ZEARALENONE: DISTRIBUTION IN WET-MILLING FRACTIONS FROM CONTAMINATED CORN[1]

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ABSTRACT

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Corn naturally contaminated with zearalenone at levels of 0.9, 4.1, and 9.4 ppm were wet milled to determine the distribution of zearalenone in milled products. Germ and starch yields were lower in the highly contaminated corn. The starch fraction was devoid of zearalenone, but the zearalenone of the whole grain was concentrated in the other fractions in the order of gluten>solubles>fiber>germ. Zearalenone is not destroyed by the laboratory steeping procedure used to condition the corn for processing, and zearalenone is not bound by gluten protein.

Zearalenone or F2 are trivial names for an estrogenic compound found in corn infected by the mold *Fusarium roseum,* which is the asexual stage of *Gibberella zeae,* the pathogen responsible for corn ear rot (1–3). Zearalenone is a metabolite of most known isolates of *F. roseum* and of several other *Fusarium* spp. (4,5). At low levels, zearalenone promotes growth of sheep (6) and chickens (5); zearanol, the tetrahydro derivative of zearalenone, is a commercial anabolic agent used to improve growth rate and feed efficiency in feedlot cattle (7). At higher concentrations (>1 mg/day), however, zearalenone adversely affects swine and causes what is known as "estrogenic syndrome." Gilts consuming corn invaded by *F. roseum* or given 5 mg zearalenone per day for five days show uterotrophic effects. The response includes enlarged vulvas, mammae, and nipples and prolapse of the vagina. In six-week-old prepubertal gilts, 1 mg zearalenone per day for eight days caused tumefaction of the vulva. Visible "toxic" effects of zearalenone, however, appear to be reversible, since the animals return to normal when put on rations containing no zearalenone (5). Corn or mixed feeds invaded by Fusarium may contain other toxin or toxins that act in concert with zearalenone to accentuate its toxic effects (8).

Several surveys on zearalenone content of corn have been made. It was detected in two lots of U.S. Sample Grade out of 283 samples from the 1967 crop (1.25 and 0.8 ppm) (9), and in four out of 293 samples, also from the 1967 crop, collected at port elevators (0.45–0.75 ppm) (10). Stoloff and Dalrymple (11) found no zearalenone in 119 samples of corn collected in the 1975–1976 marketing season (1975 crop) from 82 dry-milling establishments.

Gibberella ear rot of corn can occur in isolated areas in any one year, but excessive rain frequency and temperatures below 22°C (73°F) can cause epidemics, such as the epidemics of 1965 and 1972 in the east central corn belt (12). Experience indicates that little zearalenone is produced in infected ears in the field but that high concentrations may be produced during subsequent high-

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moisture storage of harvested ears or grain (12). Cognizant of this, Eppley *et al.* (13) sampled corn from the 1972 crop in terminal elevators during the spring of 1973. Seventeen percent (38/223) contained detectable zearalenone, and eight samples were in the physiologically important range of 1.0–5.0 ppm. In the spring of 1974, the FDA sought elevator locations with high incidence of Fusarium damage in the previous year. Of 315 samples of marketable corn (no grades given), zearalenone was found in 10% of the lots at concentrations ranging from 0.04 to 0.20 ppm (0.18 ppm average) (14).

These data indicate that zearalenone has a low level of incidence in commercial corn, and in most lots is below the physiologic threshold level. Toxicity of zearalenone in feed corn has been verified only as a reversible estrogenic effect on gilts, and only when the gilts are consuming moldy corn that would never be considered usable for any commercial purpose. Nevertheless, zearalenone has been a subject of FDA investigation to determine its fate in corn subjected to commercial processing. Bennett *et al.* (15) found that in the dry-milling process, the zearalenone concentrates in the germ and feed fractions. A similar cooperative study is herein reported in which lots of corn naturally contaminated with zearalenone were subjected to laboratory wet-milling fractionation.

MATERIALS AND METHODS

Contaminated Corn

Three different lots of naturally contaminated yellow corn were wet milled. These lots (500–600 lb each) were from the 1972 corn crop and were collected in the northern corn belt. The lots were Grade U.S. No. 2 and 4 and Sample Grade and contained 0.90, 4.1, and 9.4 ppm of zearalenone (db), respectively. A full description of these lots of corn is presented elsewhere (15).

