# Effect of Milling and Baking on Deoxynivalenol (Vomitoxin) Content of Eastern Canadian Wheats

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Ontario soft white winter wheat naturally contaminated with deoxynivalenol (DON, vomitoxin) at 0.45 mg/kg was cleaned and milled in industrial, pilot, and experimental mills. Some of the industrially milled fractions were baked commercially into a variety of food products. The samples were analyzed for DON and, in some instances, the fungal metabolite ergosterol. Milling led to a fractionation of both DON and ergosterol, with increased levels in the outer kernel (e.g., bran) portions and decreased levels in the inner flour portions. The effect of baking on non-yeast products was variable, ranging from no effect to 35% reduction, and when combined with milling and dilution effects gave an overall reduction of 66 ± 20% in DON. Experimental milling of two samples of naturally contaminated (at 1.2 and 6.9 mg/kg) Quêbec hard red spring wheat resulted in a similar fractionation of DON. A positive correlation between DON and ergosterol levels in 11 eastern Canadian wheat samples indicated that the level of DON production was directly related to the incidence of fungal growth; the milling data suggested that fungal infection was greatest at or near the kernel surface.

4-Deoxynivalenol (DON, vomitoxin, 3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one), a naturally occurring metabolite produced by the fungus *Fusarium graminearum* Schwabe on a variety of cereal grains, is a member of the trichothecene class of mycotoxins known to be associated with several diseases in animals and man (Ueno, 1980).

Following the discovery of DON at levels up to 8.5 mg/kg in some of the 1980 soft white winter wheat crops in Ontario (Scott et al., 1981; Trenholm et al., 1981), the Canadian Government set a maximum guideline level of 0.3 mg/kg in the finished product for that crop destined for use in nonstaple foods. DON also appeared in the 1981 and 1982 crops (averaging 0.7 mg/kg in 1982) as well as occurring in the 1980 and 1981 Quêbec hard red spring wheat crops. Soft winter wheat is typically used in nonstaple foods such as biscuits, cakes, cookies, and bran breakfast cereals, whereas hard spring wheat is used in staple foods such as bread and related products.

Little was known at the outset of this study concerning the fate of trichothecenes in general and DON in particular during grain processing. Collins and Rosen (1981) reported that wet milling of corn removed about two-thirds of the T-2 toxin present, and Kamimura et al. (1979) noted the effects of processing on a variety of oriental foods containing trichothecenes. Recent studies (Scott et al., 1983; Hart and Braselton, 1983) showed that milling of hard spring wheats had little palliative effect and that DON was distributed throughout the milled products. Baking the flour into bread failed to destroy the DON (Scott et al., 1983; El-Banna et al., 1983).

This paper reports the results of studies to determine the fate of DON during wheat processing. The distributions of DON in milling fractions produced from commercial, pilot, and experimental milling of soft wheats were compared, and the retentions of DON during the commercial processing of industrial soft winter wheat flour into a wide variety of food products were determined. In addition, the distribution of DON in milling fractions produced from experimental milling of soft winter wheat and hard red spring wheat was compared.

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## MATERIALS AND METHODS

**Wheat.** Samples of eastern Canadian wheat were provided as follows: two boatload samples (5.7 t each) of No. 2CE Ontario soft white winter wheat (1982 crop) by the Ontario Wheat Producers' Marketing Board, Chatham; two samples (ca. 30 kg each) of Quêbec red hard spring wheat by Dr. E. A. Chavez, McDonald College, McGill University, Montreal, Quêbec; two samples (500 g each) of No. 2CE Ontario soft white winter wheat and one sample (500 g) of Quêbec hard red spring wheat by the Laboratory Services Division of the Food Production & Inspection Branch, Agriculture Canada, Ottawa; four samples (25–100 g each) of Quêbec hard red spring wheat by the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation, Quêbec; one sample (200 g) of Prince Edward Island hard red spring wheat by the Research Station, Agriculture Canada, Charlottetown.

