Reduction in Levels of Deoxynivalenol in Contaminated Wheat by Chemical and Physical Treatment

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Ontario soft white winter wheat naturally contaminated with deoxynivalenol (DON, vomitoxin) at ca. 1 μg/g was treated with a variety of aqueous and gaseous reagents. Of those reagents investigated, aqueous sodium bisulfite effected the greatest reduction in DON levels, with the extent of reduction dependent upon concentration and contact time. Flour resulting from milling of bisulfite-treated wheat contained only low (ca. 5% of original) levels of DON. However, when this flour was baked into a variety of products, DON levels increased to 50–75% of that in the untreated wheat due to alkaline hydrolysis of the DON sulfonate intermediate. Because of detrimental effects on the rheological properties of the dough, flour resulting from the treatment level employed (33 mL of an aqueous solution containing the equivalent of 10% SO_2 (w/w) per kilogram of wheat) would not be suitable for commercial use. Although reaction between pure DON and aqueous bisulfite occurred rapidly at room temperature (half-life 5.6 min, in the presence of 10% SO_2 equivalents), DON was unaffected by treatment with gaseous SO_2.

The trichothecene mycotoxin 4-deoxynivalenol (DON, vomitoxin, 3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one) is a naturally occurring metabolite produced by the fungus *Fusarium graminearum* Schwabe on a variety of cereal grains and is known to be associated with several diseases in humans and animals (Ueno, 1983).

The majority of studies on attempted decontamination of cereals containing trichothecenes have dealt with the food-processing aspects (cf. Scott, 1984). Several recent studies on the effects of milling wheat showed that the extent of distribution of DON was related to the level of contamination. At relatively high concentrations (ca. 5 μg/g), DON was fairly uniformly distributed among the various mill portions (Hart and Braselton, 1983; Scott et at., 1983), whereas at concentrations of 0.6–1.0 μg/g, milling led to increased levels in the outer kernel (e.g. bran) portions and decreased levels in the inner flour portions (Young et at., 1984). Wet milling of corn has been reported to remove two-thirds of T-2 toxin (Collins and Rosen, 1981). The effects of further processing (e.g., baking) of wheat are variable (ranging from 0 to 50% reduction) and depend upon the mycotoxin and products (El-Banna et al., 1983; Kamimura et al., 1979; Scott et al., 1983, 1984; Young et al., 1984). Since millers usually add water to wheat prior to milling, we wanted to determine whether it was feasible to add some reagent(s) to the water during this so-called "tempering" stage and effect a substantial reduction in the DON content. The results of such studies on the effects of chemical treatment of DON-contaminated soft white winter wheat prior to milling and subsequent baking of the resultant flour are presented in this paper.

MATERIALS AND METHODS

Wheat. Samples of contaminated (225 kg) and sound (45 kg) Ontario soft white winter wheat were obtained from Reid Milling, Mississauga, Ontario, in 45-kg jute bags and stored frozen (−20 °C).

Analysis for Deoxynivalenol. Subsamples from ground blended samples were analyzed in duplicate by the gas chromatographic (GC) method of Scott et al. (1981) as modified by Young et al. (1984). GC of (heptafluorobutyryl)imidazole- (Regis Chemical Co.) derivatized extracts was carried out on a Hewlett-Packard Model 5710 GC equipped with a ^{63}Ni electron-capture detector and 183 cm × 4 mm i.d. glass column packed with 3% OV-3 on Chromosorb W-HP (80–100 mesh). The column, injector, and detector temperatures were 190, 250, and 300 °C, respectively, and the nitrogen carrier gas flow was 30 mL/min. Estimations of DON were made by comparison of peak heights from injected samples with those of standards. The results were then corrected on the basis of recoveries from samples spiked at the 0.1–0.35 μg/g level. Aliquots from reaction mixtures of treated pure DON were analyzed by high-performance liquid chromatography using a Waters Scientific Radial-PAK cartridge packed with C18 Novapak (5 μm) coupled to a Perkin-Elmer LC-85 UV detector set at 220 nm. The column was eluted at 2 mL/min with methanol–water (1:1).

