# Decomposition of Zearalenone and Deoxynivalenol in the Process of Making Tortillas from Corn[1]

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**ABSTRACT**

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Tortilla dough (masa) was prepared from two samples of corn naturally contaminated with 0.23 and 4.23 ppm zearalenone, 3.28 and 12.26 ppm deoxynivalenol (DON), and 1.49 and 9.83 ppm 15-acetyl deoxynivalenol (ADON), respectively. Control corn samples were amended with 1 ppm and 5 ppm of zearalenone and 1, 5, and 10 ppm of DON. Lime water (2% $Ca[OH]_2$) used to boil the corn contained (after boiling) trace amounts of *trans*-zearalenone but none of the other mycotoxins. The process of making tortillas from either contaminated or amended corn reduced significantly the levels of zearalenone and DON and destroyed 15-acetyl-DON completely. *cis*-Zearalenone was detected only in tortillas made from corn heavily contaminated or amended (5 ppm) with zearalenone. The percent reduction of the three mycotoxins during the preparation of tortillas ranged from 59 to 100% for zearalenone, 72 to 82% for DON, and 100% for 15-acetyl-DON. Because corn consumed by humans in most of Mexico and Latin America is largely in the form of tortillas, these findings may have some importance to public health.

Despite the great importance of grains (corn) in the form of tortillas as a principal food in the daily diet for many people in Mexico and Central America, relatively few studies have been made of the retention of mycotoxin in tortillas made from naturally mycotoxin-contaminated whole kernel corn. Mycotoxins have recently been discovered in many areas of the world on a variety of grains and foodstuffs (Mirocha et al 1976; Mirocha et al 1979; McMillian et al 1983; Yoshizawa and Hosokawa 1983; Lee et al 1985; Tanaka et al 1985a,b; Abbas et al 1986). The role of some of these as causal agents of mycotoxicosis is well established in animals and humans (Mirocha et al 1971, Yoshizawa et al 1978, Mirocha 1984, Sheehan et al 1984, Forsell and Pestka 1985, Hamilton et al 1985, Morrissey et al 1985, Bullerman 1986).

Recently researchers have focused on the retention of mycotoxins during grain processing. It has been reported in several studies that chemical and physical treatments were not effective in removing deoxynivalenol (DON) from naturally contaminated whole wheat or corn kernels or their products (Scott et al 1983,

**TABLE I**
**Corn and Tortillas Used in Zearalenone (ZEA), Deoxynivalenol (DON), and 15-Acetyldeoxynivalenol (15-ADON) Decomposition**

| Corn Samples | Corn Used to Make Tortillas[a] (g) | Tortilla Weight (g)[a] | |
|---|---|---|---|
| | | Fresh | Dry |
| Sound | 30.0 | 40.9 ± 0.5 | 30.7 ± 0.4 |
| ZEA and DON added corn 30 μg (1 ppm each) | 30.0 | 40.6 ± 2.1 | 30.2 ± 1.2 |
| ZEA and DON added corn 150 μg (5 ppm each) | 30.0 | 38.3 ± 0.6 | 29.6 ± 0.7 |
| DON added corn 300 μg (10 ppm) | 30.0 | 38.1 ± 0.9 | 29.3 ± 1.2 |
| FS#362[b] | 30.0 | 37.9 ± 1.3 | 28.9 ± 0.5 |
| FS#808[c] | 30.0 | 39.1 ± 0.5 | 29.8 ± 1.2 |

[a] Each value is the mean of five replications of each sample ± standard deviation.
[b] Corn sample stored in University of Minnesota, St. Paul.
[c] Corn sample stored in Purdue University, Lafayette, IN.

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Abbas et al 1985, Seitz et al 1985, Young et al 1986, Young 1986). Moreover, aflatoxin from naturally contaminated corn was not effectively removed in the tortilla making process (Ulloa-Sosa and Schroeder 1969, Price and Jorgensen 1985).

Because concentrations of mycotoxins are affected by methods of food preparation, further study is necessary to determine what concentrations are found in finished food. The objective of this research was to describe the effect of the tortilla-making process on the residues of *trans*-zearalenone, DON, and 15-acetyl-DON (ADON) found in naturally contaminated corn.

