Chemical Inactivation of Aflatoxins in Peanut and Cottonseed Meals[1]

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Abstract

Organic and inorganic reagents have been tested for destruction or inactivation of the aflatoxins present in peanut and cottonseed meals. The treatments were made in a special laboratory-scale reactor, and were evaluated by determination of the aflatoxins in the products by thin layer chromatography. In some instances, a larger pilot-plant scale reactor was used. Ammonia, methylamine, sodium hydroxide and formaldehyde reduced aflatoxin levels and appear practical for large scale treatments. Effects of various reaction parameters including time, temperature and moisture content on the efficiency of these reagents are presented.

Introduction

The best way of minimizing aflatoxin contamination in various agricultural commodities is to prevent or minimize mold infection by improvement in agricultural practices (1). This is of course impossible with large quantities of a feed or food already contaminated with aflatoxins. To "rehabilitate" this for its intended use, the aflatoxins must be eliminated or their concentration reduced to a value below objectionable levels. Furthermore, this reduction in level must be accomplished with minimum losses in total solids and in nutritive value. Also, no toxic or otherwise undesirable material should be added.

Two possible methods for reducing aflatoxin levels under these restrictions comprise solvent extraction of the commodity to selectively remove the aflatoxins, or subjecting the commodity to some chemical or biochemical treatment to inactivate the aflatoxins. Both solvent extraction and nonbiochemical treatments have been investigated at this Laboratory, and this discussion will be confined to the latter work. These treatments were designed to reduce or eliminate the aflatoxins in oilseed products, cottonseed and peanut meals in particular.

Earlier reports (2–4) indicated that aflatoxins in contaminated peanut meal were refractive to wet and dry heat, acids, alkalis, chlorine, sulfur dioxide, gamma rays and ultraviolet light. In a more recent report (5), autoclaving wet toxic peanut meal at 120 C lowered its aflatoxin content. Chlorine gas (6) and hydrogen peroxide (7) have been used to partially detoxify aflatoxin-contaminated peanut meals. Other reagents of potential value for detoxification include benzoyl peroxide, sodium hypochlorite, sodium perborate, chlorine dioxide and nitrogen dioxide (8). The use of ammonia for this purpose has been patented (9).

This Laboratory's program on inactivation of aflatoxins in contaminated oilseed meals consists of laboratory-scale trials chosen in part according to suggestions from the literature, considering the restrictions stated above. Based on these laboratory trials, selected treatments were performed on a larger scale in the pilot plant to provide sufficient material for biological evaluations. Laboratory-scale trials were generally confined to heat treatments with added moisture in the presence of reagents including acids, bases, oxidizing agents, ammonia and various amines. Other types of reagents also were tested.

Experimental Procedures

Equipment and Methods

Most of the laboratory-scale trials were performed in a bench-scale reactor designed and constructed at this Laboratory (10). This reactor is made of stainless steel, heated with steam or hot water, and equipped with an agitator system having sufficient torque to mix 700–800 g batches of meal. A reflux condenser was used to prevent excess loss of meal moisture during the treatment.

Larger amounts of meal, 15–25 lb. batches, were treated in a 10 gal Groen reaction kettle, heated with a steam jacket, and provided with Teflon-tipped agitator blades. The agitator was operated at a speed of 72 rpm during the treatments. The Groen cover was sealed for pressurized treatments, or vented to the atmosphere through a reflux condenser for treatments at atmospheric pressure.

In general, treatments were initiated by elevating the moisture content of the meal to the desired level by adding the calculated weight of water and blending in a Hobart mixer for 10–15 min. The moisture contents of the meals were determined by use of the infrared-heated Cenco Moisture Balance. Water-soluble reagents were dissolved in the water used to hydrate the meal, and water-insoluble reagents were finely pulverized and mixed in thoroughly, or added in solvents such as acetone and ethanol. The hydrated meal-reagent mixture was transferred to the reactor and the temperature elevated to the desired value and maintained for the specified time. The gaseous reagent (anhydrous ammonia) was added to the preheated hydrated meal in the sealed reactor until the desired pressure was reached, and the temperature maintained as described above. The charge was "dumped" and spread in a thin layer to "flash off" as much moisture as possible. Small batches were air-dried under ambient conditions, and larger batches were dried in a mechanical convection oven for about 1 hr at 55 C.

