Removal by Specific Gravity Table of Tombstone Kernels and Associated Trichothecenes from Wheat Infected with Fusarium Head Blight[1]

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ABSTRACT

Commercially grown Canada Eastern White Winter wheats infected to varying degrees by Fusarium head blight were fractionated on a specific gravity table. Visual examination by inspectors from the Canadian Grain Commission revealed that the most severely infected kernels, which were thin and shriveled (tombstone kernels), were highly concentrated in the least dense fractions for all wheats. Mycological examination of the wheats and their fractions showed that the incidence of *Fusarium* infection was greatest for the least dense fractions and least for the most dense fractions. Chemical analysis by gas chromatography using a mass selective detector verified that in all cases, the mycotoxin deoxynivalenol was highly concentrated in the least dense fractions, and the most dense fractions contained greatly reduced levels compared with the corresponding unfractionated wheats. These results suggest that specific gravity tables can be used effectively to remove tombstone kernels and associated mycotoxins from *Fusarium*-infected wheat. Removing the least dense fraction has the added advantages of promoting the visual grade and improving the milling properties of the remaining wheat relative to the corresponding unfractionated wheat.

Infection of cereal grains by Fusarium head blight is common worldwide (Osborne 1982). In western Canada, *Fusarium* molds on wheat have been reported in Manitoba and in the Peace River Region of Alberta (Sutton 1982). In the Red River Valley area of Manitoba, the classes of wheat mainly affected are Amber Durum and Prairie Spring (Tekauz et al 1986, Abramson et al 1987). In eastern Canada, severe epidemics of *Fusarium* head blight are frequent in parts of Ontario, Quebec, and the maritime provinces. On average, severe epidemics occur almost every nine years on winter wheat grown in southern Ontario (Sutton 1982). *F. graminearum* Schwabe is the most common species found on winter wheat in Ontario (Duthie et al 1986).

Deoxynivalenol (DON), or vomitoxin, a tricothecene mycotoxin usually found in *Fusarium*-infected grain, was identified in the 1980 Ontario winter wheat crop (Trenholm et al 1981) and has recurred several times at levels high enough to be of concern (Teich et al 1987). The toxicity of mycotoxins limits the marketability of *Fusarium*-infected wheat. The maximum level of DON permitted in uncleaned Canada Eastern White Winter (CEWW) wheat intended for nonstaple foods is 2.0 μg/g except for wheat destined for baby foods, in which the level is 1.2 μg/g (Scott 1990).

The presence of DON is correlated with scab damage (Teich et al 1987). *Fusarium*-infected wheat kernels are highly conspicuous; they are shriveled, light in weight, and often have pink or red patches (Seitz and Bechtel 1985). Kernels infected at the time of heading are chalky-white (Clear and Abramson 1986). In Canada, wheat kernels of this description are commonly referred to as tombstone kernels. In 1989, the maximum level of tombstone kernels allowed in grades 1–3 CEWW wheat was 1.0% (by weight). The maximum level of tombstone kernels permitted in Canada Feed grade is 5%.

Numerous studies have documented that DON is very stable during wheat processing. After milling, DON is generally more concentrated in low-grade flour streams and millfeeds, but it is still present in appreciable amounts in the most refined flour streams (Scott et al 1984, Young et al 1984, Seitz et al 1986). DON is very stable during baking, surviving temperatures of 350°C (El-Banna et al 1983). DON levels are reduced in cooked pasta and noodles because of leaching into the cooking water (Nowicki et al 1988). Some evidence indicates that DON levels may be reduced during the processing of alkaline products such as Chinese noodles (Nowicki et al 1988) and tortillas (Abbas et al 1988).

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Seitz et al (1986) showed that cleaning *Fusarium*-infected wheat by a conventional commercial flow was not particularly effective in removing DON. Huff and Hagler (1985) showed that density flotation could be used to separate *Fusarium*-infected wheat kernels, but the cost of drying the decontaminated grain limits commercial application. Specific gravity tables, which fractionate samples on the basis of density differences, effectively remove light foreign material from seeds (Peske and Boyd 1985). We recently demonstrated the effectiveness of specific gravity tables in removing shrunken, broken, and severely sprouted kernels from spring wheat (Tkachuk et al 1990, 1991) and durum wheat (Dexter et al 1991). In the present study, we investigate the potential for using a specific gravity table to lower DON levels in *Fusarium*-infected CEWW wheats by removing tombstone kernels.