**Milling.** The two boatload samples of Ontario white winter wheat were mixed, transported to Hayhoe Mills, Woodbridge, Ontario, blended, and cleaned. A 160-kg sample taken continually from the blender was set aside for smaller scale milling. The remainder was tempered to 14.4% moisture and milled to afford screenings, bran, shorts, middlings, biscuit flour, cake flour, and utility flour. An 18-kg blended sample, taken from the 160 kg, sent to the Grain Research Laboratory (GRL) Division of the Canadian Grain Commission, Winnipeg, Manitoba, was cleaned on a Carter dockage tester, put through a Forster cyclone scourer, tempered overnight to 15.0% moisture, and scoured again. The wheat was then milled in the GRL pilot flour mill (Black, 1980) to afford bran, shorts, biscuit flour, cake flour, and straight-grade flour. A 5.0-kg sample of the blended clean winter wheat was tempered overnight to 14.5% moisture and processed through a Bühler experimental flour mill to afford bran, shorts, break flour, and reduction flour.

Two separate 8.0-kg samples of clean-blended Quêbec hard red spring wheat were tempered overnight to 15.0% moisture and processed through the Bühler experimental mill to afford bran, shorts, break flour, and reduction flour.

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Table I. Moisture, Ash, Protein, Deoxynivalenol, and Ergosterol Concentrations in Industrially Milled Ontario White Winter Wheat

| sample | moisture, % | ash, % | protein, % | deoxynivalenol, mg/kg[a] GC-EC[b] | deoxynivalenol, mg/kg[a] GC-MS[c] | ergosterol[d] mg/kg[a] |
|---|---|---|---|---|---|---|
| uncleaned wheat boat sample | –[e] | – | – | 0.48 ± 0.01 | – | – |
| mill sample | 11.49 | 1.69 | 11.63 | 0.45 ± 0.02 | 0.51 | 4.00 |
| dust | 11.73 | 5.65 | 8.40 | 0.75 ± 0.02 | 0.56 | 33.8 |
| screenings | 11.43 | 2.27 | 12.37 | 1.34 ± 0.06 | 1.44 | 16.1 |
| clean wheat | 11.29 | 1.71 | 11.48 | 0.42 ± 0.02 | 0.43 | 4.55 |
| bran | 5.35 | 5.84 | 16.47 | 0.60 ± 0.03 | – | 9.99 |
| shorts | 8.19 | 4.43 | 18.40 | 0.74 ± 0.02 | – | 13.3 |
| middlings | 11.14 | 2.15 | 14.65 | 0.78 ± 0.01 | 1.02 | 7.55 |
| biscuit flour | 12.87 | 0.44 | 10.40 | 0.33 ± 0 | – | 0.96 |
| cake four | 12.53 | 0.38 | 10.05 | 0.28 ± 0.005 | 0.37 | 0.47 |
| utility flour | 11.94 | 0.74 | 11.92 | 0.34 ± 0.005 | 0.33 | 2.23 |

[a] On a fresh weight basis not corrected for recovery. [b] Determined by gas chromatography with electron capture detection. Values are means for duplicate determinations. [c] Determined by gas chromatography with mass spectrometric detection at *m/z* 884. [d] Determined by high-performance liquid chromatography with ultraviolet detection at 282 nm. [e] Not determined.

Table II. Deoxynivalenol Concentrations in Pilot Milled Ontario Soft White Winter Wheat[a]

| sample | moisture, % | ash, % | protein, % | deoxynivalenol, mg/kg[b] |
|---|---|---|---|---|
| uncleaned wheat | –[c] | – | – | 0.81 ± 0.04 |
| dockage | – | – | – | 1.17 ± 0.09 |
| cleaned wheat | 14.0 | – | – | 0.62 ± 0.05 |
| bran | – | – | – | 1.27 ± 0.04 |
| shorts | – | – | – | 1.14 ± 0.02 |
| cake flour | 12.9 | 0.36 | 7.9 | 0.44 ± 0.04 |
| biscuit flour | 12.9 | 0.53 | 10.3 | 0.54 ± 0.01 |
| straight-grade flour | 13.2 | 0.49 | 9.8 | 0.49 ± 0.01 |

[a] The overall recovery of all fractions from the mill was 97%; the flour extraction rate was 73.4%. [b] Determined by gas chromatography with electron capture detection. Values are means for duplicate determinations on a fresh weight basis not corrected for recovery. [c] Not determined.