Analysis for Sodium. Flour samples (5–10 g) were ashed at 400–600 °C for 4–6 h. The residue was dissolved in 3 M HCl and analyzed for sodium content on a Varian AA6 atomic absorption spectrophotometer.

Treatment of Wheat with Aqueous Chemical Agents. Samples of contaminated or sound wheat (1 kg) were treated with aqueous chemical reagents to achieve a final moisture content of 16%. The reagents were sprayed in four equal portions onto the wheat in a V-shaped Twin Shell dry blender. The wheat was mixed for 5 min after the addition of each portion of agent. When all of the reagent was added, the wheat was mixed for a further 1 h, transferred to a glass jar, which was sealed, and stored at 22 °C and 54% relative humidity. After 24 h, the wheat was ground on a Wiley Laboratory Mill, Model 4, and passed through a 0.5-mm screen prior to analysis. When a large volume (500 mL) of reagent was used, the wheat (1 kg) was soaked in the reagent for 24 h and air-dried for 24 h prior to grinding.

Treatment of Contaminated Wheat with Gaseous Chemical Agents. Initially, dry SO_2 gas was passed for 3 h through 1 kg of wheat in a gas-tight container. The gaseous SO_2 treatment was repeated on another sample of wheat, but the gas was bubbled through water prior to being passed through the wheat. Ozone was also used as a reagent. After being generated, the ozone was bubbled through water prior to being passed through the wheat. For all these experiments, the gases were passed through the wheat for 3 h. The wheat was then left in an atmo-

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Table I. Relative Amounts of Deoxynivalenol Remaining in Contaminated Wheat[a] after Treatment with Aqueous Reagents

reagent	concn	reagent vol,[b] mL/kg	% deoxynivalenol[c] remaining
hydrogen peroxide	5%	33	92
hydrogen peroxide[d]	6%	33	102
hydrogen peroxide[d]	6%	750	34
sodium hypochlorite	1%	33	124
sodium bisulfite	10% SO_2	750	<2
ascorbic acid	2%	600	53
ammonium hydroxide	5%	600	65
hydrochloric acid	0.1 M	600	65

[a] Ontario soft white winter wheat contaminated at ca. 1 μg/g. [b] Contaminated wheat treated for 24 h at 22 °C and ground. [c] Relative to untreated contaminated wheat analyzed simultaneously. Results are based on duplicate or triplicate analyses that showed relative standard deviations of 1–8%. [d] Sodium hydroxide (1.1%) added.

sphere saturated with the gas for 24 h prior to being analyzed.

Treatment of Deoxynivalenol with Aqueous Sodium Bisulfite. To 10 μg of DON was added 200 μL of an aqueous solution of sodium bisulfite containing the equivalent of 5, 10, or 20% SO_2 (w/w). The reactions were run at room temperature.

Milling of Wheat. To obtain a sufficient quantity of wheat for milling, separate 1 kg samples of wheat were treated with aqueous sodium bisulfite (33 mL, equivalent to 10% SO_2) until a total of 20 kg was so obtained. This wheat was mixed and milled on a Buhler pneumatic laboratory mill MLU 220. The mill afforded bran, shorts, "patent" flour (obtained by blending together the first break, second break, first reduction and second reduction flours), and "clear" flour (obtained by blending together the third break and third reduction flours). Nontreated wheat was tempered to 14% moisture prior to milling.

Baking of Flour. Only the patent flour fractions were used in the baking studies. Cookies were made by using American Association of Cereal Chemists (AACC) Method 10-50D. Cakes were made by using AACC Method 10-90. Pancakes, muffins, and crackers were made by using standard commercial recipes.

