indicates that there is, within each species, more than one mechanism responsible for the distribution of the fatty acids among the 3 positions of the glycerol molecule.

From this study and from previously reported stereospecific analyses of the triglycerides of maize (9), soya (10) and peanut oil (11,12), it may be stated that within a given species, the amount of a certain fatty acid incorporated at the different positions of the glycerol moiety of the triglycerides is related to the content of this fatty acid in the oil. The oils examined in these studies were obtained from mature seeds and therefore contain triglycerides synthesized during the development of the seeds and during the period in which the fatty acid composition of the oil of the seeds changes (13,14). It will therefore be of interest to investigate whether the mechanism responsible for the placement of the fatty acids on the 3 positions of the glycerol molecule retains its specificity during the development of the oilseeds (15).

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Destruction of Zearalenone in Contaminated Corn[1]

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ABSTRACT

Several chemical and physical treatments were investigated as possible methods for destroying zearalenone in contaminated corn. An ammoniation process which significantly lowers aflatoxin levels had no effect on zearalenone contamination in yellow corn. Also, treatments of propionic acid, acetic acid, hydrochloric acid, sodium bicarbonate and hydrogen peroxide failed to reduce toxin levels. High-temperature treatment (150 C) had no effect on zearalenone. Formaldehyde, in vapor form from paraformaldehyde crystals or in aqueous solutions, destroyed significant quantities of zearalenone in naturally contaminated yellow corn meal and in spiked corn grits and animal feed. Samples treated with aqueous formaldehyde must be dried at 50 C or more to cause effective destruction of zearalenone. Levels as high as 10 ppm zearalenone in animal feed and 8 ppm in ground corn were reduced to less than 0.5 ppm with formaldehyde. Ammonium hydroxide and formaldehyde partially destroyed zearalenone in highly contaminated ground corn. Levels as high as 33.5 ppm were reduced to 12 ppm by 3% ammonium hydroxide and to 2.1 ppm by 3.7% formaldehyde. No treatment used in this study significantly reduced zearalenone levels in whole-kernel corn.

INTRODUCTION

Zearalenone (Fig. 1), a secondary metabolite with estrogenic properties, is produced by some *Fusarium* species that colonize several cereal grains in the field and in storage. If grain infected with *Fusarium roseum* "Graminearum" is stored under conditions of high moisture (>23%) and warm days followed by cold nights, large amounts of zearalenone may be produced. When such grain is fed to animals, especially swine, a hyperestrogenic condition known as "estrogenic syndrome" (1) has been produced. Zearalenone has been detected in corn, wheat, sorghum, barley, sesame meal, oats, hay and commercial animal feeds. This toxin has been detected in cereal grains in several countries througout the world and high levels of contamination have been documented (2,3). Reviews on the chemistry of zearalenone and its derivatives (4,5) have been published; however, limited information is available on procedures or methods to inactivate zearalenone in contaminated cereal grains. This paper reports our initial efforts to destroy zearalenone and thus, detoxify contaminated corn or corn products.

EXPERIMENTAL PROCEDURES

Preliminary Tests

Initially, pure crystalline zearalenone was added to white corn grits (Quaker Quick Grits) to give toxin levels of 3.0

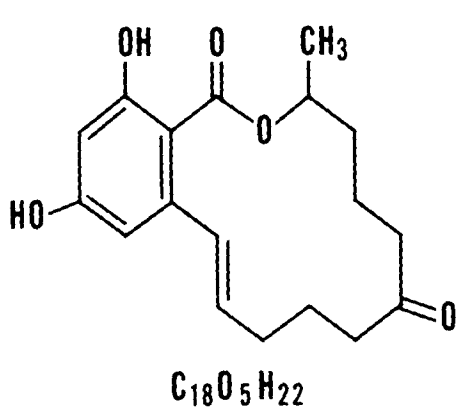

FIG. 1. Structure of zearalenone-6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcyclic acid lactone.

[1] Presented at the AOCS meeting, San Francisco, April 1979.

or 5.0 ppm. This test system was chosen because extracts obtained after treatment could be assayed directly by thin layer chromatography (TLC) without column chromatography or other clean-up procedures. If zearalenone was destroyed by a particular treatment, this treatment was then applied to naturally contaminated corn or corn product. Zearalenone was isolated from these samples by the Eppley method (6) and the levels of toxin were quantitated on TLC plates by reflectance fluorodensitometry (7,8).

