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Effect of Milling on Decontamination of *Fusarium* Mycotoxins Nivalenol, Deoxynivalenol, and Zearalenone in Korean Wheat

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Samples of wheat naturally contaminated with *Fusarium* mycotoxins were obtained from fields and mills in Korea and were milled by the Bühler test mill and an industrial-scale mill, respectively. Each of the milling fractions was analyzed for nivalenol (NIV) and deoxynivalenol (DON) by gas chromatography with an electron capture detector and for ZEN by high-performance liquid chromatography with a fluorescence detector. NIV, DON, and ZEN were found throughout all fractions, but ZEN was not detected in break and reduction flour fractions in the industrial mill. The highest concentration of NIV was found in the bran, and DON and ZEN were in the shorts. The lowest concentration of NIV was found in the reduction flour, and DON and ZEN were in the break flour. Milling was not effective in removing NIV, DON, and ZEN from the naturally contaminated wheat, but the effect on its concentration in the samples varied.

The determination of concentrations of *Fusarium* mycotoxins such as nivalenol (NIV), deoxynivalenol (vomitoxin, DON), and zearalenone (F-2 toxin, ZEN) in cereals has been carried out recently in several countries with varied results. Accumulated data have revealed DON to be the major toxicant in scabby grains in the United States (Hagler et al., 1984), Canada (Scott, 1984), England (Osborne and Willis, 1984), Austria (Vesonder and Ciegler, 1979), and South Africa (Marasas et al., 1979), while both NIV and DON were detected from cereal products in Japan (Tanaka et al., 1985c), China (Ueno et al., 1986), USSR (Ueno et al., 1986), and West Germany (Blaas et al., 1984). Regarding the occurrence of ZEN, this mycotoxin has been widely detected throughout the world in cereals, mixed feeds, and other products (Mirocha et al., 1977). *Fusarium graminearum* is the major causative fungus of NIV, DON, and ZEN contamination of grains (Ichinoe et al., 1983; Neish and Cohen, 1981). Young et al. (1984) reported that the concentration of DON was reduced during the milling for industrially milled and pilot-milled wheat. Others have also reported various concentration of DON in different milling fractions (Seitz et al., 1985; Abbas et al., 1985; Scott

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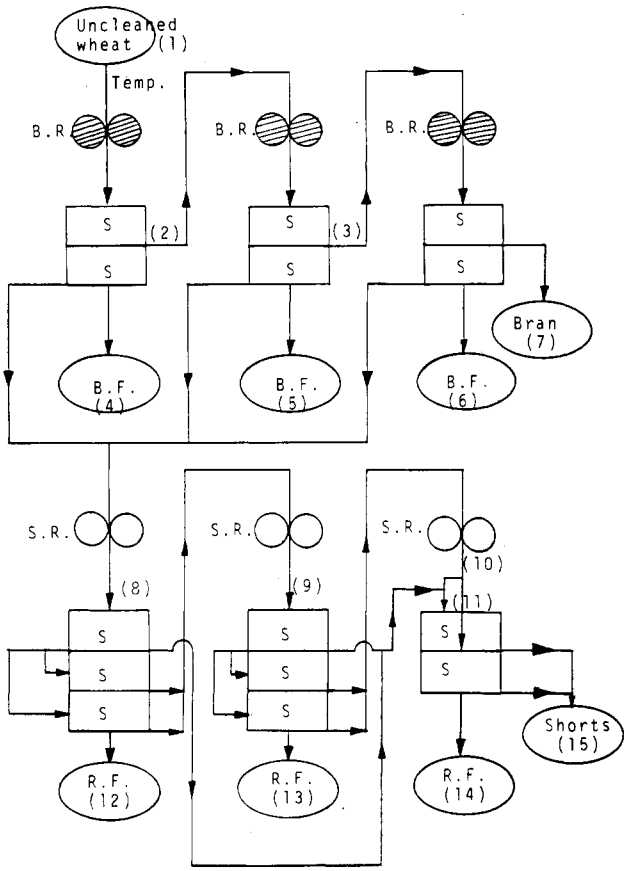

Figure 1. Milling process diagram by the Bühler test mill and sampling points: temp, tempered to 15.5% moisture; B.R., break roll; S, sifter; B.F., break flour; S.R., smooth roll; R.F., reduction flour. Sampling points in parentheses.

et al., 1983, 1984; Hart and Braselton, 1983).