## MATERIALS AND METHODS

In 1972, a 20-kg sample of refusal factor corn (FS#362) was obtained from J. Tuite, Department of Botany and Plant Pathology, Purdue University, and was placed immediately in cold storage at −8° C at the University of Minnesota and kept there for 13 years. An analogous sample (FS#808, 20 kg) collected in Indiana in 1973 from the same lot as FS#362 and stored at Purdue University for 13 years at 4° C was analyzed for mycotoxins in the Mycotoxicology Laboratory at the University of Minnesota (Abbas et al 1986). The domestic method of tortilla preparation as practiced in Mexico was followed. The samples (1 kg) were cleaned by manual removal of foreign material. These samples were blended, and representative 300-g aliquots or portions were taken and subdivided into five 60-g samples and treated as follows: 30 g of each 60-g sample was extracted directly to assay for the presence of mycotoxins before making tortillas. The remaining 30 g was used to prepare tortillas. Thirty grams of corn was placed into a 250-ml flask and solution of 2% $Ca(OH)_2$ was added to the flask so that the corn was covered totally. The contents of the flask were boiled for 5 min while stirring and allowed to soak overnight (12 hr). The corn was rinsed thoroughly with distilled water prior to grinding. Alkali-treated corn was ground using a coffee grinder (model 490-SP; Grindmaster of Kentucky, Inc., Louisville). The dough (30 g) was made into tortillas and baked on a flat plate at 110−120° C for approximately 7 or 8 min on each side. The baked tortillas were weighed, then allowed to air-dry in a ventilated hood for three days and reweighed (Table I). One sample of sound corn (300 g) was amended with 300 μg (1 ppm) of purified *trans*-zearalenone and 300 μg (1 ppm) of purified DON, another with 1,500 μg (5 ppm) of purified *trans*-zearalenone and 1,500 μg (5 ppm) of purified DON, and the third sample with 3,000 μg (10 ppm) of purified DON only. The purified mycotoxins were dissolved in 1 ml of ethanol and injected by microsyringe into more than 60% of the embryos of the corn kernels. Samples of corn and their dried tortillas were ground and divided into subsamples for subsequent analyses.

Ground corn or tortilla samples (10 g) were extracted by the method for zearalenone extraction as described by Bennett et al (1985) and analyzed by high-performance liquid chromatography (HPLC) as described by Olsen et al (1985). The chemical identity of

**TABLE II**
**Effect of Tortilla Manufacturing Process on Zearalenone (ZEA) Concentrations (ppm) in Amended and Naturally Contaminated Corn[a]**

| Sample | Corn[b] | | Tortilla | | Water Fraction[c] | | *trans*-ZEA % Reduction[d] |
|---|---|---|---|---|---|---|---|
| | *trans*-ZEA | *cis*-ZEA | *trans*-ZEA | *cis*-ZEA | *trans*-ZEA | *cis*-ZEA | |
| Sound corn | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ZEA added (1 ppm) | 0.75 ± 0.15 (22.5 ± 4.5) | 0 | 0.22 ± 0.08 (6.6 ± 2.4) | 0 | 0 | 0 | 70.7 |
| ZEA added (5 ppm) | 3.62 ± 0.75 (108.6 ± 22.5) | 0 | 0.96 ± 0.44 (28.4 ± 13.2) | 0.19 ± 0.10 | 0.02 ± 0.08 (0.29 ± 1.14) | 0 | 73.8 |
| FS#362 corn | 0.23 ± 0.08 (6.9 ± 2.4) | 0 | 0 | 0 | 0 | 0 | 100 |
| FS#808 corn | 4.23 ± 0.65 (126.9 ± 19.5) | 0 | 1.74 ± 0.47 (51.9 ± 14.0) | 0.25 ± 0.13 | 0.03 ± 0.02 (0.42 ± 0.28) | 0 | 59.1 |

[a] Calculation, based on dry weight of corn or corn product. Each value is the mean of five replications of each sample ± standard deviation. Toxins were quantitated by high-performance liquid chromatography using integrated peak area count comparisons with standards. Numbers in parentheses indicate the mean of five replications of the average of the total amount of zearalenone in the sample in micrograms ± standard deviation.
[b] Analysis of the corn was made before the tortilla-making process.
[c] The weights of the water fractions of zearalenone added (5 ppm, ZEA) and FS#808 corn were 14.3 ± 0.40 and 14.1 ± 0.47 grams, respectively.
[d] The percent reduction of *trans*-zearalenone is based on the average of the total amount of zearalenone in the corn and in the final product, tortillas.