MATERIALS AND METHODS

Wheats

Three 50-kg samples of commercially grown CEWW wheat from the 1989 crop that were naturally infected with *Fusarium* were supplied by the Grain Inspection Division of the Canadian Grain Commission in Chatham, Ontario.

Gravity Table Fractionation

The samples were cleaned with a dockage tester (Carter C-989, Simon-Day Ltd., Winnipeg, MB) to export standards and then fractionated with a specific gravity separator (SY 300, Spiroll Kipp Kelly, Inc., Winnipeg). The gravity table has a capacity of 580 kg/hr and can produce up to five fractions. In the current study, the fractions are designated F1–5 (from least to most dense). Proportions of the individual fractions are determined by airflow, deck incline, oscillation frequency, oscillation distance, and discharge splitter position.

Wheat Physical Characterization

Unfractionated wheats and gravity table fractions were inspected by inspectors from the Canadian Grain Commission, who assigned a grade to each sample and determined the proportion of tombstone kernels. Test weights and kernel weights of the wheat were determined as described by Dexter and Tipples (1987).

Milling

The unfractionated wheats and the three densest gravity table fractions recovered from each were prepared for milling and were milled in duplicate 1-kg lots by a five-stand mill (Allis-Chalmers) using procedures described by Dexter and Tipples (1987). Samples were milled in ascending order of DON contamination to prevent spurious flour DON results that could arise because of cross-contamination of stocks from successive millings. Flour yields were calculated as the proportion of wheat to first break on a constant moisture basis.

TABLE I
Properties of Canada Eastern White Winter Wheats Separated by a Specific Gravity Table

Wheat Sample	Proportion (%)	Grade	Test Weight (kg/hl)	Kernel Weight[a] (mg)	Protein Content (%)	Tombstone Kernels (%)	*Fusarium* Infection[b] (%)	Mycotoxins[c] DON[d] (μg/g)	Mycotoxins[c] HT-2 (μg/g)
Wheat 1									
UF	100	sample	73.2	27.2	11.8	5.5	14	7.96	<0.05
F1	2	sample	44.0	9.7	13.1	75.2	77	43.2	0.32
F2	11	sample	59.0	15.6	11.5	30.0	45	16.4	<0.05
F3	28	sample[e]	71.0	25.6	11.6	2.6	21	5.66	<0.05
F4	38	sample[e]	77.9	34.2	11.6	trace	8	1.43	<0.05
F5	21	CF[e]	80.5	38.5	12.1	0	7	0.87	<0.05
Wheat 2									
UF	100	CF	68.0	21.7	11.7	2.4	11	3.73	<0.05
F1	<0.01	...	...	...	...	...	...	27.8	3.08
F2	2	sample	42.2	8.9	15.4	13.4	31	22.0	0.51
F3	21	sample	59.0	14.7	13.3	6.7	9	9.34	<0.05
F4	46	3 CEWW	70.5	23.0	11.9	0	14	1.81	<0.05
F5	31	2 CEWW	75.5	30.8	10.1	0	7	0.56	<0.05
Wheat 3									
UF	100	CF	72.0	25.8	10.0	1.6	11	1.89	<0.05
F1	1	sample	55.0	12.4	11.2	18.2	43	20.8	<0.05
F2	9	sample	64.0	16.4	10.1	6.2	11	5.26	<0.05
F3	28	CF	70.0	22.5	9.9	1.0	9	1.27	<0.05
F4	40	2 CEWW	74.5	28.9	10.1	0.15	5	0.40	<0.05
F5	22	1 CEWW	78.2	34.0	10.4	trace	11	0.23	<0.05

[a] Kernel weight, protein content, and mycotoxin levels expressed on 14% moisture basis.
[b] Over 95% of infected kernels were infected by *Fusarium graminearum*. All samples positive for HT-2 contained 1–3 kernels infected by *F. sporotrichiodes*.
[c] Diacetoxyscirpenol and T-2 toxin were below detectable limit (0.05 μg/g) for all samples.
[d] DON = deoxynivalenol, UF = unfractionated, F = fraction, CF = Canada feed, CEWW = Canada Eastern White Winter.
[e] These samples were downgraded because of heated kernels. In the absence of heated kernels, F3 would be graded CF, F4 would be graded 2 CEWW, and F5 would be graded 1 CEWW. All other wheats downgraded to sample grade were downgraded because of tombstone kernels.