In previous papers (Lee et al., 1985, 1986), we analyzed the Korean cereals harvested in 1983 and 1984 to monitor for contamination of *Fusarium* mycotoxins. The results showed that Korea-grown cereals were significantly contaminated with NIV, DON, and ZEN.

During these surveys, we found that the contents and frequencies of NIV, DON, and ZEN in naked cereals were lower than the original grains; the husking process almost removed ZEN but allowed one-fifth of NIV and DON contents to remain in the naked products. Therefore, we have attempted to clarify the carry-over of NIV, DON, and ZEN during the milling of naturally contaminated Korean wheat.

MATERIALS AND METHODS

Samples and Milling. Samples A and B of naturally contaminated wheat with *Fusarium* mycotoxins were obtained from fields in Chung-buk district and mills in the milling company in Korea in 1985, respectively. Sample A (2 kg) from fields, contaminated with NIV (887 ng/g), DON (310 ng/g), and ZEN (2047 ng/g), was milled by the Bühler test mill (Bühler Brothers, Uzwil, Switzerland). The milling process and sampling points are shown in Figure 1. Sample B, contaminated with NIV (4 ng/g), DON, (68 ng/g), and ZEN (1 ng/g), was composed of 55% of U.S.-grown soft white winter wheat, 40.5% of U.S.-grown hard red spring wheat, and 4.5% of Korean wheat. The latter wheat sample was industrially milled by the milling company in Korea, and each fraction (150 g) was taken for analysis at each stage as shown in Figure 2.

Standards of Trichothecenes and Zearalenone. NIV and DON were prepared from *Fusarium sp.* Fn 2b and *F. graminearum* R2118, respectively, in our laboratory as reported in earlier papers (Ueno et al., 1971; Ishii et al., 1985). ZEN was purchased in analytically pure form from Makor Chemical Ltd., Jerusalem, Israel.

Moisture Determination. Moisture was determined in duplicate by the weight loss from heating samples (2 g) in a vacuum oven for 2 h at 135 ± 2 °C by the PSJ method (Anonymous, 1983).

Analysis and Confirmation for Trichothecenes and Zearalenone. Samples were extracted, purified, and analyzed by the methods described by Tanaka et al. (1985a, 1985b), which can be briefly summarized as follows: Each fraction was extracted with acetonitrile–water (3:1),