**TABLE III**
**Analyses of Corn and Tortillas for Deoxynivalenol (DON) and 15-acetyl(A)-DON Made from Naturally Contaminated Corn as well as Corn Amended with DON**

| Corn Sample | Mycotoxin Levels[a] (ppm) | | | | % Reduction[b] | |
|---|---|---|---|---|---|---|
| | Corn | | Tortillas | | | |
| | DON | 15-ADON | DON | 15-ADON | DON | 15-ADON |
| FS#362 | 3.28 ± 3.64 (98.4 ± 109.2) | 1.49 ± 1.07 (44.7 ± 32.1) | 0.63 ± 1.03 (18.2 ± 29.7) | 0 | 81.7% | 100 |
| FS#808 | 12.26 ± 3.01 (367.8 ± 90.3) | 9.83 ± 1.62 (294.9 ± 48.6) | 3.45 ± 1.10 (102.8 ± 32.8) | 0 | 72.1% | 100 |
| DON added corn (10 ppm) | 8.25 ± 3.78 (247.5 ± 113.4) | ND | 2.24 ± 1.22 (65.6 ± 35.7) | ND | 73.5% | ND |
| DON added corn (5 ppm) | 4.46 ± 2.75 (133.8 ± 82.5) | ND | 1.27 ± 2.01 (37.6 ± 59.5) | ND | 71.9% | ND |
| DON added corn (1 ppm) | 0.85 ± 0.66 (25.5 ± 19.8) | ND | 0.15 ± 0.05 (4.5 ± 1.5) | ND | 82.4% | ND |
| Sound corn | Neg. | Neg. | Neg. | Neg. | ... | ... |

[a] Each value is the mean of the analysis of five replications of a sample ± standard deviation. Compounds were quantitated on dry weight base by HPLC by the area counts of peak and confirmed by GC-MS. Numbers in parentheses indicate the mean of five replications of the average of the total amount of DON and 15-ADON in the sample in micrograms ± standard deviation.
[b] The percent reduction of DON and 15-ADON based on the average of the total amount of toxin in the corn and in the final product, tortillas.

*trans*-zearalenone and *cis*-zearalenone was confirmed by derivatizing with Tri-Sil BT (Pierce Chemical Co., Rockford, IL) and analyzing by combined gas chromatography-mass spectrometry (GC/MS) as described by Mirocha et al (1976). The samples were extracted and analyzed for DON and 15-ADON by the method as described by Abbas et al (1986). Briefly, the residues of the two mycotoxins were dissolved in 100 or 500 $\mu$l of mobile-phase solvent of methanol-water (30:70, v/v) for quantitation and immediately mixed on a vortex mixer for 1 min. The limits of detection by HPLC were 10 ng for DON and 500 ng for 15-ADON. The two mycotoxins were confirmed by thin-layer chromatography (TLC) by the visual comparison method with standards and by selected ion monitoring and total ion tracing (Mirocha et al 1976, Abbas et al 1984). Mass spectral confirmation (positive chemical ionization in methane) was done on a Hewlett-Packard 5987 quadrupole mass spectrometer after resolution of the mycotoxins as their trimethylsilyl (TMS) ethers on a DB-5 capillary gas chromatographic column (height, 30 cm).

Fractions of lime water (2% $Ca[OH]_2$) used to boil corn were defatted with *n*-hexane and partitioned with dichloromethane. The organic layer was drained through about 5 cm (40 g) of anhydrous $Na_2SO_4$ and glass wool in a glass funnel. The anhydrous $Na_2SO_4$ was washed with 15–20 ml of $CH_2Cl_2$. The combined $CH_2Cl_2$ extracts were evaporated to dryness on a rotary evaporator. The residue was transferred to a 20 ml Teflon-lined screw-cap vial with 3–4 washes of $CH_2Cl_2$. The contents were evaporated to dryness under nitrogen with gentle heat and redissolved in the mobile phase and analyzed by HPLC.

Concentrations of the three mycotoxins in all samples were calculated using the integrated peak area counts compared with those of authentic standards (in a biologically identical matrix).