Physical Tests on Dough. Various AACC standard tests were conducted as follows: farinograph (AACC Method 54-21), amylograph (cf. Method 22-10), extensigraph (Method 54-10), mixograph (Method 56-40), Alveograph (Method 54-30), MacMichael viscosity (Method 56-80), and cookie spread (Method 10-50D). The alkaline water retention capacity was conducted according to the method of Yamazaki (1953).

Sensory Evaluation. Cookies and muffins were baked from the patent flour fractions of sound wheat and treated sound wheat. A small profile panel (n = 4) was used to evaluate and compare the flavor of these products.

RESULTS AND DISCUSSION

Treatment of Contaminated Wheat. Of all the various aqueous treatments examined (Table I), that with sodium bisulfite resulted in nearly complete disappearance of DON. Since the other acid (hydrochloric acid), alkaline (ammonium hydroxide), oxidizing (hydrogen peroxide, hypochlorite), or reducing (ascorbic acid) reagents effected at best a 60% reduction in DON content of the wheat, they were not investigated further. The apparent increase in DON content upon hypochlorite treatment may be due to conversion of some precursor into DON (cf. Miller et al., 1983). The extent of reduction by bisulfite was dependent upon the amount of reagent employed, whether it was by

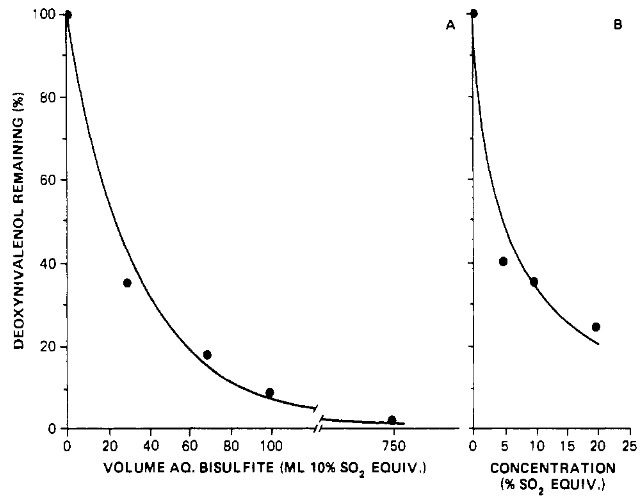

Figure 1. Reduction in deoxynivalenol content of contaminated wheat (ca. 1 μg/g) by 24-h treatment with aqueous sodium bisulfite. Treatment for 1 kg: (A) effect of volume for solution containing the equivalent of 10% SO_2 (w/w); (B) effect of concentration for 33 mL of solution.

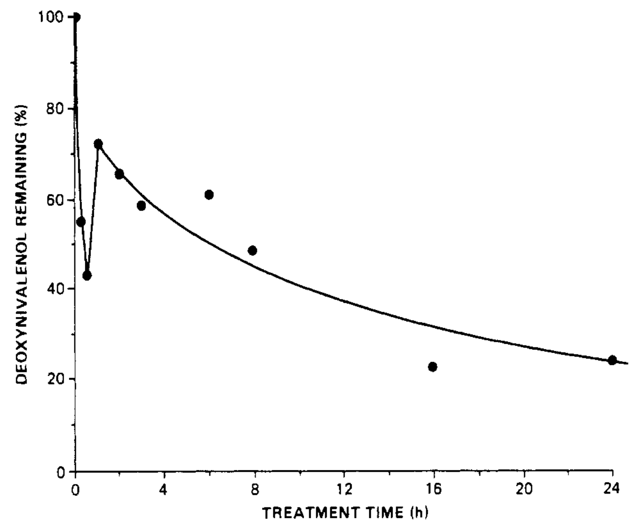

Figure 2. Reduction in deoxynivalenol content of contaminated wheat (ca. 1 μg/g) by treatment with 33 mL of aqueous sodium bisulfite containing the equivalent of 10% SO_2 (w/w).

