Inactivation of Aflatoxins in Cottonseed Meal by Ammoniation: I. Reaction Studies

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ABSTRACT

Cottonseed meal containing an average of 340 ppb total aflatoxins was treated with anhydrous liquid ammonia under varying conditions of time, temperature, and ammonia concentration. Meal moisture was held constant at 10%. A unique sampling device was constructed and used to withdraw meal at time intervals as frequent as 5 min during the reaction period, without interrupting the ammoniation process. The rate of aflatoxin inactivation occurring under a given set of treatment conditions was thus monitored. The data obtained in this study indicate that the inactivation of aflatoxins in cottonseed meal, as determined by chemical assay, may be successfully achieved by use of less stringent conditions of time, temperature, pressure, and moisture content than previous studies have indicated.

INTRODUCTION

The presence of certain toxic mold contaminants detected in animal rations in 1960 (1) was quickly recognized as a potentially serious health problem. When these toxins, now known as aflatoxins, were chemically characterized (2) in 1963, intensive research was concentrated on methods to inactivate or destroy them. Treatment of contaminated peanut or cottonseed meals with heat (3-5) was largely ineffective and tended to impair the nutritive quality of the product. Inactivation treatments of oilseed meals with sodium hydroxide, methylamine (6), hydrogen peroxide (7), ozone (8), and other chemical reagents achieved some degree of success, but generally these treatments were not economically practicable for commercial application.

The use of ammonia as a detoxifying agent for aflatoxins has met with greater success. Sargeant et al. (9), detoxified methanol extracts from contaminated peanut meal with ammonium hydroxide, and Masri et al. (10) reported that ammoniation of aflatoxin-contaminated peanut meal containing 709 μg/kg (ppb) of aflatoxin B_1, moistened to 9.6% and 14.6% at 200 F for 60 min under 20 psig anhydrous ammonia pressure, reduced the aflatoxin B_1 by 96.4% and 97.6%, respectively. Corn has also been treated with aqueous or gaseous ammonia to reduce the aflatoxin content (11). Gardner et al. (12) demonstrated the effectiveness of anhydrous ammonia for inactivating aflatoxins in cottonseed meal in large scale tests. In these experiments, cottonseed meal hydrated to 12.5% was treated for ca. 30 min at ca. 250 F under 50 psig anhydrous ammonia pressure. These conditions reduced the total aflatoxin levels from 519 ppb to less than 5 ppb. Subsequent biological tests comprising two-year rat feeding studies indicated no histopathological abnormalities or other adverse results in the animals fed this ammoniated meal (Booth, A.N., WRRC, USDA, personal communication).

Studies by Prevot (13) confirm that ammoniation seems to offer the best chance for aflatoxin inactivation on an industrial basis. Recently, Prevot and Jemmali (14) reported on pilot-plant and commercial-scale removal of aflatoxins from peanut meal in France. Their findings indicate that while ammoniation reduced the protein value of the material, as shown by tests on rats, and some supplementation of essential amino acids would be necessary, no abnormalities in the kidneys and livers of the rats after the feeding tests were revealed by histopathological examination. It is possible that the nutritive quality of other commodities, processed under different conditions, may remain unaffected by treatment with ammonia.

When it was determined at this Center that ammoniation provides an effective, economically feasible, and commercially practical method for aflatoxin inactivation in cottonseed meal, studies were initiated to determine the optimum processing conditions that might be applied to destroy aflatoxins with minimum decrease in nitrogen solubility. A reactor suitable for ammoniation was modified to permit withdrawal of samples during ammoniation without interferring with processing conditions. Thus, it was possible to monitor the progress of reaction conditions as often as every 5 min to follow the inactivation process. This paper presents the inactivation data and indicates the conditions that proved most effective in our operational system.

