Utilization of Zearalenone-Contaminated Corn for Ethanol Production[1]

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ABSTRACT

Two lots of yellow corn, severely damaged by *Fusarium* fungi and contaminated with 8.0 and 33.5 ppm zearalenone, respectively, were used for ethanol fermentations. Substrate corn (5-kg samples) was processed in a laboratory procedure similar to that used by the fermentation industry. Stillages obtained were 7.0 to 9.0% ethanol. Ethanol was recovered by distillation, residual grain solids by filtration, and solubles by concentration. No zearalenone could be detected in the ethanol fraction. Zearalenone in the original corn was concentrated in the residual solids and solubles, which are generally used for animal feed. Treatment with formaldehyde significantly reduced the level of zearalenone in fermentation solids. Ammonium hydroxide was a much less effective agent for toxin degradation.

INTRODUCTION

Zearalenone is an estrogenic metabolite produced by several species of *Fusarium* that readily colonize corn and other major cereal crops (1). Numerous animal disorders have been associated with feeding *Fusarium*-damaged corn and corn-based feeds (2,3); however, the economic importance of zearalenone-contaminated corn may be limited to the "estrogenic syndrome" (hyperestrogenism) in swine and infertility effects in cattle. Zearalenone contamination does occur in the field (4), and surveys (5-7) have shown that incidence of zearalenone in the Midwest corn belt ranges from 1-17% at levels of 0.4-5.0 ppm. Much higher levels may be produced when damaged corn is stored under improper conditions at a moisture level of 23% or more. One possible use of such contaminated corn could be the production of ethanol via the fermentation process.

This report describes experiments conducted (a) to determine the fate of zearalenone in the ethanol fermentation using naturally contaminated corn, and (b) to explore the possible use of formaldehyde and ammonium hydroxide to degrade toxin in the recovered fermentation solids, which are generally used for animal feed.

EXPERIMENTAL PROCEDURES

Corn Samples

Two lots of yellow corn containing 8.0 and 33.5 ppm zearalenone, respectively, were used in this study. Lot 1, containing 8.0 ppm toxin, was obtained as the corn was delivered to an area elevator from individual farms. Lot 2, containing 33.5 ppm, was obtained in cooperation with the University of Illinois, where the corn was inoculated in the field with mixed *Fusarium* cultures and stored on the cob over winter without protection from the weather. Samples (10-lb) were ground to pass a no. 20 sieve and blended to obtain 50-g subsamples for determining zearalenone levels in each lot.

Fermentation Procedure

The fermentation procedure used (Table I) simulates procedures generally used in the ethanol fermentation industry. The substrate corn was ground in a Straub Disc Mill (Model 4-2) to pass through a no. 10 sieve, and 5-kg samples were used for ethanol production in 50-L fermentors (New Brunswick Fermacell). Glucoamylase was provided by Hiram Walker & Sons, Peoria, IL. Yeast inoculum was prepared by growing *Saccharomyces uvarum* NRRL Y-1347 in a 2% malt medium (200 mL/500-mL flask, unshaken) for 16-18 hr at 25 C. Two flasks were used to inoculate each fermentor, and fermentations were continued for 5 days at 32 C. Samples (2-L) of fermentation medium were then removed for determining ethanol production. After ethanol was removed by steam injection into the fermentation medium, residual solids were recovered by filtration and drying. Solubles were concentrated to 1 L or less by flash evaporation and then dried in a forced-air oven.

Analytical Procedures

Zearalenone assays were done on 50-g samples of substrate corn, recovered fermentation solids, and solubles. In addition, presence of zearalenone in ethanol was determined by reducing 200 mL distilled ethanol to near dryness by flash evaporation and spotting on thin layer chromatography (TLC) plates. Assays were carried out by the Eppley method (8) as modified by Shotwell et al. (9). The hexane/acetonitrile partition of zearalenone eluates was needed to remove excess oil encountered in fermentation solids. Quantitation of toxin was accomplished by fluorodensitometry (9). The detection limit of the analysis is 0.2 ppm. Proximate analyses for starch and protein in substrate corn and fermentation solids were done by AACC Approved Methods (10). Oil contents were determined by gas chromatography (11). Levels of zearalenone in fermentation solids were confirmed on a KRATOS-MS 30 GC-MS by comparing the intensity of the parent ion (m/e 462) generated by standard TMS-zearalenone to the intensity generated by the unknowns.