**Processing and Baking.** Portions of the biscuit flour stream in the Hayhoe mill were treated with chlorine gas at rates equivalent to 0.16 and 0.50% (w/w) to afford low- and high-chlorine biscuit flours, respectively. A portion of the cake flour stream was treated at 0.10% (w/w) chlorine to afford low-chlorine cake flour.

The food manufacturers participating in this study were asked to prepare typical soft wheat products by standard commercial recipes using the bran and flour provided by us. All cookies and cakes were made from this source only. The doughnuts required some hard wheat flour, at 35–65% of the total flour, which was entirely western Canadian in origin and typically contained little or no DON. One of the bran cereals was prepared with cleaned soft winter wheat provided from a separate source by the Hayhoe mill.

**Sampling.** Special attention was paid to obtaining representative samples. Wherever possible, many small aliquots were taken from a processing stream or product line and blended well, and then samples were taken for analysis. All samples were stored at −18 °C.

**Protein, Moisture, and Ash Determinations.** Protein was determined in triplicate by the Association of Official Analytical Chemists method (Horwitz, 1980). Moisture and ash were determined in duplicate by heating samples (2 g) in a vacuum oven overnight at 60 °C and, after measurement of the weight loss, in a muffle furnace (600 °C) for 2 h.

**Analysis for Deoxynivalenol.** Samples were analyzed by the method of Scott et al. (1981) with a change in concentration of ammonium sulfate from 30 to 10%. Some samples (experimentally and pilot milled Ontario soft winter wheat) were analyzed by a modified method incorporating a partition step on a hydrophilic matrix (Health Protection Branch, 1983). Gas chromatography (GC) of (heptafluorobutyryl)imidazole (HFBI) (Pierce Chemical Co., Rockford, IL) derivatized extracts was carried out using a Varian Model 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 (100–120 mesh). In most experiments, the column, injector, and detector temperatures were 170, 230, and 300 °C, respectively, the argon–methane (95:5) flow rate was 28 mL/min, and the attenuation was $32 \times 10^{-11}$ A/mV. Estimations of DON were made by

Table III. Moisture, Ash, Protein, Deoxynivalenol, and Ergosterol Concentrations in Experimentally Milled Ontario Soft White Winter Wheat

| sample | moisture, % | ash, % | protein, % | mill run, kg | deoxynivalenol[a] mg/kg[c] | deoxynivalenol[a] total amount, mg | ergosterol,[b] mg/kg[c] |
|---|---|---|---|---|---|---|---|
| clean wheat | 10.4 | 1.49 | 11.3 | 5.00 | 0.58 ± 0.03 | 2.89 | 3.08 |
| bran | 11.6 | 4.24 | 15.2 | 1.20 | 0.98 ± 0.03 | 1.18 | 12.0 |
| shorts | 9.2 | 1.53 | 14.1 | 0.31 | 0.99 ± 0.03 | 0.31 | 6.17 |
| break flour | 10.7 | 0.36 | 8.93 | 1.06 | 0.28 ± 0.05 | 0.29 | 0.54 |
| reduction flour | 10.6 | 0.43 | 10.1 | 2.22 | 0.43 ± 0 | 0.96 | 0.59 |
| total | | | | 4.79 | | 2.74 | |
| recovery, % | | | | 96 | | 95 | |

[a] Determined by gas chromatography with electron capture detection. Values are means for duplicate determinations. [b] Determined by high-performance liquid chromatography with ultraviolet detection at 282 nm. [c] On a fresh weight basis not corrected for recovery.