## RESULTS AND DISCUSSION

Results indicated that mycotoxin concentrations were reduced during the preparation of tortillas from naturally or artificially contaminated whole-kernel corn; in the case of heavily contaminated corn (FS#808), however, zearalenone and DON (40 and 28%, respectively) were still found in the tortillas (Tables II and III).

The majority of the three mycotoxins (zearalenone, DON, 15-ADON) was removed by boiling the corn in lime water (2% $Ca[OH]_2$) (Tables II and III). In corn containing 0.23 ppm (6.9 $\mu$g/30 g) zearalenone, the tortilla-making process effectively removed all of the zearalenone; however, the tortillas made from corn containing 4.2 ppm (126.9 $\mu$g/30 g) zearalenone occurred as residues at concentrations of approximately 1.7 ppm (51.9 $\mu$g/29.8 g) *trans*-zearalenone, a reduction of 59%. The lime water remaining after boiling 4.2-ppm corn (126.9 $\mu$g/30 g) and amended corn with 5 ppm (150 $\mu$g/30 g) contained 30 ppb (0.42 $\mu$g/14.3 g) and 20 ppb (0.29 $\mu$g/14.1 g) zearalenone, respectively (Table II). Some of the zearalenone was isomerized to *cis*-zearalenone <1 ppm (5.5 $\mu$g/29.6 g or 7.5 $\mu$g/29.8 g) in heavily contaminated corn (Table II). Both *trans*- and *cis*-zearalenone are easily resolved on a capillary column, and their mass spectra are diagnostic. The retention time of *trans*- and *cis*-zearalenone in tortilla samples, FS#808, and samples amended with 5 ppm (150 $\mu$g/30 g) of purified *trans*-zearalenone were identical to those of the standards. The total ion chromatogram and total ion mass spectrum of *cis*-ZEA-TMS derivative are shown in Figure 1. The mycotoxins were not detected in tortillas or water fractions of naturally contaminated corn (FS#362) containing 0.23 ppm (6.9 $\mu$g/30 g)