The products were assayed for aflatoxins by the method of Pons, et al. (11). Briefly, the procedure involved extraction of the aflatoxins with acetone-water, (70:30 v/v), lead acetate purification, extraction into chloroform, purification of the extract on a silica gel column, thin layer chromatography on plates coated with Silica Gel GHR, and visual estimation of the fluorescence intensities of the spots under ultraviolet light. Recently, a more efficient extraction solvent [acetone-water (85:15 v/v), plus 8 ml glacial acetic acid per liter] has been used, and a Photovolt Densitometer has been employed to measure fluorescence intensities (12).

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TABLE I
Aflatoxin Contents of Peanut and Cottonseed Meals After Treatments With Acids and Anhydrides, Inorganic Bases and Oxidizing Agents

Reagents, per cent of meal	Meal[a]	Moisture %	Aflatoxins in product ppb
Acetic acid, 3%	PMA	30	83
Acetic anhydride, 2%	PMB	28	51
Ascorbic acid, 2%	PMA	30	57
Formic acid, 3%	PMA	30	50
Fumaric acid, 1%	PMB	28	63
Glycolic acid, 1% plus NaOH, 1%	PMA	30	91
Maleic acid, 1%	PMB	28	34
Maleic anhydride, 1%	PMB	28	33
Nitric acid, 2%	CM	22	102
Nucleic acid (yeast), 1% plus NaOH, 1%	PMA	30	66
Phosphoric acid, 3%	PMA	30	79
Succinic anhydride, 2%	PMB	28	15
Calcium hydroxide, 2%	PMB	28	11
Sodium carbonate, 2%	PMA	30	12
Sodium hydroxide, 2%	CM	22	11
Trisodium phosphate, 3%	CM	22	49
Ammonium persulfate, 2%	PMA	30	91
p-Benzoquinone, 2%	PMA	30	40
Hydrogen peroxide, 2%	PMA	30	68
Ozonized, esterified soybean oil, 2% plus NaOH, 1%	PMB	28	61
Potassium chlorate, 2%	PMA	30	91
Sodium bromite, 1% plus NaOH, 1%	PMB	28	35
Sodium hypochlorite, 1%	PMA	30	55
Sodium perborate, 2% plus NaOH, 1%	PMB	28	39

[a] Aflatoxin contents of original meals: PMA, 110 ppb; PMB, 99 ppb; CM, 214 ppb.

Materials

Most of the laboratory-scale trials were performed using a prepressed solvent extracted peanut meal specially chosen for its aflatoxin content. It was designated PMA and contained 70 ppb (μg/kg) aflatoxin B_1, 30 ppb aflatoxin B_2, and 11 ppb aflatoxin G_1. No aflatoxin G_2 could be detected. Another similar peanut meal, PMB, also was used in the trials. It contained 72 ppb aflatoxin B_1, 11 ppb aflatoxin B_2, 13 ppb aflatoxin G_1, and 3 ppb aflatoxin G_2. The cottonseed meal, CM, used for the heat effect experiments and for some of the screening tests contained 144 ppb aflatoxin B_1 and 70 ppb aflatoxin B_2. Aflatoxins G_1 and G_2 could not be detected in this cottonseed meal.

Larger scale treatments of peanut meal were performed using meal PMA described above. The cottonseed meal subjected to larger scale ammoniation and methylamine treatments was a blend of a highly-contaminated meal and an uncontaminated meal of high quality. This blend contained 304 ppb aflatoxin B_1 and 30 ppb aflatoxin B_2; no G_1 and G_2 could be detected.

The reagents used in the laboratory scale trials were from different sources and of varying purity, although efforts were made to obtain the highest quality available in each case. The anhydrous ammonia (The Matheson Company) was 99.99% pure; the methylamine (Eastman 527) was 40% in water; and the sodium hydroxide pellets (Mallinckrodt Analytical Reagent) assayed 97.0%.