MATERIALS AND METHODS

The cottonseed meal used was produced by prepress solvent extraction and had a moisture content of 7.06% as received. The total average aflatoxin content was 350 ppb. Other analytical data (moisture-free basis) include: 0.88% lipids, 7.33% nitrogen, 17.4% crude fiber, and 6.46% ash. The available lysine content (15) was 3.13 g/16 g N, and the nitrogen solubility in 0.02 N sodium hydroxide was 68.90%. Anhydrous ammonia having a purity (liquid phase) of 99.99% minimum was used.

Analyses for aflatoxin were conducted by the AOAC rapid method for aflatoxins in cottonseed products, 26.A09-26.A16, with the exception that the small column cleanup and extraction with hydrochloric acid suggested by Pons and Franz (16) were used for all meals.

The ammoniator, shown in Figure 1, is a pressure-vacuum reactor, completely jacketed, with an overall capacity of 7 ft^3 and a working capacity of 5 ft^3. The jacket can withstand 100 lb of steam working pressure. The shell, or reactor body, can withstand 100 psig internal pressure or full vacuum. A 10 in. diameter cover closes the reactor charge opening. Located in the center of this cover is the sampling device, Figure 2, which permits withdrawal of ca. 3-4 oz of meal at any time during operation without affecting reaction conditions. Basically, the sampling device consists of a crescent-shaped, tubular collection scoop that protrudes into the reactor when the cover is closed. Extending through the cover to the outside, the tubular collector is connected to a ball valve leading to the sample reservoir. The reservoir chamber is fitted with a quick-disconnect pneumatic fitting and is closed by a second ball valve which leads to the discharge tube.

In a typical sampling operation, rotation of the reactor is momentarily stopped with the sampling device in a vertical position above the reactor. The collection scoop then protrudes into the space above the meal charge and may contain a small quantity of meal particles, which should be removed. This is accomplished by attaching an air hose to

[1]One of the facilities of the Southern Region, Science and Education Administration, U.S. Department of Agriculture.

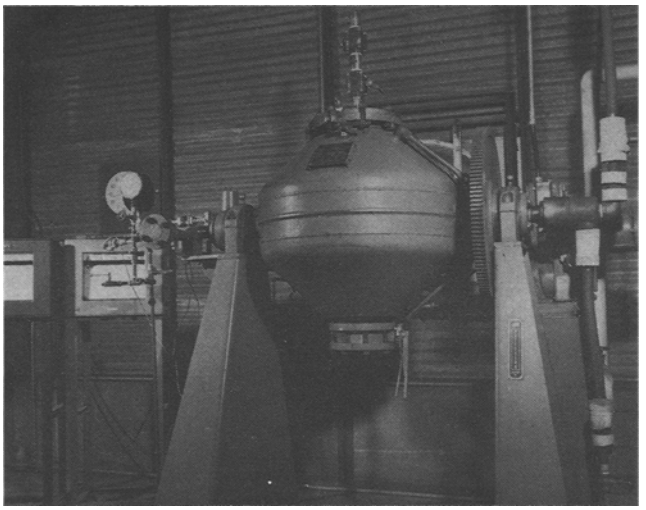

FIG. 1. Ammoniation reactor.

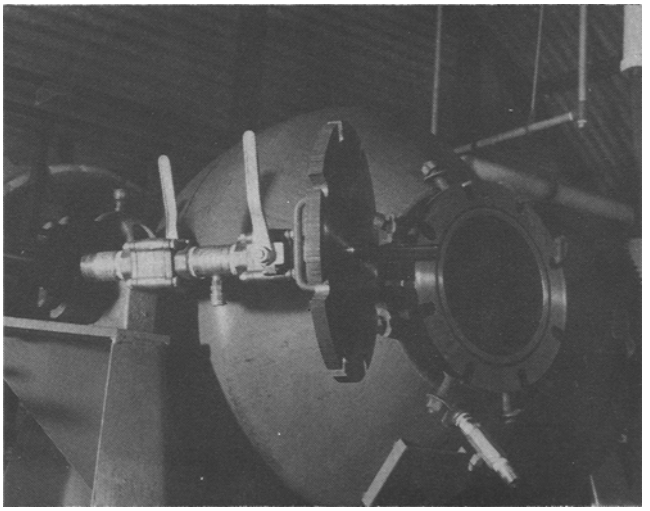

FIG. 2. Sampling device attached to cover of ammoniation reactor.