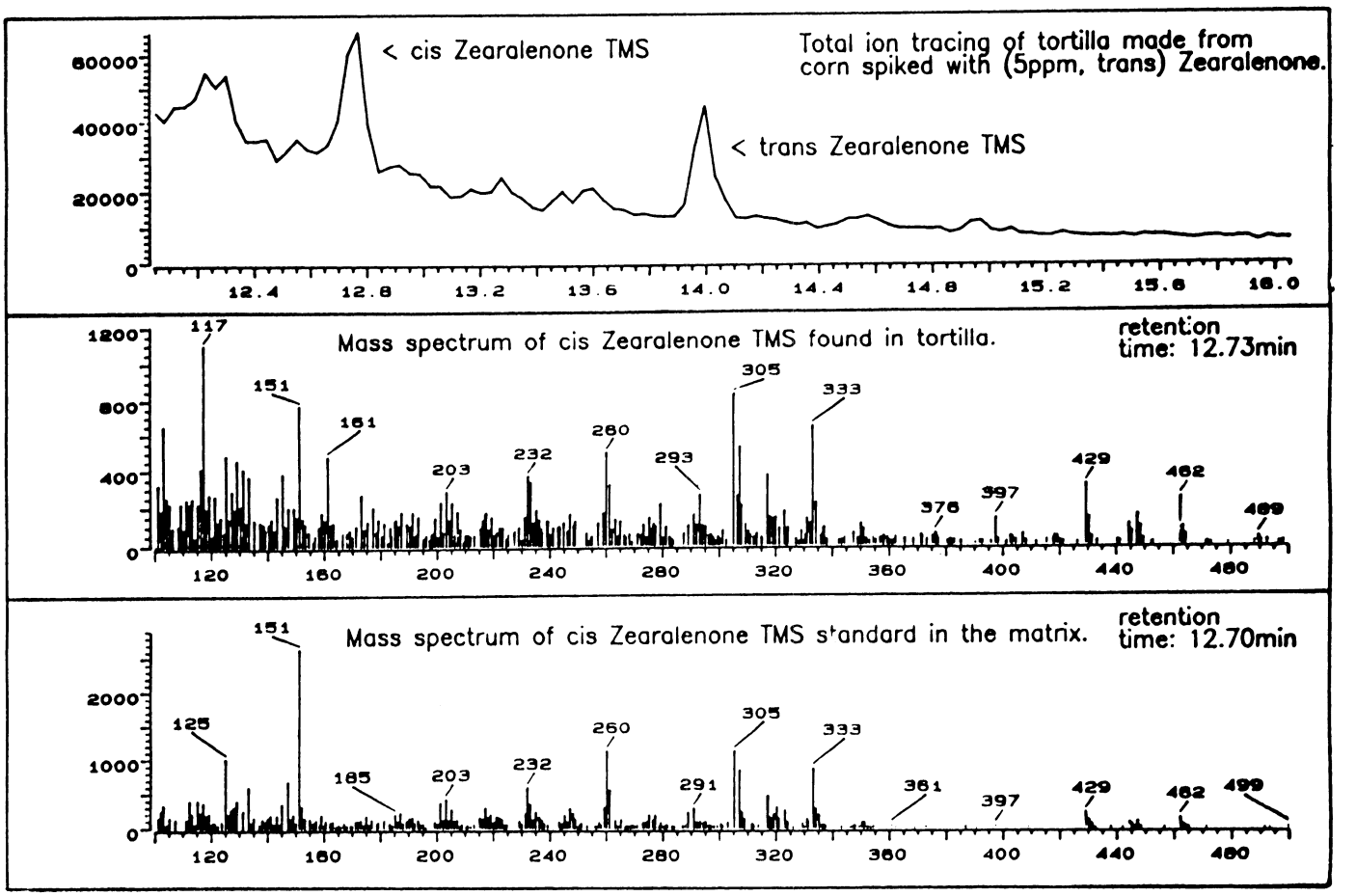


**Fig. 1. Top,** Total ion chromatogram of *cis*-zearalenone-trimethylsilyl (ZEA-TMS) and *trans*-ZEA-TMS derivatives, retention time 12.73 and 13.93 min, respectively. **Center,** Mass spectrum of *cis*-ZEA-TMS derivative for tortilla made from amended F-2 corn, retention time 12.73 min. **Bottom,** Mass spectrum of *cis*-ZEA-TMS standard, retention time 12.70 min. Mass spectrum confirms its presence.