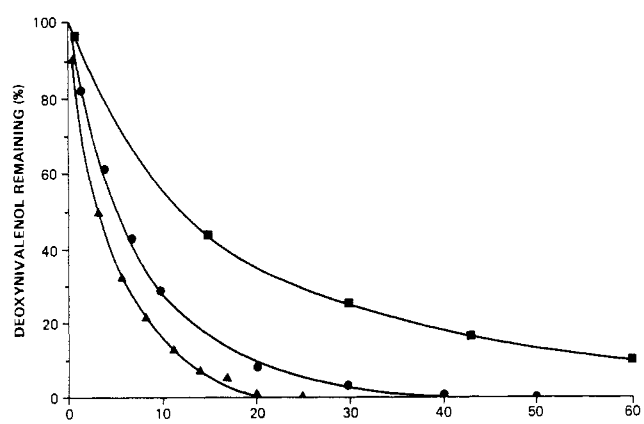

Figure 3. Reduction in amount of deoxynivalenol with different concentrations of aqueous sodium bisulfite. Treatment conditions: (■, ●, ▲) 5, 10, and 20% SO_2 equivalents (w/w), respectively.

additional solution (Figure 1A) or increased concentration (Figure 1B), or upon treatment time (Figure 2). Reaction was complete after about 16 h when bisulfite equivalent to 10% SO_2 was used at room temperature. After an initial

Table II. Deoxynivalenol Concentrations and Their Change in Experimentally Milled Ontario Soft White Winter Wheat

			% change rel to		
sample	moisture, %	DON,[a] ng/g	wheat used	contam untreated wheat	corresp untreated mill fraction
		Contaminated Untreated			
wheat	15.0	939	0		
clear flour	11.1	569	−39	−39	
patent flour	12.0	712	−24	−24	
bran	10.9	1024	+9	+9	
shorts	10.9	1177	+25	+25	
		Contaminated Treated[b]			
wheat	17.0	53	0	−94	−94
clear flour	11.9	43	−19	−95	−92
patent flour	12.4	50	−6	−95	−93
bran	12.8	45	−15	−95	−96
shorts	12.1	66	+25	−93	−94

[a] Deoxynivalenol on a dry weight basis. Average of duplicate analyses. [b] Contaminated wheat treated for 24 h at 22 °C with aqueous sodium bisulfite (33 mL (equivalent to 10% SO_2 (w/w))/kg of wheat).

rapid decrease in concentration, the DON level increased before resuming a decrease to a steady value. This study was repeated four to six times for the 15- and 60-min treatment times with identical results. Since a similar fluctuation was not observed for pure trichothecenes (Figure 3) or for DON-contaminated corn (Young, 1986a), it is not clear why this phenomenon is restricted to wheat, unless only the wheat contained a precursor that was converted to DON (vide supra) and then reacted with bisulfite. Swanson et at. (1984) observed that sodium bisulfite can destroy DON in corn after 3.5 days at 60 °C.

Although dry gaseous sulfur dioxide had no effect in reducing the DON concentration in wheat, when this gas was moistened, a 38% reduction was observed. In contrast with its effect in contaminated corn (Young, 1986a), moist ozone had little effect in wheat, presumably because the latter was much drier and the sample size much larger.

Treatment of Deoxynivalenol with Chemical Agents. The reactions of pure DON with aqueous sodium bisulfite proceeded rapidly (Figure 3) and exhibited pseudo-first-order kinetics, with half-lives of 15, 5.6, and 3.6 min for DON in 5, 10, and 20% SO_2 equivalents, respectively. By contrast, DON was unaffected by overnight treatment with gaseous SO_2, with or without the presence of water. These results suggest that the sulfite or bisulfite anion is the reactive species. Bisulfite is known to add to some unsaturated ketones (Royals, 1954). A bisulfite addition product showing physical and chemical properties consistent with the expected sulfonate has been isolated (Young, 1986b).