Table IV. Moisture, Ash, Protein, Deoxynivalenol, and Ergosterol Concentration in Experimentally Milled Québec Hard Red Spring Wheat

| sample | moisture, % | ash, % | protein, % | mill run, kg | deoxynivalenol[a] mg/kg[c] | deoxynivalenol[a] total amount, mg | ergosterol,[b] mg/kg[c] |
|---|---|---|---|---|---|---|---|
| clean wheat | 12.6 | –[d] | – | 8.00 | 8.66 ± 0.13 | 69.3 | 11.8 |
| bran | 11.3 | 4.98 | 18.6 | 1.80 | 11.3 ± 0.1 | 20.3 | 48.7 |
| shorts | 9.0 | 2.49 | 16.9 | 0.86 | 15.4 ± 0.3 | 13.2 | 52.5 |
| break flour | 10.5 | 0.52 | 14.0 | 1.06 | 8.62 ± 0.17 | 9.1 | 6.34 |
| reduction flour | 10.3 | 0.42 | 11.8 | 4.14 | 7.32 ± 0.09 | 30.3 | 5.90 |
| total | | | | 7.86 | | 72.9 | |
| recovery, % | | | | 98 | | 105 | |
| clean wheat | 11.8 | – | – | 8.00 | 0.97 ± 0.09 | 7.76 | 7.71 |
| bran | 9.9 | 4.88 | 17.1 | 1.92 | 1.17 ± 0 | 2.25 | 17.2 |
| shorts | 6.7 | 2.80 | 16.2 | 0.77 | 2.02 ± 0.02 | 1.56 | 16.7 |
| break flour | 8.9 | 0.71 | 12.3 | 0.96 | 1.07 ± 0.08 | 1.03 | 1.78 |
| reduction flour | 8.4 | 0.47 | 11.1 | 4.10 | 0.74 ± 0.05 | 3.02 | 1.31 |
| total | | | | 7.75 | | 7.86 | |
| recovery, % | | | | 97 | | 101 | |

[a] Determined by gas chromatography with electron capture detection. Values are means for duplicate determinations. [b] Determined by high-performance liquid chromatography with ultraviolet detection at 282 nm. [c] On a fresh weight basis not corrected for recovery. [d] Not determined.

Table V. Deoxynivalenol and Ergosterol Concentrations in Products Made from Industrially Milled Ontario Soft White Winter Flours and Bran

| sample | moisture, % | deoxynivalenol, mg/kg[a] GC-EC[b] | deoxynivalenol, mg/kg[a] rederivatized GC-EC | deoxynivalenol, mg/kg[a] rederivatized GC-MS[c] | ergosterol,[d] mg/kg[a] |
|---|---|---|---|---|---|
| from biscuit flour | | (0.33) | | | |
| low-chlorine treatment | 12.60 | 0.29 ± 0.04 | –[e] | – | – |
| high-chlorine treatment | 12.75 | 0.29 ± 0.01 | – | 0.31 | – |
| cookie A | 1.87 | 0.14 ± 0.02 | 0.17 | 0.16 | – |
| cookie B | 1.61 | 0.17 ± 0.02 | 0.24 | 0.23 | – |
| cookie C | 1.42 | 0.21 ± 0 | 0.20 | 0.24 | – |
| from cake flour | | (0.29) | | | |
| low-chlorine treatment | 12.29 | 0.32 ± 0.03 | – | – | – |
| yeast doughnut A | 31.74 | 0.13 ± 0 | 0.09 | 0.06 | – |
| yeast doughnut B | 32.9 | 0.11 ± 0.01 | – | – | – |
| cake doughnut | 18.86 | 0.04 ± 0.01 | 0.04 | 0.10 | – |
| white cake | 38.79 | 0.08 ± 0.01 | 0.10 | 0.13 | – |
| cookie D | 7.18 | 0.11 ± 0.01 | 0.13 | 0.22 | – |
| from bran | 6.79 | 0.67 ± 0.04 | 0.69 | 0.97 | 9.32 |
| hammermilled bran | 10.12 | 0.47 ± 0 | 0.84 | 1.05 | 9.86 |
| cooked bran | 10.66 | 0.41 ± 0.02 | 0.33 | 0.61 | 7.20 |
| bran cereal A | 1.58 | 0.40 ± 0.03 | 0.47 | 0.49 | 5.27 |
| from wheat and bran | | | | | |
| wheat | 9.45 | 0.48 ± 0.02 | 0.39 | 0.33 | 2.96 |
| wheat/bran blend | 11.68 | 0.49 ± 0.12 | 0.58 | 0.53 | 3.85 |
| cooked blend | 17.92 | 0.24 ± 0.01 | 0.35 | 0.28 | 3.20 |
| bran cereal B | 2.04 | 0.23 ± 0.03 | 0.31 | 0.36 | 2.91 |