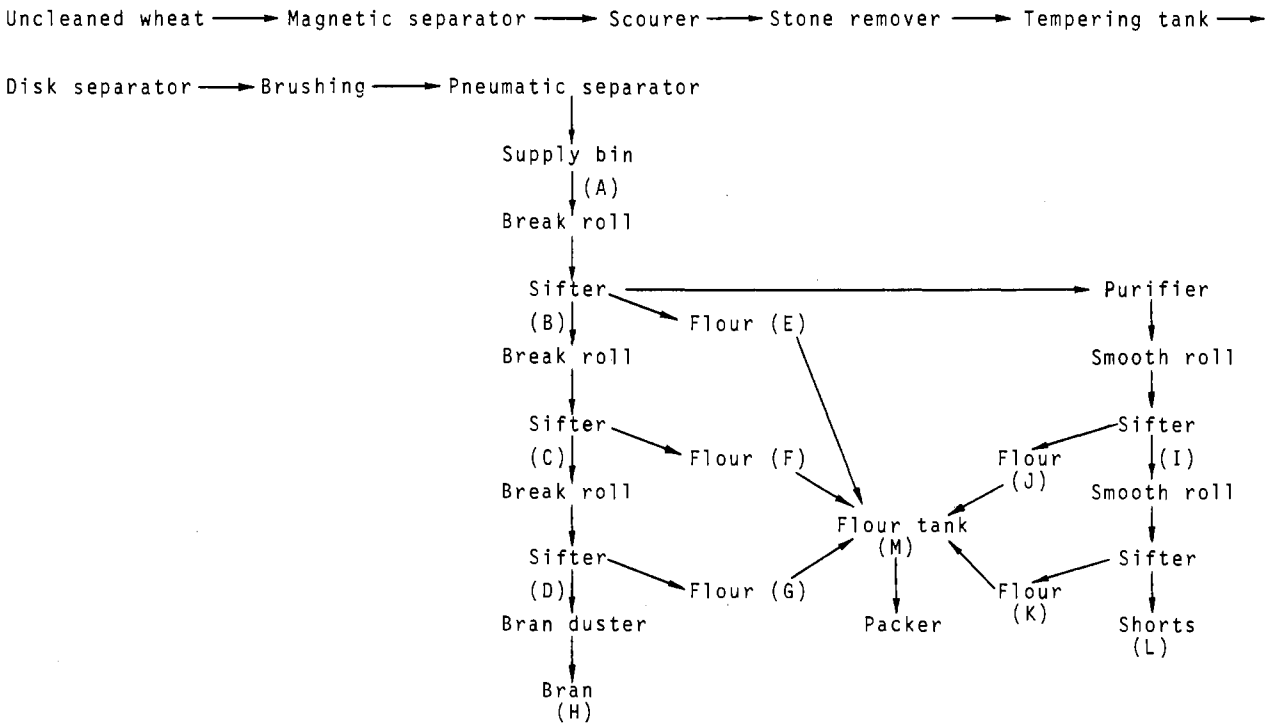

Figure 2. Milling process diagram by industrial-scale mill and sampling points. Sampling points in parentheses.

Table I. Moisture and *Fusarium* Mycotoxin Concentrations in Experimental Milled Fractions of Naturally Contaminated Wheat by Bühler Test Mill

sampling pt[a]	sample	wt, g	moisture, %	concentration,[b] ng/g			total amount, μg		
				NIV[d]	DON	ZEN	NIV	DON	ZEN
1	uncleaned wheat	2000	12.20	887	310	2047	1774	620	4094
	tempered wheat		15.50	c	...	...			
2	1st middlings	63.45	14.27	923	326	2651	58.6	20.7	168.2
3	2nd middlings	66.21	13.64	1639	385	2968	108.5	25.5	196.5
4	1st break flour	151.7	13.11	705	234	752	107.0	35.5	114.1
5	2nd break flour	106.21	13.11	515	202	698	54.7	21.5	74.1
6	3rd break flour	78.28	12.88	542	184	730	42.4	14.4	57.1
7	bran	264.83	13.38	1770	469	3834	468.8	124.2	1015.4
8	1st redn	66.86	13.78	783	328	2125	52.4	21.9	142.1
9	2nd redn	66.72	12.75	872	359	2334	58.2	24.0	155.7
10	3rd redn	65.86	12.44	992	409	2664	65.3	26.9	175.5
11	red dogs	65.62	12.55	1260	509	3232	82.7	33.4	212.1
12	1st redn flour	262.41	12.55	275	188	784	72.2	49.3	205.7
13	2nd redn flour	270.69	12.12	315	203	865	85.3	55.0	234.2
14	3rd redn flour	136.90	12.25	420	233	1065	57.5	31.9	145.8
15	shorts	210.02	12.10	1707	579	4785	358.5	121.6	1005.0
total		1875.78					1671.9	605.7	3901.4
rec, %		93.79					94.24	97.70	95.30

[a] Sampling point shown in Figure 1. [b] Means of duplicate analyses; uncorrected for recovery and moisture differences. [c] Not determined. [d] Key: NIV, nivalenol; DON, deoxynivalenol; ZEN, zearalenone.