Milling of Sodium Bisulfite Treated Wheat Contaminated with Deoxynivalenol. Milling of contaminated wheat (Table II) effected the expected distribution (cf. Young et al., 1984) of DON into the various mill streams with relatively higher levels in the bran and shorts fractions and lower levels in the flour portions. Bisulfite treatment of the contaminated wheat effected a 94% reduction in DON levels; the remaining DON was distributed as per the untreated contaminated wheat, and each mill fraction continued to show a 92–96% reduction (Table II). Residual levels of sodium were highest in the outer bran (1290 μg/g) and shorts (4520 μg/g) fractions of treated contaminated wheat with lower levels in the clear (490 μg/g) and patent (700 μg/g) flours; untreated wheat contained 170, 160, 40, and 20 μg/g of sodium in the corresponding fractions, respectively.

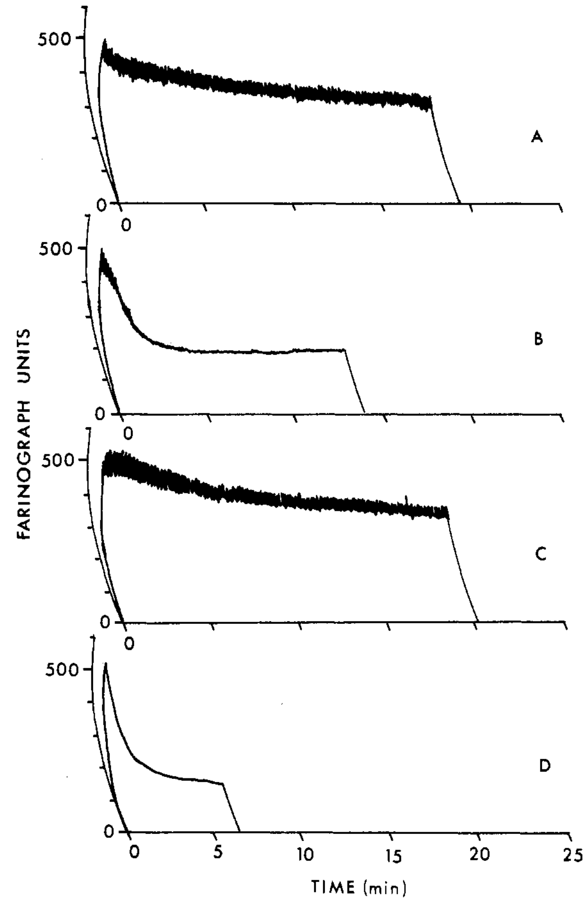

Figure 4. Farinograph curves for Ontario soft white winter wheat flour as determined by the American Association of Cereal Chemists test Method 54-21: (A) sound untreated flour; (B) sound flour treated with 33 mL of aqueous sodium bisulfite (equivalent to 10% SO_2 (w/w))/kg wheat; (C) contaminated untreated flour from wheat containing ca. 1 μg/g of DON; (D) DON-contaminated flour treated with aqueous sodium bisulfite.

Baking of Flour Derived from Sodium Bisulfite Treated Wheat Contaminated with Deoxynivalenol. Baking of contaminated flour into cookies and muffins showed (Table III) only modest changes in the DON levels (−19 and +31%, respectively), consistent with results from an earlier study (Young et al., 1984).

On the other hand, baking of contaminated flour derived from sodium bisulfite treated wheat resulted in a 3–6-fold increase in the DON levels, relative to the unbaked treated flour for the five products investigated (Table III). Despite these increases on baking, the observed DON levels are still lower than those relative to the untreated flour or corresponding untreated product.

In another experiment, cookies were baked with contaminated treated flour (DON at 70 ng/g). The regular formula, which included sodium bicarbonate and gave a dough of pH 8.1, yielded a final DON concentration of 369 ng/g (dry weight basis corrected for percent flour in the product), whereas omission of sodium bicarbonate gave a dough of pH 5.5 and a final DON concentration of 169 ng/g. Thus, the increase in DON levels may be due to partial alkaline hydrolysis of the DON sulfonate product back to free DON in the dough during baking. Such conversions under alkaline conditions have also been observed for pure DON sulfonate (Young, 1986b).

