were TeA producers, suggest that TeA is responsible for most of the lethal effect observed in laboratory feeding tests of *Alternaria* isolates grown on grain substrates. However, ATX-I also may have contributed to the toxicity we observed. Isolate HRS-5 was a stronger producer of ATX-I than FN-8442-5 which may explain why D-50 and E-50 diets were equally lethal to chicks, and D-50 was more toxic than E-50 to rats, even though E-50 had the higher TeA concentration. Production of ATX-I by *A. alternata* isolates is not always associated with production of TeA.

Alternariols measured in weathered sorghum grain may have been produced by several separate invasions of the maturing seeds and of mature seeds as they were repeatedly wetted and then dried during rainy periods. Most infections probably were actively growing for only a short time and perhaps not long enough for a late-produced metabolite, such as ALT (Burrough et al., 1976), to accumulate appreciably. If TeA and ATX-I are also late-produced metabolites, this would explain the absence of TeA and the trace amounts of ATX-I in the weathered sorghum samples. Meronuck et al. (1972) did not find TeA in a sorghum sample heavily invaded by *Alternaria.* There have been two reports of TeA in crops: in rice plants (Umetsu et al., 1973) and in tobacco plants (Mikami et al., 1971). The steady, high moisture contents of susceptible green plants permits continuous growth of the fungus for many days.

Although no toxic effects were observed from feeding *Alternaria* isolates that do not produce TeA or ATX-I, results of a short-term feeding trial do not answer questions about the effects of long-term ingestion of grain heavily invaded by *A. alternata.*

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", 12th ed, Washington, D.C., 1975, Sect. 7.016.

Burroughs, R., Seitz, L. M., Sauer, D. B., Mohr, H. E., *Appl. Microbiol.* **31**, 685 (1976).

Christensen, C. M., Nelson, C. H., Mirocha, C. J., Bates, F., *Cancer Res.* **28**, 2293 (1968).

Doupnik, B., Sobers, E. K., *Appl. Microbiol.* **16**, 1596 (1968).

Forgacs, J., Carll, T. W., *Adv. Vet. Sci.* **7**, 273 (1962).

Gitterman, C. O., *J. Med. Chem.* **8**, 483 (1965).

Hamilton, P. B., Lucas, C. B., Welty, R. E., *Appl. Microbiol.* **18**, 570 (1969).

Harvan, D. J., Pero, R. W., *Adv. Chem. Ser.* **No. 149** (1976).

Meronuck, R. A., Steele, J. A., Mirocha, C. J., Christensen, C. M., *Appl. Microbiol.* **23**, 613 (1972).

Mikami, Y., Nishijima, Y., Iimura, H., Suzuki, A., Tamura, S., *Agric. Biol. Chem.* **35**, 611 (1971).

Palyusik, I., Szep, A., Szake, F., *Acta Vet. Acad. Sci. Hung.* **18**, 363 (1968).

Pero, R. W., Posner, H., Blois, M., Harvan, D., Spalding, J. W., *Environ. Health Perspect.*, 87 (June 1973).

Seitz, L. M., Mohr, H. E., *Anal. Biochem.* **70**, 224 (1976).

Seitz, L. M., Sauer, D. B., Mohr, H. E., Burroughs, R., *Phytopathology* **65**, 1259 (1975).

Smith, E. R., Fredrickson, T. N., Hadidian, Z., *Cancer* Chemother. *Rep.* **52**, 579 (1968).

Umetsu, N., Kaji, J., Tamari, K., *Agric. Biol. Chem.* **37**, 451 (1973).

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Aflatoxin Inactivation in Corn by Ammonia Gas: Laboratory Trials

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An ammonia (NH_3)–air mixture was recycled at 25 °C through a glass column containing aflatoxin-contaminated, shelled corn until uniform distribution of NH_3 in the corn bed was achieved. After ammoniation and sealed storage at 17.6% moisture and 25 °C for 14 days total reaction time, aflatoxin B_1 level was reduced from 1000 μg/kg (ppb) to 10 μg/kg. Effects of varying the NH_3 addition level, corn moisture, reaction time, initial aflatoxin level in corn, and recycle gas flow rate were investigated. A pilot-scale experiment wherein 4.86 metric tons of 11% moisture corn was treated with 1.1% of NH_3 reduced the corn's B_1 level from 90 μg/kg to a nondetectable level during 7 months of storage indoors. Swine feeding tests on this ammoniated corn gave good results as reported elsewhere. In laboratory experiments, aqua and gaseous NH_3 appeared equally effective for inactivating the aflatoxin in corn.