Results and Discussion

Effects of Heat

Because of apparent contradictions in the literature concerning the effects of heating on the aflatoxins in oilseed meals, a study was made of the influence of moisture content, temperature and time of heating on the aflatoxin content of contaminated cottonseed and peanut meals. This has been reported in detail elsewhere (13), and can be briefly summarized here by Figure 1 which shows the effect on the aflatoxin B_1 content of time and moisture when heating at

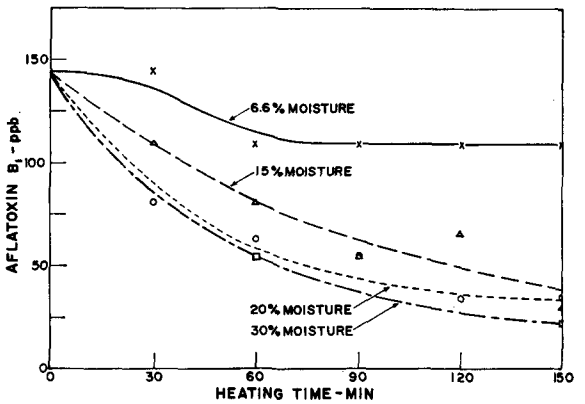

FIG. 1. Rate of aflatoxin B_1 destruction in cottonseed meal at 100 C (data from Ref. 13).

100 C, the cottonseed meal initially having 144 ppb of this toxin.

In general, more aflatoxin B_1 was eliminated by longer periods of heating for a given moisture content, and elevation of moisture also led to decreased aflatoxin levels, particularly after longer heating periods. Although greatest reductions were obtained at 30% moisture, treatments at this or higher moisture levels were impractical because the meal tended to form a tough, plastic mass. Increasing the temperature above 100 C or extending the period of heating beyond 120 min also appeared undesirable because of excessive darkening of the meal. Hence the lowest level of aflatoxin B_1 obtained under conditions deemed practical (120 min at 100 C, 20% moisture) was 33 ppb. The meal heated under these conditions also contained 11 ppb aflatoxin B_2. Since aflatoxins G were not detected in this meal, the total aflatoxin content was 44 ppb, a level higher than the maximum value of 30 ppb for peanuts and edible protein supplements established by a Protein Advisory Group, sponsored by FAO/WHO/UNICEF, for those parts of the world where the need for protein is very urgent (14).

Similar experiments with peanut meal (110 ppb total aflatoxins) revealed that a moisture content of 30% could be used without serious plastic mass formation, but heating with this moisture level for 120 min at 100 C yielded a product containing 73 ppb total aflatoxins. The aflatoxins in peanut meal appear to be more refractory towards these heat treatments than the cottonseed meal aflatoxins.

Laboratory Scale Trials

Based on the moist heat treatments outlined above, laboratory scale trials were performed for 120 min at 100 C. The cottonseed meal was adjusted to a moisture content of about 15% or 20%, and the peanut meal to about 30%. The reagents generally were added at the rate of 2% (or less) of the meal being treated, the addition being performed as described earlier. The results of some of the trials are presented in Tables I–III.

As shown in Table I, acids, anhydrides and oxidizing agents generally failed to yield products containing less than 30 ppb total aflatoxins, the one exception being succinic anhydride. The inorganic bases calcium hydroxide, sodium carbonate and sodium hydroxide appear quite promising, but the action of calcium hydroxide and sodium carbonate appeared erratic upon subsequent tests. Sodium hydroxide was more consistent in its ability to lower aflatoxin levels and hence was selected for further testing. In some treat-

TABLE II
Aflatoxin Contents of Peanut and Cottonseed Meals After Treatments With Nitrogen Compounds

Reagents, per cent of meal	Meal[a]	Moisture %	Aflatoxins in product ppb
Acetonitrile, 1% plus NaOH, 1%	PMA	30	90
Aniline, 1% plus NaOH, 1%	PMA	30	3
Betaine, 2%	PMA	30	80
Choline, 1%	CM	22	26
Cystosine, 0.5% plus NaOH, 1%	PMB	28	13
Ethanolamine, 1%	PMA	30	33
Ethylenediamine, 2%	PMA	30	ND[b]
D-Glucosamine hydrochloride, 1% plus NaOH, 1%	PMA	30	26
Glycine, 1% plus NaOH, 1%	CM	15	65
Guanine, 1% plus NaOH, 1%	PMB	28	13
Hydroxylamine, 2%	CM	22	23
Lysine hydrochloride, 1% plus NaOH, 1%	PMA	30	29
Methylamine, 1.25%	PMA	30	ND
Pyrrole, 2%	PMA	30	85
Thiourea, 2%	PMA	30	32
Trimethylamine, 1.25%	CM	15	29
Uracil, 1% plus NaOH, 1%	PMB	28	13
Urea, 2%	PMA	30	19
Ammonium carbamate, 2% plus NaOH, 1%	PMA	30	10
Ammonium carbonate, 1% plus NaOH, 1%	PMA	30	33
Poly(ammonium phosphate), 2%	PMA	30	29
Potassium cyanate, 2% plus NaOH, 1%	PMA	30	56

[a] Aflatoxin contents of original meals: PMA, 110 ppb; PMB, 99 ppb; CM, 214 ppb.
[b] ND, None detected.

ments, this base was added together with other reagents in effort to enhance the effectiveness of the latter.