Wet Milling

A laboratory procedure was used for wet milling three contaminated samples and one control sample (Dekalb XL-45). To provide adequate quantities of each wet-milled fraction for zearalenone analyses, fractions were composited from four to five separate millings of 400-g lots of corn. The corn was steeped at 49° C for 48 hr in dilute solutions of sulfur dioxide and lactic acid adjusted to pH 4 (16). Milling was accomplished as previously described (17). The steepwater was decanted and the corn was ground in fresh water in a Waring Blendor to release the germ. Subsequent separations were germ by flotation on liberated starch, fiber by screening, and starch and gluten by tabling. All fractions were washed with fresh water and dried at 49° C. Solubles in filtrates, washes, and steepwater were combined and concentrated to about 60% total dry substance. Fractions produced were: germ, coarse and fine fiber, gluten, starch, and solubles. The quantity of corn solubles in the solubles fraction was determined by subtracting the contribution made by steep acid solubles from the total dry solids. Wet-milling fractions as percentage weight of whole corn were determined.

Zearalenone Analyses

Zearalenone assays were done according to Eppley's procedure as modified by Bennett *et al.* (15). The solubles fractions were extracted by combining a volume of the solubles fraction that contains 50-g corn solubles with 100 ml of aqueous

sodium chloride (20%) and 250 ml of chloroform. This mixture was blended for 3 min with a Waring Blendor and centrifuged at 3,000 rpm for 30 min. Fifty-milliliter aliquots of the chloroform layer were taken for column chromatography. Fiber fractions were extracted with 350 ml of chloroform instead of 250 ml due to solvent adsorption by these fractions. Chloroform extracts were vacuum filtered and the residues were washed with two 100-ml vol of chloroform. The combined filtrates and washes were concentrated to 250 ml, and a 50-ml aliquot was taken for column chromatographic isolation of zearalenone. Amounts of zearalenone in column eluates were determined by visual comparisons of zones produced by samples with those produced by standard zearalenone on thin-layer chromatography (TLC) plates. Confirmatory analyses were done by gas chromatography-mass spectroscopy of the trimethylsilyl derivative of zearalenone in sample extracts.

Steeping Experiments

Quantities of each lot of zearalenone-contaminated corn were steeped in lactic acid and sulfur dioxide solution to determine the stability of zearalenone to steeping. Corn samples (1,500 g) were placed in insulated bottles with 2,800 ml of steep solution (1.5% lactic acid and 0.05% sulfur dioxide; pH adjusted to 3.7 with sodium hydroxide). Steep water was circulated for 48 hr at 51–51.5°C. The steep was decanted and the corn dried in a forced-air oven at 54°C overnight. Dried corn was ground, blended, and assayed for zearalenone. Recovered steepwater was concentrated to 400 ml, and 100 ml was taken for analysis.

Gluten-Zearalenone Experiments

Commercially processed (12% moisture) gluten (CPC, Pekin, IL) was spiked with zearalenone to determine if the zearalenone was binding with components of the gluten fraction. One hundred-gram samples were spiked with a benzene solution to give 5,000 μg of zearalenone per kilogram. Samples were equilibrated at room temperature for up to eight weeks, with one sample assayed each week.

RESULTS AND DISCUSSION

The results of the wet-milling study are summarized in Table I. Visually, all corn samples had good degermination with clean, whole germ and little fiber contamination. Lots A and B had good starch-gluten separation, whereas lot C, which contained the highest level of zearalenone (9.4 ppm), had high loss of starch to gluten and a higher squeegee yield. Gluten and squeegee were combined as a single fraction (gluten) for analyses. The gluten fraction from zearalenone-contaminated corn was darker and less yellow in color than the starch fraction, indicating a loss of xanthophylls. Low germ and high gluten-squeegee yields from the highly contaminated corn are typical of mold-damaged corn (18,19). Excellent recoveries of dry substance were obtained from the three contaminated lots of corn: 100.1, 99.6, and 99.9%.

The starch fraction, which is the largest and most important product for food purposes, was essentially free of zearalenone, even from highly contaminated corn (Table I). The germ fraction, from which edible oil is obtained, contained one to two times the level of zearalenone in the whole corn and accounted for 9–11% of the total zearalenone. The fiber fractions had concentrations about

TABLE I
Zearalenone Distribution Among Wet-Milled Corn Fractions[a]