Various acids, alkalies, oxidizing agents, aldehydes, and nitrogen compounds have been tested for the ability to chemically inactivate the aflatoxin present in contaminated feeds and foods (Dollear, 1969; Mann et al., 1970; Detroy et al., 1971; Goldblatt, 1971; Beckwith et al., 1976). For large-scale detoxification, the chemical with greatest practical potential appears to be ammonia (NH_3). NH_3 inactivation of the aflatoxin in oilseed meals, particularly cottonseed meal, has been reported by Masri et al. (1969), Dollear (1969), Mann et al. (1970, 1971), Gardner et al. (1971), and McKinney et al. (1973). For pilot- and plant-scale tests on cottonseed meal, NH_3 gas under pressure and elevated temperature (typically 30 min at 110–120 °C and 310-345 kPa, i.e., 45–50 psig in the plant test) was used.

This paper reports on laboratory experiments completed at room temperature to study the effect of several process variables upon the degree of aflatoxin inactivation obtained by treating a stationary bed of corn with a recycling mixture of NH_3 and air as proposed by Lancaster et al. (1975). Process variables investigated included: ammonia

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addition level, corn moisture content and distribution, initial aflatoxin content of the corn, recycle gas flow rate and time, and treatment of cracked vs. whole corn.

One pilot-scale test was also made to obtain additional design data and to prepare a quantity of ammoniated corn for a feeding trial to determine animal acceptability of the material.

Under its current action guideline, the Food and Drug Administration (FDA) has set 20 ppb (i.e., μg/kg) total aflatoxin content (B_1, B_2, G_1, G_2) as the maximum permissible in foods and feeds (Rodricks, 1975). Aflatoxin B_1 constituted 83% or more of the total aflatoxin content in a series of corn samples analyzed previously (Brekke et al., 1977a). Based on this proportion of B_1 to the total aflatoxin content and a relative standard deviation (RSD) of 37% for a single aflatoxin determination (Shotwell et al., 1975a), we set 10 μg of aflatoxin B_1/kg of corn as the maximum acceptable level in the ammoniated corn.

EXPERIMENTAL SECTION

Corn. Individual lots of yellow or white dent corn naturally contaminated with aflatoxin B_1 at levels varying from 30 to 1200 μg/kg were used. Total aflatoxin content (B_1, B_2, G_1, G_2) varied from 32 to 1300 μg/kg; aflatoxin B_1 constituted 83 to 94% of the total. Aflatoxin B_1 levels for individual lots are given in subsequent data tables.

NH_3. Liquid anhydrous ammonia, 99.99% minimum purity, was used.

Ammoniation of Corn on Laboratory Scale. Shelled corn (5.1 to 6.1 kg) was tempered to moisture contents ranging from 14 to 20% (wet basis) or used as is at 11 to 13% and then treated with an upflowing ammonia–air mixture in a glass column, 9.7 cm i.d. × 114 cm long. Except for use of a larger column and two or more aquarium air pumps operating in parallel to provide the desired recycle gas flow rate, the equipment and procedure were the same as described by Lancaster et al. (1975).

To determine the distribution of ammonia, moisture, and residual aflatoxin content within the column before and after steady-state conditions were reached, the column was emptied by inverting and tapping it so that the ammoniated corn could be removed as a plug, which was then segmented for analysis.

Temperatures at four points within the corn bed were obtained during ammoniation using a temperature recorder connected to four thermocouples located as near the column center as possible and spaced approximately 23, 48, 73, and 98 cm above the bed support. Total bed depth normally was 102–109 cm. Ammoniation experiments were conducted in a room maintained at 25 °C and 50% relative humidity. Ammonia concentrations in gas leaving the column were determined by the GC procedure described by Lancaster et al. (1975).

Ammoniation of Corn on a Pilot Scale. Approximately 4.68 metric tons (184 bu) of a white dent corn were ammoniated indoors in a closed plywood bin. The corn contained 11% moisture, was U.S. sample grade [9% broken corn and foreign material (BCFM) and 15% total damaged kernels], and had a total aflatoxin content of 110 μg/kg of corn (90 μg of aflatoxin B_1, 16 of B_2, 3 of G_1, and trace of G_2; average of two determinations). The bin measured 2.32 m long × 1.71 m wide × 2.13 m high above the slotted metal false bottom, all inside dimensions. Interior of the bin was painted with epoxy paint, seams were caulked, and the cover was fitted with a sponge rubber gasket. Liquid NH_3 was vaporized in a steam-heated heat exchanger and added to the recirculating NH_3–air mixture. NH_3 was added during the initial 5-h period and a 2-h period near the end of the first 24 h of operation. Analyses of the ammoniated corn indicated a total of 1.1 g of NH_3/100 g of corn dry matter was absorbed. The NH_3–air mixture was recirculated by a rotary lobe gas pump at a rate of 0.71 $m^3\ min^{-1}\ (m^3\ corn)^{-1}$ (i.e., 0.89 cfm/bu) for a period of about 72 h. The gas flow was down through the stationary bed of corn.