the quick disconnect fitting and applying two short bursts of 25 psig compressed air into the reservoir chamber. The combined force of the air and gravity causes adhering meal particles to fall into the main charge, clearing the sampler. The reactor then is rotated 180°, and meal falls through the collection scoop and into the reservoir, now located below the reactor. The upper ball valve is then shut to maintain reactor pressure, and the meal sample contained in the pressurized interlock reservoir is discharged and collected in a suitable container by opening the lower ball valve. When the sample has been obtained, the lower ball valve is closed and rotation is resumed. The entire sampling procedure is accomplished in a period of about 30-45 seconds.

Other features of the reactor include a ¼ in. thermowell and a piezoresistive pressure sensor. The conical shape of the reactor assures direct contact between the meal and the heated surface of the interior so that heat is transferred rapidly by conduction. Also, rotation of the reactor causes the meal to cascade through the ammonia atmosphere, assuring thorough solid-gas interaction. Pressure is monitored constantly by the pressure sensor and transmitted to a strip chart recorder. Temperature is recorded with a copper-constantan thermocouple attached to a strip chart recorder.

Hydration of Cottonseed Meal

All experiments described in this study were conducted with cottonseed meal having a moisture content of 10%. The hydration was accomplished by adding calculated quantities of water to 75 lb of cottonseed meal and blending the mixture in a Hobart M-802 mixer equipped with a 110 qt capacity bowl. The blending was accomplished in 10 min with the Hobart "B" Flat Beater at a rotational speed of 55 rpm. Two such blendings provided 150 lb of hydrated meal, which was sealed in a polyethylene liner to equilibrate, and stored in a 55-gal steel drum until ready for use.

Ammoniation Procedure

Fifty lb of hydrated meal was used in each experiment, and this was charged into the reactor. A polyethylene bottle containing predetermined quantities of liquid ammonia transferred from a 15 lb anhydrous ammonia cylinder was implanted upright in the meal within the reactor. The bottle was quickly uncapped and the reactor port sealed. By this means, precise percentages of ammonia in relation to meal charge were used. As the reactor began to revolve (7 rpm), the liquid ammonia spilled from its container and quickly vaporized on the relatively warm walls of the interior. Heat was simultaneously applied, and sampling (described earlier) was begun. A typical ammoniation run was completed in ca. 60-65 min. Half of this period was utilized in attaining reaction temperature, which was then maintained for an additional 30 min.

TABLE I

Effect of Ammoniation of Aflatoxin Content of Cottonseed Meal of Varying Particle Sizes

Sample no.	Reaction conditions[a] Time in reactor min	Temperature at sampling F	Ammonia pressure at sampling psig	Total aflatoxin content, μg/kg Meal retained on 16 mesh screen (>1.2 mm)	Meal retained on 35 mesh screen (0.5 to 1.2 mm)	Meal passed 35 mesh screen (<0.5 mm)
Control	0	---	---	251	247	266
1	5	87	15	273	226	269
2	10	110	20	236	216	232
3	15	135	26	164	120	147
4	20	159	32	80	75	97
5	25	171	32	47	43	35
6	30	178	31	24	25	20
7	35	179	30	24	15	11
8	45	180	28	16	10	7
9	55	179	27	6	8	6
10	65	184	30	5	7	5

[a]Ammonia, 4% based on meal charge.