TABLE I

Ethanol Fermentation Protocol

1. Substrate corn (5 kg) and ground malt (26 g) added to 18 L of water (52 C) in 50-L fermentor.
2. Premalt (stir 15 min).
3. Cook (90 C for 5 min).
4. Mash (121 C for 20 min).
5. Conversion (cool to 64 C, add 52 g malt, stir 30 min).
6. Add 3 L cool water (32 C), adjust pH to 5.0 with lactic acid.
7. Add 600 units glucoamylase.
8. Add yeast suspension (NRRL 1347 grown in 2% malt medium for 16-18 hr at 25 C).
9. Add water to final vol of 26 L and continue fermentation for 5 days at 32 C with agitation rate of 50-75 rpm.
10. Distill ethanol by steam injection.
11. Screen fermentation medium through cheesecloth (50 mesh) and dry residual solids.
12. Concentrate filtrate to 1 L on flash evaporator.

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TABLE II

Ethanol Content of Stillages and Zearalenone Levels in Substrate Corn and Products from Fermentations of *Fusarium*-Damaged Corn

Corn lot	Grade	Ethanol[a] (%, v/v)	Zearalenone[b] (ppm)			
			Substrate	Solubles	Solids	Ethanol
1	Sample	7.0-9.0	8.0	10-12	18-20	0
2	Ungraded	7.0-7.5	33.5	14.20	50-62	0

[a]Percentage ethanol determined hydrometrically on stillage distillate.

[b]Values are ranges obtained from duplicate assays on products from 3 fermentations of lot 1 and 4 of lot 2.

Detoxification Attempts

We have previously determined that zearalenone levels in ground corn and feed can be significantly reduced by formaldehyde and, to lesser degree, by ammonium hydroxide (12). These reagents were tested on residual solids to determine if toxin levels could be effectively reduced. Test samples (50 or 25 g, dry weight) were treated in extraction flasks with equal volumes of formaldehyde (3 and 6%, wt/wt) and ammonium hydroxide (3, 6 and 12%, wt/wt). After equilibrating for 15-20 min, the samples were placed at 50 C in a forced-air oven for 16-18 hr and then assayed for zearalenone.

RESULTS AND DISCUSSION

The fermentation procedure used in this study was designed so that adequate weight of products could be recovered and assayed to ascertain the fate of zearalenone during the fermentation process. Also, portions of recovered products were used to determine if the concentration of zearalenone could be reduced or eliminated by treatment with ammonium hydroxide or formaldehyde.

Two lots of contaminated corn were used. One lot, containing 8.0 ppm zearalenone, was obtained from commercial channels. This level could be expected to occur in corn colonized by *Fusarium* and stored on the cob without adequate drying. The second lot of corn contained 33.5 ppm zearalenone and would not be encountered in commercial channels due to extensive damage by *Fusarium* mold. This lot of corn was intentionally stored under adverse conditions to encourage toxin formation. These lots provided a basis for comparison of the distribution of zearalenone in fermentation products obtained from moderately and highly contaminated corn. Only 25-28% of the weight of the starting corn was recovered as solids.

Ethanol levels obtained from 4 fermentations of lot 2 corn and 3 of lot 1 are shown in Table II. The high degree of damage that had occurred in lot 2 had some effect on ethanol production, but the 7.0-7.5% (vol/vol) levels are only slightly less than those obtained with sound corn (12).