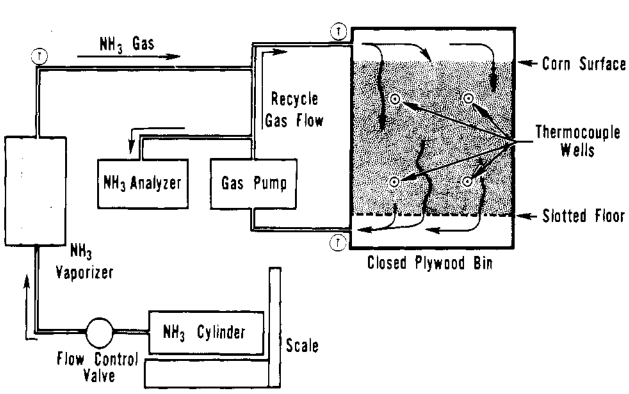

Figure 1. Flow diagram for recycle gas-phase ammoniation of corn in bin. T = location of thermometers.

Eight thermocouples placed four at each of two levels, 28 and 120 cm above the false bottom, were used to record temperatures in the corn bed (150 cm deep) during the experiment.

Figure 1 provides additional information on the equipment and flow used.

Perforated plastic bottles containing approximately 200 g of corn were buried at levels approximately 35, 70, and 100 cm above the false bottom at several places in the bin to check uniformity of NH_3 distribution throughout the corn bed.

After 7 months, corn was removed from the bin in two approximately equal parts through a sidewall port, bagged, and was used for a swine feeding test (Jensen et al., 1977).

Composite samples (23 kg) were prepared from a series of spot samples taken as the bin was filled initially and emptied 7 months later. Composites (7–8 kg) obtained from spot samples taken by probing the bin vertically were used to follow the reduction in aflatoxin content of the corn.

ANALYSES

Ground (approximately 98% through U.S. 20 mesh) ammoniated samples (150 g) were neutralized to pH 5.7 before analysis of 50 g for aflatoxin content (Brekke et al., 1977a). Reported values are based on a single determination (AOAC, 1970, 1972) unless otherwise noted. For a single determination on samples of ground, unammoniated corn, Shotwell et al. (1975a) have reported the RSD as 37%, which decreases to 25% for two determinations and to 15% for five determinations. Sensitivity of the method is 1–3 μg/kg (Shotwell et al., 1975b).

Methods used to determine total nitrogen content and the various forms of ammonia in the corn [i.e., ammoniacal nitrogen (NH_3-N) and water-extracted NH_3] were those of Lancaster et al. (1974) and Uhl et al. (1971). The term "free" ammonia as proposed by Lancaster et al. (1974) is now referred to as water-extracted ammonia (WE-NH_3). Sample size was 20 g. Moisture content of the corn was taken as loss in weight when ca. 200 g of whole kernel corn was heated in a 103 °C forced-draft oven for 72 h (U.S. Department of Agriculture, 1959) or when ca. 10 g of ground corn were heated for 30 min in a Brabender moisture oven set at 130 °C. If ammonia was present, the weight loss is reported as moisture and volatile matter (M & VM). Moisture or M & VM content of the corn is

Table I. Reproducibility among Duplicate Runs and Effect of Several Process Variables

item	run no. A	B	C	D	E	F
corn lot no.	125F	125F	107R	107F	108R	108R
corn moisture, % w.b.	14.3	13.7	15.2	14.0	11.8	11.8
NH_3 added, g/100 g dry matter	2.0	2.0	2.1	2.1	1.5	1.5
recycle gas rate, $m^3\ min^{-1}\ (m^3\ corn)^{-1}$	0.78	0.77	0.46	0.43	0.25	0.25
recycle time, h	24	24	48	48	75	78
NH_3 in corn as % of NH_3 added						
WE-NH_3	55	60	63	70	76	72
NH_3, based on NH_3-N content	56	70	98	90	98	99
NH_3 addition time, h	4.0	4.1	4.3	4.3	8+8	55
NH_3 breakthrough time, h	0.5	0.6		0.6	1.5	3.0
max corn temp during NH_3 addition, °C	34	35	42	31	28	28
aflatoxin reaction conditions						
temp, °C	25	25	25	25	25	25
time, days	30	30	60	30	60	39
aflatoxin B_1 content, μg/kg						
initial	1000	1000	120	150	370	370
final	16	12	ND[a]	5	14	11

[a] None detected.

reported on wet basis (w.b.) and ammonia content on dry basis (d.b.).