defatted with *n*-hexane, and purified by a chromatographic procedure using a Florisil column. After the derivatization to trimethylsilyl ethers of the column eluates with a trimethylsilylating agent consisting of *N*-(trimethysilyl)-imidazole–trimethylchlorosilane–ethyl acetate (1:0.2:9), the amounts of NIV and DON were estimated by gas chromatography (GC); Shimadzu Model GC-8 AE; Shimadzu Ltd., Kyoto) utilizing ^{63}Ni electron capture detection (ECD). A portion of the Florisil column eluates was subjected to analysis of ZEN by high-performance liquid chromatography (HPLC; Shimadzu Model LC-4A) with fluorescence detection (FD). The chromatographic separation was carried out on a silica gel column (Nucleosil 50-10), and the elution solvent was 90% water-saturated chloroform–cyclohexane–acetonitrile–ethanol (50:15:2:1). The detection limits of these methods employed were 2 ng/g for NIV and DON and 1 ng/g for ZEN. The recoveries from wheat were 89% for NIV and DON and 87% for ZEN. The NIV, DON, and ZEN in all positive samples were confirmed by GC–mass spectrometry (MS; Hitachi Model M-80A; Hitachi Ltd., Tokyo). The operating conditions were as follows: glass column, 200 cm × 3.0 mm i.d., packed with 1.5% OV-17 on 80–100-mesh Gas-Chrom Q; carrier gas, helium 30 mL/min; temperature, injector 250 °C, column 230 °C, ion source 180 °C; ionizing voltage, 20 eV; total emission, 100 μA; multiplier gain, 1.5 kV; vacuum, 3×10^{-7} torr. Fragment ions monitored were m/z 510 and 379 for NIV, m/z 512 and 422 for DON, and m/z 462 for ZEN.

RESULTS AND DISCUSSION

Distribution of *Fusarium* Mycotoxins. Various milling fractions were sampled and analyzed for NIV and DON by GC-ECD and for ZEN by HPLC-FD. Average concentrations of NIV, DON, and ZEN in the naturally contaminated wheat and various milling fractions are shown in Tables I and II. The contents of NIV, DON, and ZEN were variable, ranging from 1770 ng/g (bran) to 275 ng/g (reduction flour), 579 ng/g (shorts) to 184 ng/g (break flour), and 4785 ng/g (shorts) to 698 ng/g (break flour) in experimentally milled fractions. The recoveries of NIV, DON, and ZEN for the mill run of the naturally contaminated wheat were 94.2%, 97.7%, and 95.3% to total amounts from all mill fractions, respectively. In the industrially milled fractions, NIV, DON, and ZEN were detected, ranging from 8 ng/g (bran) to not detected (reduction flour), 102 ng/g (shorts) to 40 ng/g (reduction flour), and 2 ng/g (bran, shorts) to not detected (break, reduction and whole flour). NIV, DON, and ZEN were mostly detected in all fractions. The highest concentration of NIV was found in the bran, while DON and ZEN were in the shorts at these milling fractions. The lowest concentrations of these mycotoxins were found in break and reduction flour. According to these results, it may be assumed that the greater portion of NIV, DON, and ZEN is distributed in the outer layer such as bran and shorts, whereas the inner layer such as break and reduction flours contains less. As for mycological origin of these three mycotoxins, Ichinoe et al. (1983) have proposed two chemotypes of *F. graminearum*; one is NIV type and the other is DON type. NIV-type *F. graminearum* produces NIV and 4-Ac-NIV, and DON-type produces DON and 3-Ac-DON. ZEN is produced by these two types, and there are some regional difference in their distribution.

Table II. Moisture and *Fusarium* Mycotoxin Concentrations in Industrially Milled Fractions of Naturally Contaminated Wheat by the Milling Company

sampling pt[a]	sample	moisture, %	concentration,[b] ng/g		
			NIV[d]	DON	ZEN
A	clean wheat	15.00	4	68	1
B	1st middlings	14.14	4	70	1
C	2nd middlings	13.87	6	75	2
D	3rd middlings	13.87	7	87	2
E	1st break flour	13.51	2	50	nd[c]
F	2nd break flour	13.48	2	46	nd
G	3rd break flour	13.48	2	40	nd
H	bran	10.36	8	89	2
I	redn	12.67	4	70	1
J	1st redn flour	12.60	2	40	nd
K	2nds redn flour	12.53	nd	42	nd
L	shorts	11/57	6	102	2
M	whole flour	13.42	2	42	nd

[a] Sampling point shown in Figure 2. [b] Means of duplicate analyses; uncorrected for recovery and moisture differences. [c] Not detected. [d] Key: NIV, nivalenol; DON, deoxynivalenol; ZEN, zearalenone.