Physical Tests on Dough. The results in Table IV and Figure 4 show that treatment of sound or contaminated wheat with sodium bisulfite had profound detrimental effects on many of the measured rheological and baking

Table III. Deoxynivalenol Concentrations and Their Changes in Products Made from Patent Flour Derived from Untreated and Sodium Bisulfite Treated Ontario Soft White Winter Wheat

sample	moisture, %	% wheat in final product	DON, ng/g A[a]	DON, ng/g B[b]	% change rel to flour used	% change rel to untreated flour	% change rel to corresp untreated flour
			Sound Untreated				
cake	25.0	25	30				
cookie	3.0	55	30				
cracker	2.0	76	20				
muffin	21.0	37	20				
pancake	59.0	24	20				
			Contaminated Untreated				
flour	12.5	100	624	633	0		
cookie	3.0	55	274	513	−19		
muffin	21.0	37	242	827	+31		
			Contaminated Treated[c]				
flour	11.9	100	61	70	0	−89	
cake	25.0	25	58	308	+340	−51	
cookie	3.0	55	233	436	+520	−31	−15
cracker	2.0	76	281	378	+440	−40	
muffin	21.0	37	90	308	+340	−51	−63
pancake	59.0	24	48	488	+600	−23	

[a] Deoxynivalenol on a dry weight basis coated for recovery. [b] Deoxynivalenol on a dry weight basis corrected for recovery, moisture, and percent flour in product. [c] Contaminated wheat treated for 24 h at 22 °C with aqueous sodium bisulfite (33 mL (equivalent to 10% SO_2 (w/w))/kg of wheat).

Table IV. Effects of Sodium Bisulfite Treatment of Soft White Winter Wheat Flour on Physical Properties of Dough

test	wheat (treatment): sound (untreated)	sound (sodium bisulfite)[j]	sound (buffer)	contam[l] (untreated)	contam (sodium bisulfite)[j]
farinograph[a]					
abs, %	49.7	49.8		50.4	50.1
arrival time, min	1.00	0.75		1.00	0.50
dev time, min	1.25	nd[m]		1.75	0.55
stability, min	0.5	nd[m]		2.50	nd[m]
breakdown time, min	1.75	1.00		3.00	0.75
tolerance index, BU	90	290		80	370
amylograph[b]					
pasting (°C)	59.5	61.0		62.5	61.0
viscosity at 95 °C, BU	390	600		240	550
peak visc, BU	700	845		590	715
visc first holding, BU	170	200		145	180
visc at 50 °C, BU	1005	1140		750	990
visc second holding, BU	1600	1920		1280	1650
cookie spread[c]					
spread factor	82	79	70		
alkaline water retentn capacity[d]					
weight gain, %	63.5	62.5	70.3		
Alveograph[e]					
peak, mm	20.45	nd[n]	20.40		
length, mm	97.5	nd[n]	102		
peak/length	0.21	nd[n]	0.20		
W, 10^{-4} J	55.13	nd[n]	43.95		
MacMichael viscosity[f]					
visc,[g] (°M)	23	14	17.5		
visc,[h] (°M)	65	17.5	53		
visc,[i] (°M)	73	17.5	62		

[a] American Association of Cereal Chemists (AACC) test Method 54-21. [b] AACC Method 22-10. [c] AACC Method 10-50D. [d] Method of Yamazki (1953). [e] AACC Method 54-30. [f] AACC Method 56-80. [g–i] 0, 2, and 4 mL of 1 N lactic acid added, respectively. [j] Aqueous sodium bisulfite added (33 mL (equivalent to 10% SO_2 (w/w))/kg of wheat). [k] pH 1.90 KCl/HCl buffer added (33 mL/kg wheat). [l] DON ca. 1 μg/g. [m] Not determined because of extremely low stability and high stickiness of the dough. [n] Not determined because dough was excessively sticky and lacking elasticity.