Corn samples were graded by the USDA standard procedure (U.S. Department of Agriculture, 1970).

Safety Aspects. Because aflatoxin can act as a potent toxin, carcinogen, teratogen, and mutagen (Ciegler, 1975), certain safety precautions should be followed. Dust created in grinding contaminated samples should be collected and disposed of properly; respirators should be used to avoid breathing contaminated dust; protective clothing such as disposable gloves and laboratory coats should be worn; and exposed skin, laboratory surfaces, and equipment should be decontaminated. Information on decontamination is given by Goldblatt (1969) and Detroy et al. (1971).

Handlers and users of ammonia are or should be familiar with its characteristics and recommended safety practices. Such information is available in the literature and need not be reviewed here.

RESULTS AND DISCUSSION

All results relate to the laboratory studies unless specified for the pilot-scale experiment.

Reproducibility of Repetitive Runs. Excellent reproducibility in residual aflatoxin content was obtained among four ammoniation runs, all of which were made on portions taken from one lot of corn that had been preconditioned to 17.6% moisture. For the treatment, 1 g of NH_3 was added per 100 g of corn dry matter and the NH_3-air mixture was recycled for 24 h at the rate of 0.78 $m^3\ min^{-1}\ (m^3)^{-1}$ corn before the column was emptied. The contents were stored at 25 °C, and after a 30-day reaction period (including 1 day in the column), aflatoxin B_1 content for three of the samples had been reduced from 1000 to 4.9, 4.1, and 4.9 μg/kg (SD = 0.7) for three successive runs. After a 15-day reaction period, the fourth sample assayed 6.2 μg/kg. Samples taken immediately after the column was emptied in the first three runs had an average NH_3-N content of 0.74% d.b. (SD = 0.04) and an average WE-NH_3 content of 0.56% d.b. (SD = 0.07).

While reproducibility in the runs discussed above was exceptionally good, it was also satisfactory in the other runs—even when the sublots were individually moisture conditioned. Comparable values were obtained in paired runs when the corn was analyzed for aflatoxin B_1, WE-NH_3, and NH_3-N (Table I). The table also includes data on the process conditions and the percent NH_3 accounted for in the corn. The NH_3 can be added over varying periods of time (compare run no's. E and F with others).

Table II. Effect of Recycle Gas Flow Rate and Recycle Time Upon Gradient of Water-Extracted Ammonia in Corn within Column

flow rate				
m^3 gas min^{-1} $(m^3)^{-1}$ corn	cm/min[b]	cfm/bu	recycle time, h	WE-NH_3 gradient,[a] %
0.072	8	0.09	336	24
0.25	28	0.31	24	27
0.25	28	0.31	47	18
0.25	28	0.31	60	7
0.25	28	0.31	75	3[c]
0.45	50	0.56	48	5[d]
0.78	86	0.96	24	5[e]

[a] Water-extracted ammonia. Gradient is based on maximum difference between column segments divided by weighted average. Three column segments were analyzed. [b] Superficial velocity through empty column. [c] Average of three experiments. [d] Average of four experiments. [e] Average of two experiments.

Effect of Recycle Gas Flow Rate and Recycle Time upon Uniformity of Ammonia Distribution in Corn Bed. Recycle gas flow rates of 0.07, 0.25, 0.45, and 0.78 $m^3\ min^{-1}\ (m^3\ corn)^{-1}$ (ca. 0.1, 0.3, 0.6, and 1.0 cfm/bu) were used in the experimental tests. With the lowest flow rate, uniform NH_3 distribution (i.e., column gradient $\leq$10%) was not attained even after a 336-h recycle period (Table II). With a flow rate of 0.25 $m^3\ min^{-1}\ (m^3)^{-1}$, 60 h was required to bring the column gradient down to 7%. The gradient remained at about 5% when the gas was recycled at 0.45 $m^3\ min^{-1}\ (m^3)^{-1}$ for 48 h or 0.78 $m^3\ min^{-1}\ (m^3)^{-1}$ for 24 h. These data indicate that flow rates of 0.25 to 0.78 $m^3\ min^{-1}\ (m^3)^{-1}$ can be used in the column when the recycle period is made sufficiently long to obtain adequate ammonia distribution. No investigation was made of the effect of bed depth-to-diameter ratio upon the minimum acceptable gas velocity.