Fraction	Control	Lot A				Lot B				Lot C			
			Zearalenone				Zearalenone				Zearalenone		
	% of Corn	% of Corn[b]	ppm[c]	Weight (μg)	% of Sum	% of Corn	ppm	Weight (μg)	% of Sum	% of Corn	ppm	Weight (μg)	% of Sum
Corn as milled			0.9				4.1				9.4		
Germ	6.9	6.9	1.7	117	9.1	6.0	3.6	216	10.2	6.2	7.5	465	10.9
Fiber	7.1	9.0	2.7	243	19.0	8.6	3.6	310	14.7	9.7	6.8	660	15.4
Gluten	11.4	9.7	6.8	660	51.5	7.7	13.4	1,032	48.8	11.8	20.4	2,407	56.3
Starch	68.6	67.8	ND[d]	0	0	71.2	ND	0	0	65.2	Tr[e]	0	0
Solubles	6.8	6.7	3.9	261	20.4	6.1	9.1	555	26.3	7.0	10.6	742	17.4
Total	100.8	100.1		1281	100.0	99.6		2113	100.0	99.9		4,274	100.0
				% Recovery 142				% Recovery 52				% Recovery 45	

[a]Fractions from wet milling four to five 400-g portions from each lot.
[b]Average of four determinations.
[c]Assayed in duplicate; values reported on a dry basis.
[d]ND = not detected.
[e]Tr = trace, less than 0.1 ppm.

one to three times that of the original corn and accounted for 15–19% of the total zearalenone. Two fractions, gluten and solubles, had high contamination levels. The gluten fractions contained two to seven times the concentration found in the original corn and accounted for 49–56% of the total. Milling solubles contained one to four times that found in the original corn and accounted for 17–26% of the total zearalenone. Together, these two fractions accounted for 72–75% of the zearalenone, although they accounted for only 14–19% of the corn.

Recoveries of zearalenone from the wet-milling fractions differ for the three lots of corn. As concentration in the whole corn increased, concentration in the milling fractions also increased. The percentage of zearalenone recovered, however, is high for the corn with the lowest contamination (142%) and low for the corn with the highest contamination (45%). Similar results, although not as pronounced, were reported in a collaborative study on the determination of zearalenone (20). Corn samples spiked at 0.3 ppm showed recoveries of 129%, whereas samples spiked at 3.0 ppm showed recoveries of 88%. Greater discrepancies can be expected from naturally contaminated samples due to the uneven distribution of highly contaminated corn kernels throughout a corn lot. This problem is known to cause severe sampling problems with aflatoxin-contaminated corn (21). Another source of error is the interfering substances that are not fully resolved from zearalenone on the TLC plate, especially in the gluten fraction. Corn and corn products appear to contain material or materials that enhance the fluorescence of zearalenone at low levels and result in high recoveries (>100%). This enhancement is not apparent when zearalenone levels exceed 1 ppm.

We have calculated zearalenone distribution both on the basis of initial corn analysis and on the sum of zearalenone in the separate fractions. The latter calculations give more consistent results with respect to distribution of contamination among the fractions. Both methods of calculation show that the gluten and solubles fractions contain most of the zearalenone contamination. These data are unlike the data obtained from the wet milling of aflatoxin-contaminated corn (22). Aflatoxin was found primarily in the solubles fraction (40% of total) and fiber fractions (38% of total). These differences in distribution are undoubtedly due to the differences in chemical structures and thus solubilities of the two mycotoxins.

To investigate the possibility of zearalenone destruction during steeping, contaminated corns were steeped in lactic acid and sulfur dioxide solutions. Levels of zearalenone in three samples of corn were 0.8, 3.5, and 6.3 ppm before steeping. After steeping, the levels were 0.7, 2.8, and 5.2 ppm, respectively. Zearalenone was detected in steepwater from these samples of corn at 0.087, 0.23, and 0.37 mg/L, respectively. Zearalenone appears to be stable to the steeping procedure used in the wet-milling process.

Zearalenone added to gluten was quantitatively recovered at 5.0 ppm for up to eight weeks after the gluten was spiked with zearalenone. Although these conditions do not resemble conditions that exist in the intact kernel, zearalenone does not appear to bind to components in the gluten fraction.

SUMMARY AND CONCLUSIONS

Three lots of zearalenone-contaminated corn (Grade U.S. No. 2, 4, and Sample Grade) were processed by wet milling. The starch fractions were free of

detectable levels of zearalenone. Zearalenone was found in all other fractions: gluten>solubles>fiber>germ. Gluten and solubles accounted for 49–56% and 17–26%, respectively, of the zearalenone in the corn fractions. This study shows clearly that should corn contaminated with zearalenone be processed accidentally in a wet-milling plant, no contamination would be expected in starch-containing food products. Feed products, however, would contain much greater zearalenone concentrations than would those found in the original corn. A survey of the literature revealed that incidence of zearalenone in commercial corn at concentrations used in this study is rare.

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