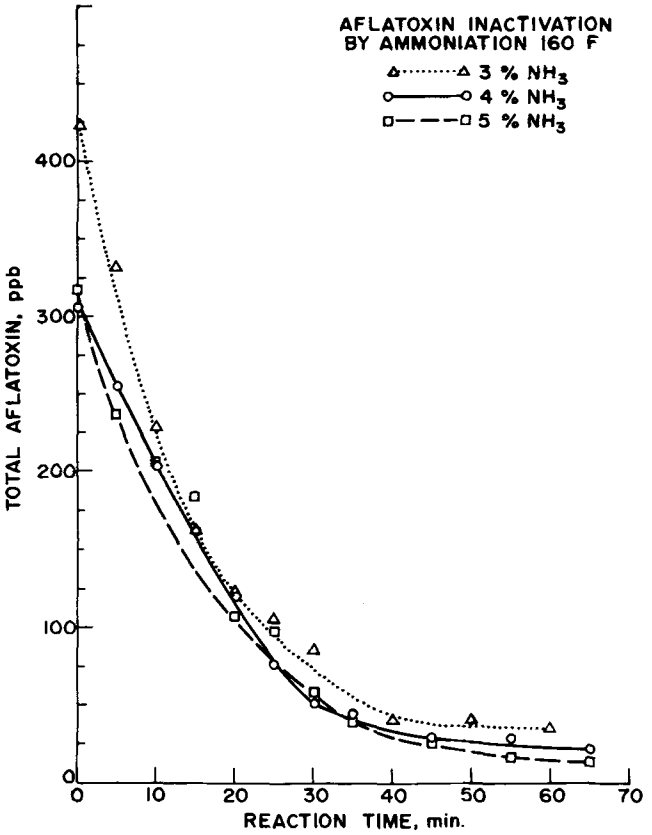

FIG. 3. Effect of temperature (160 F) and ammonia concentration on inactivation of aflatoxins in cottonseed meal.

RESULTS AND DISCUSSION

In examining the effect of ammonia on aflatoxin inactivation in cottonseed meal, an experiment was designed to study the rate of the inactivation process relative to meal particle size. This was accomplished by ammoniating a 50 lb charge of meal by conditions that experience had shown to be effective for destruction of essentially all of the aflatoxins. Samples, double in size from a normal sampling, were taken every 5 min for the first 35 min of processing, and every 10 min for the remaining 30 min of the reaction. The larger sample enabled each sample to be subsequently fractionated by sieving with 16 and 35 mesh wire screens. Thus, three ranges of meal particle size, ammoniated simultaneously under identical conditions, were obtained and assayed for aflatoxin content. The reaction conditions and the results obtained are shown in Table I.

Under the parameters of this study, no major difference of inactivation rate due to meal particle size was noted. It would appear that aflatoxin inactivation occurs as readily with the large, discrete particles retained on the 16 mesh screen as it does with the fine, powdery material that passed the 35 mesh screen. Diffusion of the ammonia gas into meal particles in this range is not the rate-limiting factor in the inactivation process; accordingly, meal particle size was not treated as a process variable.

It has been shown previously (12) that substantially severe ammoniation conditions will readily inactivate aflatoxins in cottonseed meal. It was, therefore, the specific intent of this study to examine the possibility of using milder treatment conditions to achieve equal inactivation results. Processing at lower ammonia pressure and temperature, coupled with reduced meal moisture, has potential for producing a finished product with minimal nutritional impairment. Early in the experimentation process, it was determined that a meal moisture of 10% was adequate and