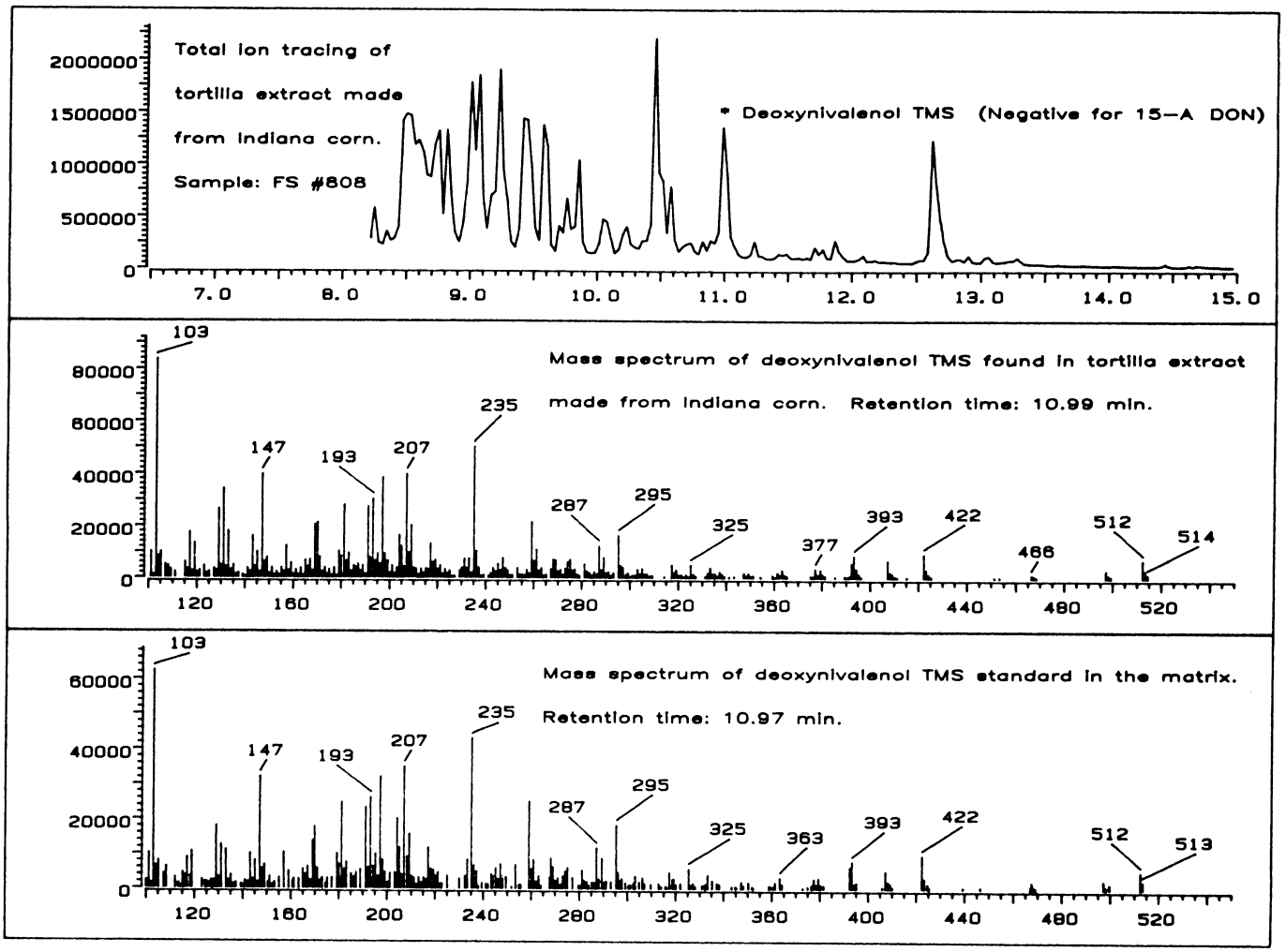


**Fig. 2. Top,** Total ion chromatogram of tortilla extract made from corn (FS#808) contaminated naturally with deoxynivalenol (DON) and 15-acetyl-ADON showing DON but not 15-ADON. **Center,** Mass spectrum of DON-trimethylsilyl (TMS) derivative from tortilla, retention time 10.99 min. **Bottom,** Mass spectrum of DON-TMS standard, retention time 10.97 min. Mass spectrum confirms DON presence but not 15-ADON.

zearalenone. Alpha- and beta-zearalenol were not detected in the tortilla samples.

DON levels were reduced in the preparation of tortillas from contaminated or amended whole-kernel corn. The percent reduction found in the preparation of tortillas ranged from 71.9 to 82.4% (Table III). The DON derivative 15-ADON was totally destroyed in the preparation of tortillas from contaminated corn (Table III). The total ion chromatogram and total ion mass spectrum of DON-TMS and 15-ADON-TMS derivatives are shown in Figure 2. The mycotoxins were not detected in water fractions of naturally contaminated or amended corn. The toxins 3-ADON, nivalenol, and fusarenon-X were not detected in either tortilla sample.

Boiling of the corn in lime water prior to preparation of the corn dough and cooking of the corn dough (masa) as tortillas caused some decomposition of the mycotoxins (ranging from 59 to 100% for zearalenone, from 72 to 82% for DON, and 100% for 15-ADON). These reduction recoveries were determined based on the mycotoxin content in the starting corn and the final product tortillas (Tables II and III). We suggest that this could be attributable in part to treatment with $Ca(OH)_2$, which attacks the lactone in zearalenone. As an example, addition of base causes a bathochromic shift in the ultraviolet spectrum of zearalenone, shifting the absorption maximum from 276 to 250 nm; this can be reversed by addition of acid if done quickly enough (Mirocha et al 1967).

The method of making tortillas in this study has been used in Mexico for centuries. The corn is boiled in lime water to facilitate peeling and softening of the kernels before they are ground for the preparation of tortillas and other foods, but the procedure also appears to increase the healthfulness of the final food product. Although the tortilla-making process reduces the concentration of zearalenone and DON in tortillas, we have no data to suggest what effect this may have on public health in the consumption of tortillas. The concentrations of these mycotoxins after cooking are too low to suggest any adverse effects. However, it is comforting to know that the tortilla-making process does help purify the final product from mycotoxin residue. Similarly, aflatoxin is reduced in quantity by base in tortillas (Ulloa-Sosa and Schroeder 1969, Price and Jorgensen 1985).

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