Propionic acid (3%), acetic acid (3%), hydrochloric acid (1.85%), sodium bicarbonate (10%), formaldehyde (3.7%), ammonium hydroxide (3%) and hydrogen peroxide (3%) were applied to duplicate 50-g samples of white corn grits spiked with either 3.0 or 5.0 ppm zearalenone. These treatments were tested for 3 days at room temperature or at 50 C for 16 hr. The effect of heat was determined by heating spiked grits at 150 C for 44 hr. After these times, the samples were extracted and assayed as we already described. Spiked grits treated with water served as controls.

Since formaldehyde is very reactive in the gaseous state, the effect of formaldehyde vapors was tested on corn grits (2 kg) and laboratory rat feed (4 kg), each spiked to contain 10 ppm zearalenone. The spiked substrate was placed in a 10-kg capacity Patterson-Kelly twin shell blender (P-K Co., Inc., E. Stroudsburg, PA) modified so that vapors could be circulated through the blender by means of an air pump. Vapors were generated by heating paraformaldehyde crystals to 90-100 C. The blender was connected to a timer which rotated the mixing shells 7-8 times each 20 min. Samples (50 g) were taken from the blender after 1, 3, 7 and 10 days and assayed. The rat feed required column chromatography clean-up of the zearalenone extract prior to TLC separation.

Treatment of Naturally Contaminated Ground Corn

Naturally contaminated corn samples containing different levels of toxin (3.5, 8.0 and 33.5 ppm) were treated with 3.7% and 0.75% formaldehyde solutions. These samples (50 ml reagent and 50 g meal) were heated overnight (16 hr) at 50 C. Zearalenone was extracted with chloroform/water, isolated by column chromatography and quantitated by TLC. Also, corn containing 33.5 ppm zearalenone was treated with ammonium hydroxide (3%) and hydrogen peroxide (3%) as already described.

Treatment of Naturally Contaminated Whole Kernel Corn

A laboratory-scale apparatus used by Brekke et al. (9) to study the action of ammonia on aflatoxin in corn was used to treat zearalenone-contaminated corn. Whole-kernel corn (7 kg) which contained 3.5 ppm toxin was placed in the glass cylinder and treated with gaseous ammonia for 48 hr. This results in ca. 1.5% wt/wt treatment. The treated corn was allowed to equilibrate in a closed container for 14 days at room temperature. A second lot of corn was treated and allowed to equilibrate for 5 days at 45 C. Each lot of corn was ground and blended and 150-g portions prepared for assay as described by Brekke et al. (9). A 1-kg sample of untreated corn was ground, blended, slurried, filtered, dried and 50-g subsamples assayed to determine toxin level in the starting corn. Also, filtrates from the acidified slurries were extracted with benzene (3-100 ml vol) and zearalenone content determined.

Whole-kernel corn (5 kg) containing 3.5 ppm zearalenone was placed in a glass cylinder (20.5 x 45 cm), and paraformaldehyde vapors were circulated through the corn by means of an air pump. The column of corn was raised from the bottom of the cylinder by a steel mesh support. Treatment was continued for 10 days at room temperature. Samples (1 kg each) were taken from the top and bottom of the cylinder, ground and blended. Subsamples (50 g) were assayed for zearalenone. Naturally contaminated whole kernel corn containing 33.5 ppm zearalenone were treated with 6% ammonium hydroxide and 6% hydrogen peroxide (200 ml reagent on 2 kg corn samples). After standing at room temperature for 2 hr, the treated samples were dried for 16 hr at 50 C, ground, blended and assayed for zearalenone.

RESULTS AND DISCUSSION

The effects of the various reagents tested on corn grits spiked to contain 3.0 or 5.0 ppm zearalenone are shown in Table I.

Exposure to 150 C heat for 44 hr did not cause degradation of pure zearalenone or zearalenone in ground corn. Roasting has been reported to cause a reduction of aflatoxin in corn (10). Temperatures of 145-165 C resulted in a 48-81% reduction of aflatoxin in naturally contaminated corn. Heating large quantities of corn is an expensive procedure, and use of low heat and proper chemical treatment would be desirable for detoxification.

None of the acid treatments used in these studies caused a reduction in zearalenone levels in corn grits. These acids and others have been used as grain preservatives against *F. graminearum*, a common zearalenone producer; however, wheat grain treated with 1 ppm (equivalent to 0.1% wt/wt) propionic acid was not protected from fungal invasion after 10 weeks' storage (11).

