that destined to be used intact for animal feeds. Moreover, the substantial reductions in mycotoxin contamination would compensate for some of the losses in protein.

CONCLUSIONS

In conclusion, dehulling, using a Scott-Strong dehuller, shows promise as a method for decontaminating grain predominantly contaminated with mycotoxins near the surface. This typically occurs in light infestations of *Fusarium* fungi and hence light to moderate DON and ZEN contamination. By use of this method prior to milling, the more lightly contaminated inner seed portion and hence flour fractions could be separated from the more heavily contaminated outer portions of the grain. Moreover, combining commercial dehulling with sieving to remove particles of less than +16-mesh size achieves a greater degree of decontamination, at least for barley. Unfortunately, due to relatively large losses of material during sieving, this technique would be advantageous only during conditions of extensive mycotoxin contamination of grain crops. In general, these decontamination procedures fit some of the criteria for a good, decontamination process (Young, 1985): they are effective in reducing mycotoxin contamination presumably without creating new toxins; they do not drastically alter the nutritional parameters of the grain; they are simple and relatively inexpensive and use existing technology.

ACKNOWLEDGMENT

We thank Maxine Ifill for assistance in conducting mycotoxin analysis.

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Received for review June 19, 1990. Accepted September 11, 1990.

Registry No. DON, 51481-10-8; ZEN, 17924-92-4.