[a] On a fresh weight basis not corrected for recovery. [b] Determined by gas chromatography with electron capture detection. Values are means for duplicate determinations. [c] Determined by gas chromatography with mass spectrometric detection at $m/z$ 884. [d] Determined by high-performance liquid chromatography with ultraviolet detection at 282 nm. [e] Not determined.

comparison of peak heights from injected samples with those of derivatized standards. The data reported in Tables I–V were not corrected for recovery; for the samples that were milled on the industrial and small scales, recoveries of DON from wheat flour averaged ca. 80 and 100%, respectively. Additional aliqouts were taken from a number of the sample extracts that had been stored in the dark at room temperature for 2 weeks, then derivatized, and analyzed.

Confirmation of the GC-EC results was accomplished using a Varian Model 3700 GC equipped with a splitless injector and a 12 m × 0.2 mm i.d. fused silica SP2100 capillary column connected to a Finnigan MAT Model 312 mass spectrometer (MS). The GC column was programmed from 40 to 150 °C immediately upon injection and at 5 °C/min for ca. 15 min thereafter; injector and transfer line temperatures were 230 and 220 °C, respectively. At a helium flow of 1 mL/min the HFBI derivative of DON eluted at 185 °C; head pressure was 8 psi. The MS was operated in the single ion monitoring mode at $m/z$ 884 at a resolution of 1000 (5% valley), 70 eV, and an ion source temperature of 230 °C.

**Analysis for Ergosterol.** Samples were extracted and cleaned up according to the procedure of Seitz et al. (1979) except that pentane was used for the final extraction. The combined pentane extracts were evaporated to dryness, and the residues were taken up in methylene chloride–2-propanol (99:1) and filtered through a Waters Scientific Clarification Kit. The filtrate was dried under nitrogen and made to volume with methanol–methylene chloride (1:1). High-performance liquid chromatographic analysis was carried out with a Waters Scientific Model SDA-6000

pump system and a Merck Hibar II column (25 cm × 4 mm) packed with LiChrosorb RP-18 (5 μm) coupled to an LDC Model Spectromonitor I variable-wavelength detector. With a solvent gradient of acetonitrile to acetonitrile–methanol (1:1) over 10 min at 2.0 mL/min, ergosterol eluted at 14 min and was detected at 282 nm.

**Calculations of Percentage Change in Deoxynivalenol Levels upon Milling and Baking.** Calculations to determine DON in starting and mill products were based on fresh weights. Percent changes were calculated on a dry weight basis with compensation for any dilution effects.