Results of zearalenone assays on substrate corn, ethanol, and fermentation solids and solubles are also shown in Table II. Since no toxin was detected in the ethanol fractions, fermenting corn in this manner with high levels of zearalenone does not represent a hazard of carryover to the distilled product. However, zearalenone levels in recovered solids are about twice the levels in substrate corn. This concentration effect, due both to conversion of starch in the corn to ethanol and to loss of solids to the solubles fraction, suggests that little of the toxin is destroyed by the fermentation process. The solubles fractions from lot 1 contained 10-12 ppm toxin, and those from lot 2 contained 14-20 ppm toxin. Assay of recovered solids for zearalenone was more difficult than assays of substrate corn because of a significant increase in oil content. Analyses by gas chromatography showed that oil content of the starting corn lots was 2.8% and of the recovered solids were 11.7% and 12.0% for lots 1 and 2, respectively. No starch was detected in the recovered solids, and protein content of these solids was 19% compared to 9.5% protein in the starting corn.

Table III shows the effects of treating recovered solids with formaldehyde and ammonium hydroxide. Fermentation solids containing 19.7 ppm toxin contained only 2.0 ppm after exposure to 3% formaldehyde for 16-18 hr at 50 C. Similar samples treated with 6% formaldehyde contained a zearalenone level no greater than 0.2 ppm. Treatment of highly contaminated samples (60.0 ppm) with 6% formaldehyde caused a significant reduction down to 6.7 ppm. Although the zearalenone is not completely destroyed by these treatments, the residual levels probably would not be harmful to poultry, which have a high tolerance for zearalenone (13,14).

Ammonium hydroxide was less effective in reducing toxin levels in fermentation solids. The 3% treatment had no effect on toxin level; this may be due to the significant amount of oil in these solids. Zearalenone in solids recovered from the highly contaminated corn was not reduced below 12.1 ppm even when treated with 12% ammonium hydroxide. These data show that ammonium hydroxide is not effective, at a concentration practical for use, in reducing toxin levels.

These studies show that zearalenone in corn is not destroyed by the ethanol fermentation process. The toxin concentrates in the residual solids and solubles that are generally used as animal feed; should such solids be fed to swine or breeding cattle, the potential for animal disorders would be heightened in comparison to the original corn. Formaldehyde shows considerable promise as a treatment to detoxify zearalenone-contaminated fermentation products.

TABLE III

Effects of Formaldehyde and Ammonium Hydroxide on Zearalenone Levels in Fermentation Solids

Corn lot	Treatment[a]	Zearalenone (ppm)
1	None	19.7
	H_2CO, 3%	2.0
	H_2CO, 6%	ND[b]
	NH_4OH, 3%	20.0
	NH_4OH, 6%	10.0
2	None	60.0
	H_2CO, 6%	6.7
	NH_4OH, 6%	19.2
	NH_2OH, 12%	12.1

[a]All samples heated for 16-18 hr at 50 C.

[b]ND = not detected (<0.2 ppm).

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REFERENCES

1. Bennett, G.A., and O.L. Shotwell, JAOCS 56:812 (1979).
2. Mirocha, C.J., and C.M. Christensen, in "Mycotoxins," edited by I.F.H. Purchase, Elsevier Press, Amsterdam, 1974, Ch. VI.
3. Christensen, C.M., in "Conference on Mycotoxins in Animal Feeds and Grains Related to Animal Health," edited by W. Shimoda, Bureau of Vet. Med., Rockville, MD, (1979).
4. Caldwell, R.W., and J. Tuite, Phytopathology 64:752 (1974).
5. Shotwell, O.L., C.W. Hesseltine, E.E. Vandegraft and M.L. Goulden, Cereal Sci. Today 16:266 (1971).
6. Eppley, R.M., L. Stoloff, M.W. Trucksess and C.W. Chung, J. Assoc. Off. Anal. Chem. 57:632 (1974).
7. Stoloff, L., S. Henry and O.J. Francis, Jr., Ibid. 59:118 (1976).
8. Eppley, R.M., Ibid. 51:71 (1968).
9. Shotwell, O.L., M.L. Goulden and G.A. Bennett, Ibid. 59:666 (1976).
10. AACC Approved Methods, Starch method 76-20. The Association, St. Paul, MN.
11. Black, L.T., G.G. Spyres and O.L. Brekke, Cereal Chem. 44: 152 (1967).
12. "Ethyl Alcohol Production and Use as a Motor Fuel," edited by J.K. Paul, Noyes Data Corp., Park Ridge, NJ, 1979, p. 257.
13. Chi, M.S., C.J. Mirocha, H.J. Kurtz, G.A. Weaver, F. Bates, T. Robison and W. Shimoda, Poult. Sci. 59:531 (1980).
14. Chi, M.S., C.J. Mirocha, G.A. Weaver and H.J. Kurtz, Appl. Environ. Microbiol. 39(5):1026 (1980).