properties of the dough. Large changes were noted in the farinograph, amylograph, Alveograph, and MacMichael viscosity tests. In addition, the mixograph test showed that bisulfite reduced dough development times by 86% and dough strength by 43%, and the extensigraph test could not be done because of extremely poor handling properties. On the other hand, the cookie spread and alkaline water retention capacity tests did not reveal any extensive differences. To determine whether the effects observed were due to the action of bisulfite or pH, sound wheat was treated with a buffer solution (KCl/HCl) of the same pH (1.90) as the sodium bisulfite solution and milled and the patent flour so obtained tested. While the buffer treatment did alter the rheological properties somewhat, the changes were not consistent with those observed for the bisulfite-treated flour. The presence of DON contamination in untreated wheat also had effects on some rheological properties (Table IV), although the extent of these effects was much less dramatic than those resulting from bisulfite treatment.

Sensory Evaluation. The cookies and muffins made with untreated flour had normal flavors. However those made with bisulfite-treated flour were perceived as having slight off-flavors (fruity and watermelon rind) by two panelists; the other two panelists did not detect any off-flavors.

Conclusions. This study demonstrated that it is possible to reduce the level of DON in contaminated wheat by chemical and physical treatment. Considerable reductions in DON levels were achieved by tempering contaminated wheat with sodium bisulfite (equivalent to ca. 540 $\mu g/g$ added) prior to milling, although rheological and baking properties indicated that the resulting flour for this treatment level would be unsuitable for general commercial use. Milder treatment conditions for sodium bisulfite or some of the other treatments may have the potential for affording flour of normal commercial utility while still offering sufficient reduction in DON to below the current guideline limit (Canadian) of 2 $\mu g/g$ in uncleaned soft wheat, should the natural level be above this value. These treatments also show promise for use in decontaminating grains destined for animal feeds.

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Registry No. DON, 51481-10-8; $NaHSO_3$, 7631-90-5; SO_2, 7446-09-5; HCl, 7647-01-0; NH_4OH, 1336-21-6; H_2O_2, 7722-84-1; NaOCl, 7681-52-9; ascorbic acid, 50-81-7.

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Reduction in Levels of Deoxynivalenol in Contaminated Corn by Chemical and Physical Treatment

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Field corn artificially inoculated with the fungus *Fusarium graminearum* was treated by various chemical and physical means. Moist ozone (1.1 mol %) in air effected a 90% reduction in deoxynivalenol (DON, vomitoxin) levels, at ca. 1000 $\mu g/g$, after 1 h, while dry ozone effected only a 70% reduction after the same treatment time. Total destruction was achieved after 0.5 h with 30% chlorine (v/v in nitrogen). Prolonged (18 h) exposure to ammonia was required to obtain an 85% reduction in DON levels. Thermal treatment, by microwave or convection, achieved 50–90% reductions. The inclusion of ammonium carbonate with heat treatment afforded only slight improvements in destruction of DON. Nearly complete reduction could be achieved by addition of aqueous sodium bisulfite with the extent dependent upon concentration and contact time.

The trichothecene mycotoxin 4-deoxynivalenol (DON, vomitoxin, $3\alpha,7\alpha$,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one) is a naturally occurring metabolite produced by the fungus *Fusarium graminearum* Schwabe on a variety of cereal grains and is known to be associated with several diseases in animals and is related to other trichothecenes that affect humans as well (Ueno, 1983).

Although chemical and physical treatments of grains contaminated with other mycotoxins such as aflatoxin (Doyle et al., 1982) have been investigated in some detail, there have been few reports on such treatments where DON has been present. Bleaching of flour with chlorine in a commercial mill afforded only a modest 10% reduction in DON concentration (Young et al., 1984). In a preliminary study, Swanson et al. (1984) reported some lowering

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