**Safety Note.** Deoxynivalenol should be handled with caution and contact with skin should be avoided.

## RESULTS AND DISCUSSION

**Analyses for Deoxynivalenol.** The data in Tables I–V show that there was excellent agreement (correlation coefficient $r = 0.99$) between the duplicate GC-EC results for DON. Samples extracts (prior to derivatization) stored in toluene–acetone (95:5) at room temperature in the dark for 2 weeks were stable and gave similar results ($r = 0.88$) when additional aliquots were derivatized and analyzed (Table II). Further, the good agreement ($r = 0.91$) between electron capture detection and mass spectral single ion monitoring at $m/z$ 884 (parent ion for the heptafluorobutyryl derivative) confirmed the nature of the substance quantitated by the GC-EC determinations (Tables I and V).

**Fungal Infection and Mycotoxin Production.** Ergosterol is a metabolite that has been used as an indicator of fungal biomass in grains (Seitz et al., 1977, 1979, 1982; Miller et al., 1983). For the soft winter wheat (Tables I and III) and two hard spring wheat samples (Table IV) that were milled, the elevated ergosterol levels in the bran and shorts fractions and decreased levels in the flours suggest that the fungal infections were greater at or near the surface of the kernels. A similar partitioning of DON between the outer and inner fractions (Tables, I, III, and IV) was observed. Typically, there is a decrease in ash and protein concentration from the outer, subaleurone region of wheat endosperm to the central (mid) endosperm portion of the grain. Thus, protein and ash values of the various flours are a crude indication of flour origin, and in all instances both DON and ergosterol values were highest in the high-ash, high-protein flours and lowest in the low-ash, low-protein flours. The positive correlation ($r = 0.80$) between ergosterol and DON levels in the various fractions (Tables I and II) indicates that the mycotoxin is produced at the site of fungal growth rather than transported from the kernel surface to the interior. This is supported by microscopic analysis of several wheat samples, which showed invasion of the starchy endosperm by the fungus (Fulcher, 1983).

Data from 11 different eastern Canadian wheat samples (Table VI) showed a positive correlation ($r = 0.99$) between ergosterol and DON levels in the whole grain, further confirming that increased fungal infection leads to a higher incidence of mycotoxin. Québec spring wheat sample G came from an experimental plot where seeds highly contaminated by *F. graminearum* had been planted; all other samples were from naturally infected sources.

**Effects of Storage, Transportation, and Milling of Soft Winter Wheat.** The data in Table I show that in the short term, storage and handling of the unclean winter wheat had no significant effect on the level of DON since there was little change in the samples taken from the hold of the ships compared with those from the commercial mill 6 weeks later. Interkernel abrasion during handling created a dust that had higher DON and ergosterol levels. Removal of the dust and screenings by cleaning gave a modest (7%) reduction in DON. Three other soft white winter wheat samples cleaned at the same mill showed an average reduction of 23%.

Table VI. Deoxynivalenol and Ergosterol Concentrations in Various Eastern Canadian Wheat Samples

| source and type | deoxynivalenol | ergosterol |
|---|---|---|
| Ontario, winter | | |
| A | 0.03[a] | 0.86[a] |
| B | 0.27 | 1.30 |
| C | 0.45 | 4.00 |
| Québec, spring | | |
| A | 0.02[b] | 0.94 |
| B | 0.11[b] | 6.91 |
| C | 1.16 | 10.3 |
| D | 1.61 | 12.0 |
| E | 2.88[b] | 5.20 |
| F | 6.93 | 21.2 |
| G[c] | 172[b] | 174 |
| Prince Edward Island, spring | | |
| A | 0.64 | 9.72 |

[a] mg/kg. [b] Samples analyzed by Ministère de l'Agriculture, des Pêcheries et de l'Alimentation, Québec. [c] Experimental sample.

These data also show a fractionation of DON into the various mill streams. For reasons discussed above, the outer bran, shorts, and middling fractions contained increased (37–87%) levels, whereas the flours contained lower levels (17–31%) with respect to the whole wheat.

The data in Tables II and III show that milling of the same wheat in pilot and experimental mills also gave the expected fractionation of DON. By using the weights of the experimental mill fractions (96% recovery overall), one can account for essentially all (95%) of the DON in these fractions.