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Water Imbibition by Normal and Hard Soybeans[1]

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ABSTRACT

By observing the imbibition of dyed water, soybeans were classified into damaged (break in seed coat), normal and hard beans. Hard soybeans were unique in having a long, variable lag time before starting imbibition, but once water uptake was started, the rate was similar to that of normal beans. Soaking hard beans in methanol or ethanol for 24 hr at 20 C made them permeable to water. The cuticle was the most likely site of the water barrier in the seed coat of soybeans.

INTRODUCTION

For production of soymilk, soybeans usually are soaked in water, and after imbibing water, the soaked soybeans are heated to inactivate lipoxygenase and trypsin inhibitors. If, upon soaking, the soybean does not imbibe water, the subsequent heating may not inactivate antinutritional factors which could cause poor quality in the final product.

Soybeans that do not imbibe water (hard beans) have been studied (1-4), but there is no agreement as to the cause of failure to imbibe water, nor is there any extensive study of conditions that would cause hard beans to imbibe.

Smith and Nash (4) observed that the seed coat was the principal barrier to water imbibition and defined hard beans as those that do not absorb enough water in 16 hr to soften normally when cooked in steam. They also observed that hard beans usually were smaller and drier than soybeans that imbibe normally.

Saio (3) studied the seed coat of hard and normal soybeans and concluded that hard beans had more fiber and Ca in their seed coats than normal soybeans. Also, she observed that the micropyle seemed to be closed (when observed by scanning electron microscopy) in hard beans, and this fact may account for the failure to imbibe water.

Growing conditions are known to affect water imbibition by soybeans. Baciu-Miclaus (1) found that soybeans grown under conditions of low relative humidity tended to produce more hard beans than when grown at high relative humidities. Also, Smith and Nash (4) indicated that development of hardness is partly the result of hot dry weather during ripening.

Duangpatra (2) studied some of the beneficial aspects of hard soybeans and found hard seed (after mechanical scarification) had better total germination than normal seed. He hypothesized that the seed coat, except for the hilar region, was water-impermeable and that a suberin layer under the hilum prevented water from entering in hard beans.

In a study of textural differences in legumes, Sefa-Dedeh and Stanley (5) observed that normal soybeans imbibe water faster and to a greater extent than other legumes studied (cowpea, white beans, pinto beans, adzuki beans and U.S. black-eyed peas). They had no explanation based on structural differences in seed coats that would cause different rates of water imbibition.

To learn about the cause of hardness in soybeans and how to control it, we studied the imbibition of water by normal and hard soybeans under a variety of conditions.

MATERIALS AND METHODS

The soybeans used in these experiments were Amsoy 71 and were of seed quality. For soaking in dyed water, a blue food dye (FD and C blue No. 2, indigotine) was used at 0.075%.

Weight increase was calculated on an "as is" basis, with the moisture content of "as is" soybeans ranging from 10 to 13%. The individual beans were weighed, immersed in distilled water for the time indicated, removed from the water, blotted, weighed and returned to the water.

Hard beans were selected by sieving seed beans with a No. 16 round mesh sieve. About 1 kg of small beans passed the screen for every 28-kg bag. The small beans were soaked in distilled water for 24 hr at 5 C. All beans that did not take up water during this time were labeled hard beans and saved for further experiments.

For soaking in organic solvents, 10 hard beans were placed individually in small vials and covered with each of the 5 solvents: hexane, chloroform, acetone, ethanol (95%), and methanol. There were 2 beans exposed to one solvent

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