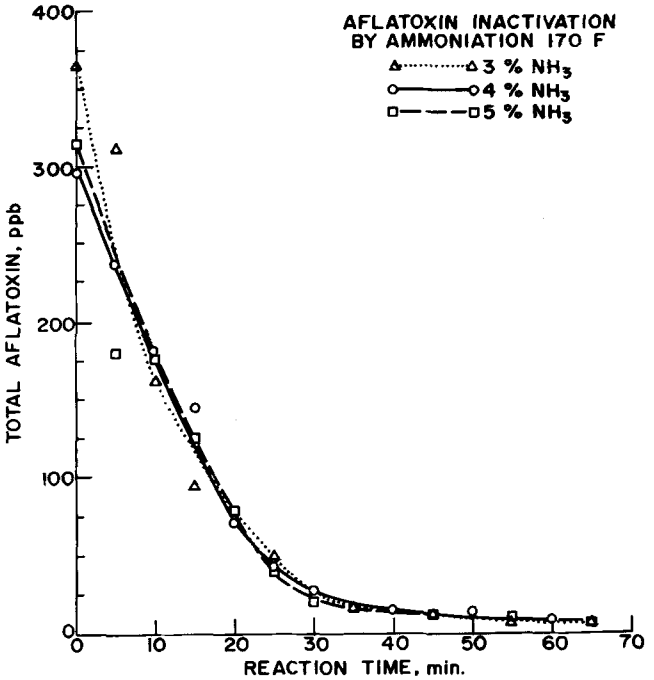

FIG. 4. Effect of temperature (170 F) and ammonia concentration on inactivation of aflatoxins in cottonseed meal.

practical for this work; therefore, all experiments were conducted at this hydration level. The three main process variables considered in this study were ammonia concentration, reactor temperature, and reaction time. Preliminary experiments with ammonia concentrations at the 3, 4 and 5% levels (based on hydrated meal charge) and a temperature of 170 F produced maximum ammonia pressures of 25, 33 and 36 psig, respectively. These parameters appeared to encompass a useful spectrum for investigation and thus constitute the general range of conditions examined in this study.

Figure 3 shows the data obtained from three runs conducted at 160 F with 3, 4 and 5% ammonia concentrations. The peak pressures recorded at these concentrations were 22, 25 and 31 psig, respectively. At this relatively low temperature, after 60 to 65 min processing, neither the 3% nor the 4% ammonia concentrations reduce total aflatoxin levels below 25 ppb. The 5% level appears only slightly more effective, approaching FDA guideline levels of 20 ppb after 65 min of processing. As indicated under "Ammoniation Procedure," the reaction temperatures shown are attained in ca. 30 min and maintained for an additional 30 min for each run; therefore, considerable inactivation of aflatoxins occurs during the "heat up" phase, as demonstrated in Figure 3. In only 10 min, at all three ammonia concentrations and with temperatures below 110 F, aflatoxin concentrations have been reduced a minimum of 100 ppb. Figure 4 shows similar data for cottonseed meal treated with 3, 4 and 5% ammonia, and held at 170 F for 30 min. The peak pressures recorded at these concentrations were 25, 33 and 36 psig, respectively. The curves are remarkably similar and clearly demonstrate the pattern of aflatoxin inactivation that occurs under these ammoniation conditions. Although the aflatoxin is not completely eliminated, the total concentrations remaining in these runs are all below 10 ppb.

Examination of the reduction in aflatoxin content that takes place during the first 5-10 min of processing (before significant reactor temperature has been attained) raised the question of whether such a reduction rate would continue progressively without additional heat. An experi-

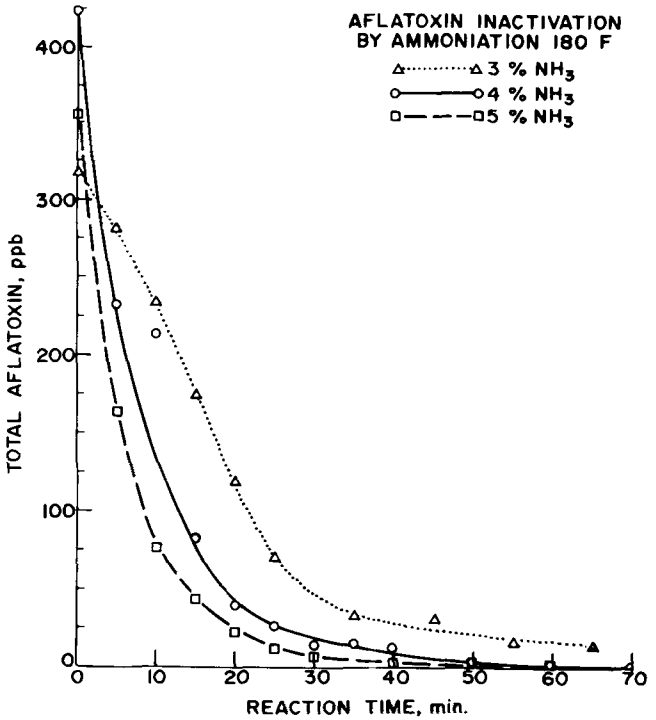

FIG. 5. Effect of temperature (180 F) and ammonia concentration on inactivation of aflatoxins in cottonseed meal.