A comparison (Table VII) of the percentage changes in DON levels resulting from the three different mills shows quite good agreement. This suggests that the experimental mill with a kilogram capacity can reproduce the results from those mills requiring much larger (up to 10 t) amounts.

**Effects of Milling Hard Spring Wheat.** Previous studies (Scott et al., 1983; Hart and Braselton, 1983) on milling of highly contaminated (ca. 4–7 mg of DON/kg) hard spring wheat indicated that there was little or no reduction of DON in the resultant flours. The data in Table IV show that DON was present in lower concentrations in the reduction flour, which comes from the central portion of the kernel. The degree of reduction of DON (Table VIII) on experimental milling was less in the more contaminated sample, probably because of greater fungal penetration and concomitant mycotoxin formation. Extraction of total DON from the different experimental mill fractions was essentially quantitative (Table IV). Hart and Braselton (1983) reported a 2-fold recovery of DON in their study and attributed the increase to a week-long tempering of the wheat (to raise the moisture content) prior to milling; in this study, tempering times were always less than a day.

**Effects of Processing Soft Winter Wheat Mill Fractions.** The levels of DON in a variety of products made from industrially milled soft winter wheat are given in Table V. A summary of the percentage changes is given in Table IX.

Treatment of DON contaminated corn with air containing 5–100% chlorine afforded nearly complete destruction of DON dependent upon concentration and time

Table VII. Changes in Deoxynivalenol Concentrations in Industrially, Pilot, and Experimentally Milled Ontario Soft White Winter Wheat

| | % change due to milling | | | | | |
|---|---|---|---|---|---|---|
| | industrial | | pilot | | experimental | |
| processing | relative to wheat used | relative to unclean wheat | relative to wheat used | relative to unclean wheat | relative to wheat used | relative to unclean wheat |
| uncleaned wheat to | | | | | | |
| dust | +68 | +68 | | | | |
| screenings | +198 | +198 | | | | |
| dockage | | | +44 | +44 | | |
| cleaned wheat | −7 | −7 | −24 | −24 | −29 | −29 |
| cleaned wheat to | | | | | | |
| bran | +34 | +25 | +106 | +57 | +71 | +22 |
| shorts | +71 | +59 | +85 | +41 | +72 | +22 |
| middlings | +87 | +74 | | | | |
| cake flour | −31 | −36 | −29 | −46 | | |
| biscuit flour | −20 | −26 | −12 | −33 | | |
| utility flour | −17 | −23 | | | | |
| straight-grade flour | | | −19 | −39 | | |
| break flour | | | | | −52 | −66 |
| reduction flour | | | | | −25 | −47 |

Table VIII. Changes in Deoxynivalenol Concentrations in Experimentally Milled Québec Hard Red Spring Wheat

| | % change due to milling | |
|---|---|---|
| processing step | sample A | sample B |
| clean wheat to | | |
| bran | +30 | +21 |
| shorts | +78 | +108 |
| break flour | 0 | +10 |
| reduction flour | −15 | −24 |

(Young, 1983). However, the use of chlorine levels (0.1–0.5%) typical for mill conditions had very little effect on DON levels in the biscuit and cake flour.

DON levels in cookies made from biscuit and cake flours decreased by up to 35% during the processing step and about 50% overall from the uncleaned wheat. The batter for the cookie containing ammonium carbonate showed the greatest reduction, possibly because this substance releases ammonia on heating and ammonia has been shown to reduce DON levels in contaminated corn (Young, 1983). Cake doughnuts showed a 34% reduction in the baking step whereas there was no effect on processing into white cake.

The unanticipated and dramatic increase in DON levels for yeast doughnuts was confirmed in a duplicate run. This was the only product in the study that involved biological processing. The increase may be due to the enzymatic conversion of some precursor into DON; the identity of such a precursor is unknown since the analytical method was specific for DON only. Further studies to shed light on this phenomenon are under way.