Wheat and Flour Analyses

All analytical tests were performed in duplicate and adjusted to 14% moisture basis. The moisture content of all samples was determined by rapid moisture tester (C. W. Brabender Instruments, South Hackensack, NJ) as outlined in the instruction manual.

Protein content was determined by the modified Kjeldahl procedure of Williams (1973); ash content was determined by AACC method 08-01 (AACC 1983). Flour color was determined by Simon Colour Grader Series IV (Henry Simon, Stockport, U.K.) as outlined in the instruction manual.

Mycological Screening

Surface sterilized seeds (100) of each unfractionated wheat and the gravity table fractions were plated onto agar and incubated to induce sporation of fusaria for identification as described by Nowicki et al (1988).

Determination of Trichothecene Mycotoxin

Samples of wheat, millfeed, and flour were screened to determine the presence of DON, diacetoxyscirpenol, HT-2 toxin [15-acetoxy-3*a*,4-dihydroxy-8*a*-(3-methylbutyryloxy)-12,13-epoxytrichothec-9-ene], and T-2 toxin [4*β*,15-diacetoxy-3*a*-hydroxy-8*a*-(3-methylbutyryloxy)-12,13-epoxytrichothec-9-ene] at levels equal to or greater than 0.05 μg/g. Before extraction, wheat samples and millfeed samples were ground in a coffee grinder such that 90% of the ground product passed through a no. 20 U.S. standard sieve. The unfractionated wheats and the three densest wheat fractions (F3–5) were analyzed in triplicate. F1 and 2 fractions were analyzed singly because of limited sample size. Flours from each of the duplicate millings were analyzed in duplicate; millfeeds were analyzed singly.

Samples were extracted as described by Ware et al (1986). Cleanup was performed by gel permeation chromatography using a GPC/Autovap (Analytical Bio-Chemistry Laboratories Inc., Columbia, MO) with a column containing 50 g of BioBeads SX-3 swelled in methylene chloride-cyclohexane (15/85 v/v). The heptafluorobutyrate derivative was prepared immediately before gas chromatography after the procedure of Ware et al (1986) was performed. DON, diacetoxyscirpenol, and HT-2 and T-2 toxins were determined by capillary column gas chromatography using mass selective detection with selected ion monitoring (Nowicki et al 1988).

Recovery of DON from wheat samples fortified at 2.1 μg/g averaged 99.7%, with a coefficient of variation of 7.9% based on duplicate sets of six samples. The coefficient of variation for replicated wheat gravity table fractions and flours was 7–25% (average, 10%).

RESULTS

Wheat Physical Properties

The broad range of density among the gravity table fractions resulted in the expected range in wheat physical properties (Table I). Because the tombstone kernels were so thin and shrunken, they were concentrated in the least dense fractions from each wheat, which were downgraded to sample because of the tombstone kernels. The remaining fractions from each wheat qualified for the milling grades of CEWW wheat except for wheat 1, which contained heated kernels. In the absence of heated kernels, the two densest fractions from wheat 1 would have been graded No. 1 and No. 2 CEWW, respectively.

Wheat Mycological Properties

The proportion of *Fusarium*-infected kernels was highly concentrated in the least dense fractions (Table I). As expected, the predominant *Fusarium* species identified was *graminearum* Schwabe, which accounted for over 95% of infected seeds. One to three kernels of 100 tested were infected by *F. sporotrichioides* Schwabe in the unfractionated wheats and in the least dense fraction from each. In several other samples, single kernels (of 100 tested) were infected by *F. poae* Schwabe, *F. acuminatum* Schwabe, and *F. crookwellense* Schwabe.

Wheat Mycotoxin Analyses

Tricothecene mycotoxin analyses of the unfractionated wheats and the gravity table fractions confirmed that removing most of the tombstone kernels in the least dense fractions resulted in reduced mycotoxin levels in the denser fractions (Table I). The predominant mycotoxin identified was DON. HT-2 toxin was

TABLE II
Properties of Flour from Eastern White Winter Wheats Separated by Specific Gravity Table