ment with 3% ammonia without heat was conducted with cottonseed meal containing 302 ppb total aflatoxins. After 5 min of processing in the ammonia atmosphere under ambient conditions, total aflatoxins in the meal were lowered to 258 ppb. However, after 55 min further treatment, the aflatoxin level was lowered only slightly, to 240 ppb.

We believe that the initial rapid reduction in aflatoxins is the result of localized heating of minute areas of meal resulting from adsorption of ammonia. The internal energy of the ammonia molecule is significantly reduced by adsorption, and this energy is released in the form of heat. Very high localized temperatures in the presence of ammonia and aflatoxin inactivate the aflatoxin in the immediate vicinity of the adsorption site. As the heat of adsorption is dispersed throughout the meal particle by conduction, the temperature falls below the level where any further significant reaction can occur. Thus, heat is an essential factor in the inactivation process.

Figure 5 presents data obtained from three runs conducted at 180 F with 3, 4 and 5% ammonia concentrations. The highest pressures recorded in these runs were 27, 38 and 47 psig, respectively. As noted in the previous runs, aflatoxin concentrations drop quickly, and at the 4 and 5% ammonia levels they are reduced to nondetectable in 60 min. All the reaction temperatures in this study were attained slowly (over a period of 30 min) and were then maintained for an additional 30 min. For practical application in a continuous process where temperature conditions may be attained more rapidly, it is quite likely that shorter ammoniation treatment times may be used. In general, it would appear from the data that treatment of aflatoxin-contaminated cottonseed meal, hydrated to a level of 10%, with anhydrous ammonia at 30 psig and 180 F for 30 min, should reduce the aflatoxin content to well below guideline levels of 20 ppb, and in many instances may result in nondetectable levels. These conditions are closely simulated by the 4% ammonia concentration run shown in Figure 5. Table II shows the processing conditions and analytical data for this run and for one reported previously (12), in which higher temperature and pressure were used. Both sets of treatment conditions appear equally effective for inactivating aflatoxins. However, nitrogen solubility, which is frequently considered an index of nutritional quality for ruminants, is reduced nearly 18% with the higher temperature and pressure compared to 12% with the milder conditions. Epsilon amino-free (EAF) lysine values are reduced by 15% with the more rigorous treatment, and by 9% with the less stringent conditions. As expected, nonprotein nitrogen is increased ca. 1.5% with higher temperature and pressure, and ca. 1% with milder conditions.

A subsequent manuscript, in preparation at this Center, will attempt to describe mathematically the effects of ammonia treatment conditions as they relate to detoxification of aflatoxins and to identify the rate-limiting step or steps in the process.

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A.O. Franz, Jr., and L.P. Codifer, Jr. conducted the analyses for aflatoxin.

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TABLE II

Ammoniation Conditions and Analytical Data of Aflatoxin-Contaminated Cottonseed Meal

Ammoniation conditions			Analytical data							
Peak ammonia pressure psig	Peak temperature F	Reaction time[a] min	Total aflatoxins ppb		Nitrogen solubility % (0.02 N NaOH)		Epsilon amino-free lysine g/16 g N		Nitrogen % (MFB)[b]	
			B[c]	A[d]	B	A	B	A	B	A
47	235	38	545	ND[e]	68.2	50.6	2.89	2.45	6.90	8.43
30	180	30	425	ND	66.9	55.0	3.55	3.22	7.40	8.43

[a]At peak conditions.
[b]Moisture-free basis.
[c]Before ammoniation.
[d]After ammoniation.
[e]Nondetectable.

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