Although one of the bran cereal products showed an overall reduction in DON levels from the starting uncleaned wheat, both showed significant reductions during the processing of the bran itself.

For the nine non-yeast products, the average reduction in concentration of DON from the starting wheat due to processing effects only was 36 ± 21%. Inclusion of dilution effects due to non-flour ingredients gave an average total reduction of 66 ± 20% for all products examined.

It should be noted that based on the results of a number of milling and processing studies and animal feeding trials, the Canadian Government recently concluded that

Table IX. Changes in Deoxynivalenol Concentrations in Products Made from Ontario Soft White Winter Wheat, Flour, and Bran

| | | % change | | |
|---|---|---|---|---|
| | | | relative to unclean wheat | |
| processing step | % soft wheat in product | relative to flour used | processing only | processing and dilution |
| biscuit flour to | | | −26 | −26 |
| low chlorine treated flour | 100 | −14 | −36 | −36 |
| high chlorine treated flour | 100 | −14 | −36 | −36 |
| cookie A | 63 | −35 | −51 | −69 |
| cookie B | 56 | −8 | −31 | −61 |
| cookie C | 58 | +10 | −18 | −52 |
| cake flour to | | | −36 | −36 |
| low chlorine treated flour | 100 | +12 | −28 | −28 |
| yeast doughnut A | 16 | +189 | +86 | −71 |
| yeast doughnut B | 18 | +118 | +40 | −78 |
| cake doughnut | 22 | −34 | −58 | −91 |
| white cake | 29 | −4 | −39 | −82 |
| cookie D | 48 | −20 | −49 | −76 |
| bran to | | | +42 | +42 |
| hammermilled bran to | 100 | −27 | +3 | +3 |
| wheat/bran blend to | 100 | +1 | +4 | +4 |
| cooked blend to | 82 | −36 | −33 | −45 |
| bran cereal B | 82 | −19 | −44 | −56 |
| cooked bran to | 76 | +14 | +18 | −10 |
| bran cereal A | 76 | −11 | +6 | −20 |

guideline levels of 2.0 and 1.0 mg/kg DON in uncleaned soft wheat intended for use in nonstaple foods would not pose a health hazard to adults or infants, respectively, assuming an estimated overall 40% reduction in DON levels upon manufacturing products from uncleaned soft wheat.

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# Worker Reentry Studies for Captan Applied to Strawberries in California

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Captan was applied to strawberries in fields along the coastal region of central California to determine the level of exposure to residues by harvesting crews. Residues of captan and its metabolite, tetrahydrophthalimide (THPI), were determined as dislodgeable residues on foliage and fruit. Air samples were also collected and analyzed during the harvest operations. Field workers, including the applicator/loader/mixer, were monitored for exposure by determining the amount of residues adsorbed to patches attached to the workers' clothing and from the amount present on their gloves. Respirator pads worn by the applicator/loader/mixer were also analyzed. As a biological index, urine of the workers was examined for the presence of THPI. Though the applicator/loader/mixer was exposed to higher dermal levels of captan, no THPI could be detected in the urine while the urine of the pickers had detectable levels.

Captan [*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide] is a widely used foliar fungicide for the control of scabs, blotches, rots, mildew, and various other fungal diseases on fruits, vegetables, and ornamentals. It has been estimated that since 1978 over 8.5 million lb/year have been used in commerical agriculture. Because of the large quantity applied, the potential of exposure to captan residues has been a matter of concern to various state and federal regulatory agencies. Reports of worker dermatitis from spraying apples, allergic dermatitis in pickers, mutagenic effects in bacteria in human embryonic lung cells and in cell lines derived from the kidney of the kangaroo rat, teratogenic effects in developing chicken embryos, increased tumor incidence in mice, and gene-mutation studies in flies have led to a rebuttable presumption against captan (Environmental Protection Agency, 1980). Apparently those at the highest exposure risk are agricultural workers, who are pesticide applicators, mixers, or

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