Wheat Sample	Yield[a] (%)	Ash Content[b] (%)	Grade Color (units)	Protein Content (%)	DON[c,d] Millfeed (μg/g)	DON[c,d] Flour (μg/g)
Wheat 1						
UF[d]	70.5	0.54	6.5	9.7	8.79	6.52
F3	70.0	0.56	7.5	9.8	7.10	4.53
F4	71.2	0.46	3.6	9.6	2.29	0.68
F5	72.9	0.52	2.0	10.2	1.65	0.44
Wheat 2						
UF	67.5	0.54	2.1	9.8	5.98	2.83
F3	60.1	0.66	4.4	11.7	10.03	8.39
F4	69.1	0.52	0.3	9.8	2.91	0.94
F5	72.0	0.50	−0.3	9.0	1.17	0.21
Wheat 3						
UF	69.8	0.39	0.8	8.6	3.04	2.35
F3	70.2	0.38	0.2	8.4	1.85	1.09
F4	70.9	0.40	−0.2	8.4	0.77	0.28
F5	70.9	0.44	0.1	8.8	0.48	0.16

[a] Proportion of clean wheat on a constant moisture basis.
[b] Expressed on 14% moisture basis.
[c] Diacetoxyscirpenol, HT-2 toxin, and T-2 toxin were below detectable limit (0.05 μg/g) for all samples.
[d] DON = deoxynivalenol, UF = unfractionated, F = fraction.

found above detectable limits (0.05 μg/g) in the least dense fractions from wheats 1 and 2. All wheat fractions that contained detectable HT-2 toxin contained one or more kernels (of the 100 tested) infected by *F. sporotrichioides*.

Wheats 1 and 2 were well above the Canadian DON food-use tolerance (2.0 μg/g). The two densest fractions from wheat 1, which represented about 60% of the total wheat, would contain only about 1.2 μg of DON per gram if recombined. This is well within food tolerance and less than 20% of the 7.96 μg of DON per gram found in the unfractionated wheat 1. Similarly, for wheat 2, the two densest fractions (which represent almost 80% of the total wheat) would contain only about 1.2 μg of DON per gram (about one third of the 3.73 μg/g found in the unfractionated wheat).

Wheat 3, at 1.89 μg of DON per gram, was barely within the Canadian food-use tolerance. Removal of the two least dense fractions, which represent 10% of the total wheat, would reduce the DON level of the remaining wheat to less than 0.6 μg/g.

Wheat Milling Properties

The densest fractions recovered from all the CEWW wheats exhibited superior milling properties (higher flour yield and brighter color) compared with the corresponding unfractionated wheats (Table II). For two of the three wheats, the densest fractions also exhibited lower ash content than did the unfractionated wheats. This result corroborates previously reported results for Canada Western Red Spring wheats (Tkachuk et al 1990, 1991) and verifies that promoting the densest wheat fractions to the milling grades is justified on the basis of improved milling performance.

Analyses of Flour and Millfeed Mycotoxins

The only tricothecene mycotoxin detected in the flour and millfeed of the CEWW wheats was DON (Table II). DON was concentrated in the millfeed for all samples, which is in agreement with numerous reports (Scott et al 1983, 1984; Young et al 1984; Seitz et al 1985, 1986; Nowicki et al 1988). The DON values of the flours and millfeeds verified the accuracy of DON estimates in the unmilled wheats. Combined recovery of DON in the flour and millfeed was 80–109% of the DON found in the corresponding wheat.

DISCUSSION AND CONCLUSIONS

Results from this study demonstrate that tombstone kernels can be effectively removed from *Fusarium*-infected CEWW wheat by a specific gravity table. The tombstone kernels and associated DON are heavily concentrated in the least dense gravity table fractions. As a result, a relatively large proportion of wheat with safe DON levels can be isolated from wheats containing DON levels well in excess of food-use tolerances.

A disadvantage of gravity table fractionation of *Fusarium*-infected wheat is that the lightest fractions contain such high levels of infection and associated mycotoxins that they would not be suitable for feed or food. However, the resulting loss of value of the total wheat package would be compensated at least partially by promoting the denser fractions to higher grades. An advantage of gravity table separation is that it would not be necessary to blend off wheats containing high DON levels with a large excess of wheat containing low DON levels. Sufficient quantities of wheat with very low DON levels for blending are not always readily available.

The current study made only one pass over the gravity table, and the gravity table was not adjusted for optimum performance between samples. If two or more gravity tables were used in series and if gravity table settings were optimized, then a greater proportion of wheat with DON values within food-use tolerances probably could be recovered from *Fusarium*-infected wheat.

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