Sodium bicarbonate (10%) and hydrogen peroxide (3%) had no effect on zearalenone under the mild conditions used. Shipchandler (5) reported that base hydrolysis of zearalenone with sodium bicarbonate results in the cleavage of the macrolide ring, followed *by* decarboxylation. Exposure to 50 C did not cause this reaction to occur. Hydrogen peroxide has been reported to destroy *Fusarium* toxins by oxidation when 3% or 6% solutions are applied to whole kernel corn which is then dried to 10% moisture (12).

Formaldehyde, in aqueous form or as vapors, appears to be an effective agent for the destruction of zearalenone. Zearalenone could not be detected in corn grits which had been spiked to contain 5.0 ppm toxin after the formaldehyde-treated grits were heated for 16 hr at 50 C. In

TABLE I

Summary of Treatments and Their Effect on Zearalenone Added to Corn Grits[a]

Treatment	Conditions	Zearalenone destroyed (%)
Propionic acid	3%, 3 days at R.T.[b]	0
Acetic acid	3%, 3 days at R.T.	0
Hydrochloric acid	1.85%, 3 days at R.T.	0
Sodium bicarbonate	10%, 16 hr at 50 C	0
Hydrogen peroxide	3%, 3 days at R.T.	0
Hydrogen peroxide	3%, 16 hr at 50 C	0
Formaldehyde, solution	3.7%, 16 hr at 50 C	100
Formaldehyde, vapors[c]	10 days at R.T.	96
Ammonium hydroxide	3%, 16 hr at 50 C	80
Heat	150 C for 44 hr	0

[a]Corn grits spiked to contain 3.0 or 5.0 ppm zearalenone.

[b]R.T. = room temperature, 19-23 C.

[c]Grits treated with formaldehyde vapors spiked with 10 ppm zearalenone.

TABLE II

Effects of Chemical Treatments on Zearalenone in Naturally Contaminated Ground Corn[a]

Reagent	Concentration (%)	Zearalenone (ppm)
Control corn		33.5
H_2O_2	3.0	32.0
NH_4OH	3.0	12.0
HCHO	3.7	2.1
HCHO	0.7	5.3

[a]All samples heated 16 hr at 50 C.

contrast, spiked grits treated with formaldehyde and allowed to stand 16 hr at room temperature still contained 50% or more of the toxin. Grits and laboratory feed were effectively treated with vapors of formaldehyde over a 10-day period. TLC plates examined by fluorodensitometry showed only small broad peaks at the Rf value for zearalenone. These may be from traces of toxin; the zearalenone values obtained (0.34-0.48 ppm) were calculated from these apparent peaks.

Ammonium hydroxide was less effective in destroying zearalenone in spiked grits. One advantage of ammonium hydroxide treatment is that no residual odor can be detected after heating; however, formaldehyde odor can be detected and may affect animal acceptance. Only formaldehyde was effective in reducing significantly the toxin level in ground corn. These results are summarized in Table II. Ammonium hydroxide was a less effective agent. Hydrogen peroxide failed to reduce toxin level in the ground corn under the conditions used. Zearalenone in highly contaminated corn meal (33.5 ppm) was not totally destroyed by formaldehyde within 16 hr at 50 C; longer exposure may further reduce the toxin levels.

Ammonium hydroxide and hydrogen peroxide have been reported to detoxify whole corn when applied at the 3% or 6% level (12). However, the report did not state the level of zearalenone contamination in the starting corn. Treatment of whole corn with 6% solutions of hydrogen peroxide, ammonium hydroxide and formaldehyde resulted in no reduction of toxin.

Gaseous ammonia failed to reduce the level of zearalenone in whole-kernel corn under conditions which do detoxify aflatoxin-contaminated corn. Control corn (untreated) contained 2.8-3.1 ppm toxin and the treated corn contained 2.6-3.3 ppm toxin. Also, small amounts of zearalenone (representing 0.04-0.07 ppm in the original 150-g sample) were detected in the filtrates from the acidified slurries of ground corn.

The reaction product(s) formed by the action of formaldehyde and ammonium hydroxide have not been isolated and characterized. Interestingly, Bolliger and Tamm (13) reported that traces of 5-formylzearalenone have been produced by cultures of *Gibberella zea*. Work is continuing to determine the minimal conditions required for the complete destruction of zearalenone by formaldehyde and ammonium hydroxide and to determine if treated corn is acceptable and safe for animal consumption.

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