Table II indicates that, in general, lower aflatoxin levels were obtained using nitrogen compounds as compared with the reagents described in Table I. The outstanding reagents appear to be ethylenediamine and methylamine yielding products in which no aflatoxins could be detected. Considerations based on cost and probable toxicity led to the choice of methylamine for further examination. Similar considerations led to the rejection of other treatments (aniline plus sodium hydroxide, cystosine plus sodium hydroxide, etc.) which appeared to lower the aflatoxin level markedly. Erratic results were obtained when urea was used in other treatments.

Most of the aldehydes examined are listed in Table III.

Formaldehyde was selected for further testing since it appeared to eliminate aflatoxins completely. The other treatments which seemed effective (crotonaldehyde plus sodium hydroxide, 2-furaldehyde plus sodium hydroxide, etc.) were deemed impractical for commercial purposes because of cost.

It has been observed in this Laboratory that ammonia acts most effectively when applied to aflatoxin-contaminated meals under pressure at elevated temperatures. The data in Table IV were obtained from a series of larger scale ammoniation experiments using contaminated peanut and cottonseed meals. These data show the effects of moisture, ammonia pressure and temperature on the lowering of aflatoxin content by this treatment. Compared with peanut meal, it appears that more drastic ammoniation conditions were required to inactivate the aflatoxins in cottonseed meal.

Data on methylamine and sodium hydroxide treatments of contaminated peanut and cottonseed meals as given in Table V. In laboratory scale treatments of peanut meal with sodium hydroxide alone, 2.0% of this reagent and 30% moisture were required to markedly reduce the aflatoxin content. Similar results were obtained when contaminated cottonseed meal was treated with sodium hydroxide. Treatment

TABLE III
Aflatoxin Contents of Peanut Meals After Treatments With Aldehydes

Reagents, per cent of meal	Meal[a]	Moisture %	Aflatoxins in product ppb
Acetaldehyde, 2%	PMA	30	102
Acrolein, 1%	PMA	30	91
Aldol, 1%	PMA	30	57
Benzaldehyde, 1%	PMA	30	90
Butyraldehyde, 1% plus NaOH, 1%	PMA	30	93
Crotonaldehyde, 1% plus NaOH, 1%	PMA	30	11
Dialdehyde starch, 1% plus NaOH, 1%	PMA	30	102
Formaldehyde, 2%	PMA	30	ND[b]
2-Furaldehyde, 1% plus NaOH, 1%	PMA	30	5
Glutaraldehyde, 1%	PMA	30	91
DL-Glyceraldehyde, 2% plus NaOH, 1%	PMA	30	90
Glyoxal, 0.5% plus NaOH, 1%	PMA	30	76
Heptaldehyde, 1% plus NaOH, 1%	PMA	30	73
Isobutyraldehyde, 2%	PMB	28	49
Paraldehyde, 1% plus NaOH, 1%	PMA	30	20
Phenylacetaldehyde, 1% plus NaOH, 1%	PMA	30	24
Propionaldehyde, 1% plus NaOH, 1%	PMA	30	81

[a] Aflatoxin contents of original meals: PMA, 110 ppb; PMB, 99 ppb.
[b] ND, None detected.

of the peanut meal with 0.60% methylamine plus 1.0% sodium hydroxide seemed more effective than either reagent acting alone. In larger scale treatments of cottonseed meal with methylamine and sodium hydroxide, practical considerations led to lowering the moisture contents to 15% or less, and shortening the reaction periods to 15 or 30 min. With this meal under these conditions, a higher concentration of methylamine, 2.0%, was required for drastic lowering of aflatoxins, and addition of sodium hydroxide seemed necessary to reach nondetectable levels.