Effect of Milling on Decontamination of *Fusarium* Mycotoxins. As shown in Table I, the milling of wheat brought about 20–69% reduction of NIV concentrations, 24–41% of DON, and 48–66% of ZEN in flour fractions intended for human consumption by the test mill, while bran and shorts fractions for animal feed increased about

2-fold in NIV, 1.5–1.9-fold in DON, and 1.9–2.3-fold in ZEN. By the industrial-scale mill, NIV was reduced 50–100%, DON 26–41%, and ZEN 100% in flour fractions, but NIV increased 1.5–2-fold, DON 1.3–1.5-fold and ZEN 2-fold in bran and shorts (Table II). These results showed that concentrations of *Fusarium* mycotoxins in naturally contaminated wheat give an influence on decontamination by milling but was not effective on removal of NIV, DON, and ZEN. In industrial-scale milling, milling was likely to be effective on removal of ZEN, but we think not effective because it is caused by the low concentration of the naturally contaminated wheat; in other words, its concentration is near the detection limit (1 ng/g). Young et al. (1984) reported that the outer layer such as bran and shorts fractions contained increased levels (by 37–87%) of DON, whereas the inner layer such as break and reduction flours contained lower levels (by 17–31%) with respect to the whole wheat, and the lowest concentration of DON was present in the reduction flour, which comes from the central portion of the kernel. Abbas et al. (1985) also reported that the concentrations of DON during milling reduced 31.4–74.6% in reduction flour and 51.6–84.6% in break flour. Similar results with hard red spring wheat (Scott et al., 1984) and soft wheat (Seitz et al., 1985) on little or no reduction of DON concentration in flour should be referred to also. According to our results, we think that NIV, DON, and ZEN in the naturally contaminated wheat were not completely removed by milling, and concentrations of toxins remaining were about 30–80% in NIV, 60–75% in DON, and 30–50% in ZEN in flour fractions intended for human consumption. In bran and shorts used as animal feed, these mycotoxins were increased about 2-fold in raw wheat for milling.

Simple Dilution Not Effective for Removal of the *Fusarium* Mycotoxins. We have already reported the significant contamination of NIV in Korean wheat (Lee et al., 1985, 1986). In this report, the Korean wheat (A) not mixed with the U.S.-grown wheat was also contaminated with NIV (887 ng/g), DON (310 ng/g), and ZEN (2047 ng/g), while no NIV was detected from the U.S. wheat (Tanaka et al., 1985). Since wheat B, composed from the U.S.- and Korean-grown wheat, was also contaminated with 4 ng/g (NIV), we presumed that NIV of this wheat (B) was originated from Korean wheat mixed, and its concentration in the original Korean wheat was supposed to be about 90 ng/g. The results on decontamination of these mycotoxins by milling showed that the reduction percent of highly contaminated wheat (A) was almost similar to that of the wheat diluted with the U.S. wheat. Therefore, the effect of milling on removal of these mycotoxins in the contaminated wheat is not effective in spite of dilution with clean wheat. In this respect, we propose that raw wheat has to be analyzed before dilution and milling and the highly contaminated wheat has to be eliminated.

As a result, the removal of *Fusarium* mycotoxins in the naturally contaminated wheat by milling is impossible because these mycotoxins are distributed through all fractions of the milled wheat and more attention to food sanitation has to be paid during the milling of naturally contaminated wheat because the flour and feed fractions intended for human and animal consumption can contain NIV, DON, and ZEN.

Registry No. NIV, 23282-20-4; DON, 51481-10-8; ZEN, 17924-92-4.

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