Data on the laboratory scale treatments of contaminated peanut meal with formaldehyde and formaldehyde donors are presented in Table VI. The effectiveness of formaldehyde appeared enhanced by increasing moisture content and reaction times, and by the addition of 1.0% sodium carbonate. Similar tendencies have been noted in some of the treatments previously described. Formaldehyde treatments also lowered aflatoxin levels in contaminated cottonseed meal.

All treatments darkened the meals to some extent, the effect being greater with cottonseed meal. Ammonia and methylamine yielded darker products from cottonseed meal than did sodium hydroxide and formaldehyde, but the discoloration did not seem excessive. Meal texture was seemingly unaltered by the treatments. Of the four reagents, only methylamine yielded products with off odors, a slight "fishy" aroma noticed when higher concentrations of the reagent were used.

Contaminated peanut meal has been treated with ammonia, methylamine and sodium hydroxide, and the products subjected to preliminary biological eval-

TABLE IV
Effect of Ammoniation on Aflatoxins in Peanut and Cottonseed Meals

Moist %	Time min	Aflatoxins, ppb				
		66 C		93 C		
		15 psig	30 psig	15 psig	30 psig	45 psig
Peanut meal (110 ppb)						
8.6	15	36	30	41	5	
	30	44	18	24	ND	
15.0	15	41	ND[a]	5	ND	
	30	26	10	5	ND	
Cottonseed meal (334 ppb)						
10	15			....	....	12
15				81	69	6
10	30			....	....	5
15				....	....	4

[a] ND, None detected.

TABLE V

Effect of Methylamine and Sodium Hydroxide Treatments on Aflatoxins in Peanut and Cottonseed Meals (Treatment temperature 100 C)

Methylamine %	NaOH %	Moisture %	Time min.	Aflatoxins ppb
Peanut meal (110 ppb)				
0.0	0.0	22	120	75
0.0	0.0	30	120	73
0.0	1.0	22	120	34
0.0	1.0	30	120	22
0.0	2.0	22	120	18
0.0	2.0	30	120	4
0.0	3.0	22	120	4
0.0	3.0	30	120	7
0.70	0.0	30	120	32
1.25	0.0	30	120	ND[a]
0.60	1.0	30	120	10
Cottonseed meal (334 ppb)				
1.0	1.0	15	30	55
1.5	0.0	15	15	53
1.5	0.0	15	30	53
1.5	1.0	15	15	29
1.5	1.0	15	30	27
2.0	0.0	15	15	16
2.0	0.0	15	30	17
2.0	1.0	15	60	ND
2.0	1.0	15	30	ND
2.0	1.0	15	15	16
2.0	1.0	15	15	38[b]
2.0	1.0	10	30	32

[a] ND, None detected.
[b] Treatment temperature 85 C instead of 100 C.

uations. Details of the treatments, the composition of the products, and the biological evaluations have already been published (15), and the evaluations may be summarized as follows: (a) the treated meals did not produce symptoms of aflatoxicosis in ducklings; (b) the treatments did not seem to introduce any markedly toxic agents; and (c) some reductions in protein efficiency ratios (rat) resulted from the treatments, ranging from 18.1% for ammoniation to 28.0% for methylamine treatment. The formaldehyde-treated product has not been subjected to preliminary biological evaluation.

Ammoniation yielded a product of very low aflatoxin content in the shortest period of treatment at the lowest temperature, with only moderate alterations in meal properties. Ammoniation, however, requires a pressurized reactor capable of handling solids, equipment not generally found in oilseed mills. The methylamine and sodium hydroxide treatments do not require pressurized equipment, and hence might be performed in readily available apparatus such as the "five high" cooker. There is considerable reason to believe that these detoxification treatments might be performed on a commercial scale.

TABLE VI

Effect of Formaldehyde and Formaldehyde Donor Treatments at 100 C on Aflatoxins (110 ppb) in Peanut Meal

Reagents, per cent of meal	Moisture %	Time min	Aflatoxins ppb
Formaldehyde, 0.5%	30	60	18
Formaldehyde, 0.5% plus	15	30	34
	15	60	37
	15	120	37
Sodium carbonate, 1.0%	30	30	26
	30	60	8
	30	120	Trace
Paraformaldehyde, 0.5% plus	15	120	25
Sodium carbonate, 1.0%	30	30	16
	30	60	13
	30	120	11
Dimethylol urea, 0.5% plus Sodium carbonate, 1.0%	30	60	21
	30	120	19

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