Detoxification of Corn in a Bed Having Two Moisture Levels. The need for conditioning the entire corn bed to a uniform moisture content for good detoxification was demonstrated in two runs wherein separate zones of the corn bed differed in their moisture content. Average moisture content of the bed was 16% in each run. In one run, corn of 20.5% moisture content was in the top half of the column and 11.2% in the bottom half; the zones were reversed for the second run. Some redistribution of

Table III. Detoxification of Corn With Two Moisture Zones Within the Stationary Bed[a]

constituent	location of high-moisture corn within column: top half	location of high-moisture corn within column: bottom half
final M & VM, % w.b.		
top quarter	20.8	12.1
third quarter	18.5	12.7
second quarter	12.8	17.9
bottom quarter	14.5	19.6
average[b]	16.6	15.8
WE-NH_3, % d.b.		
top quarter	1.60	0.97
third quarter	1.58	1.13
second quarter	1.07	1.45
bottom quarter	1.17	1.74
average[b]	1.36	1.34
ammoniacal N, % d.b.		
top quarter	1.52	1.07
third quarter	1.51	1.20
second quarter	1.13	1.49
bottom quarter	1.17	1.50
average[b]	1.34	1.34
aflatoxin B_1 content, μg/kg[c]		
top quarter	7	150
third quarter		100
second quarter	120	12
bottom quarter	28	
average[b]	42	60

[a] Corn moisture was adjusted to 11.2 or 20.5%, column loaded with approximately equal quantities (dry weight basis) of corn tempered to the specified moisture levels, then 2.0 g of NH_3 added per 100 g of dry matter in corn. After ammonia-air mixture was recycled for 48 h at rate of 0.52 m^3 min^{-1} (m^3 $corn)^{-1}$, column contents were removed in segments and sampled for content of total volatiles and ammonia. Remainder of contents was held at 25 °C for an additional 28 days before sampling for aflatoxin analyses. [b] Weighted. [c] Initial aflatoxin B_1 content was 1000 μg/kg.

moisture, based on M & VM values, occurred during the recycle operation (Table III). The quarter-column segments remained near their initial moisture values but limited migration of moisture from high-moisture to low-moisture corn was evident. Average values for WE-NH_3 and NH_3-N contents of the high- and low-moisture zones are in good agreement for the two runs. Because WE-NH_3 and NH_3-N contents of the individual column segments differed and their levels followed that of the total volatiles pattern, differences in the residual aflatoxin B_1 content of the column segments should be expected. However, the extent of these differences was considerable. Both portions of the high-moisture corn were reduced to low aflatoxin levels. Residual aflatoxin content of the four segments of low-moisture corn varied over a fivefold range. Among these four segments, the greatest aflatoxin reduction occurred in the segment whose volatiles content increased from 11.2 to 14.5%. Thus, these data demonstrate that moisture has a strong effect upon the NH_3–aflatoxin reduction. The large aflatoxin differences also demonstrate amply the need for uniform kernel-to-kernel moisture conditioning for the best detoxification results in a stationary bed.

Table IV. Effect of Corn Moisture and Ammonia Addition Level Upon Residual Aflatoxin B_1 Content[a]

NH_3 added, % d.b.	corn moisture, % w.b.: 14	17	20
	B_1 content, μg/kg		
0.5	77	23	16
1.0	45	6[b]	8
1.5	24	9[c]	14
2.0	11	11	12

[a] Initial aflatoxin B_1 content was 1000 μg/kg of corn. Ammonia-air mixture was recirculated through the column for 48 h at rate of 0.78 m^3 gas min^{-1} (m^3 $corn)^{-1}$. After 48 h, column contents were removed and held at 25 °C for an additional 12 days. [b] Interpolated from value of 4 μg/kg at 22 days. [c] Average of two runs.

Table V. Response of Various Contaminated Corn Lots to Uniform Ammoniation Treatment[a]

lot no.	total time, days	aflatoxin B_1, μg/kg: initial	aflatoxin B_1, μg/kg: final
102	30	140	ND
103	30	140	ND
107	30	150	5
123	48	170	ND
108	30	220	6
104	30	260	11
105	61	450	ND
125	30	1000	10[b]
124	30	1200	10

[a] Corn moisture was adjusted to 14% wet basis, then 2.0 g of ammonia gas was added per 100 g of dry matter in corn. Ammonia-air mixture was recycled through the corn bed at rate of 0.45 m^3 min^{-1} (m^3 $corn)^{-1}$ for 48 h, then column contents were removed and held at 25 °C for an additional period. [b] Average of four runs; SD = 4.5.

Effect of Corn Moisture and Ammonia Levels on Aflatoxin Inactivation. In a 4 × 3 factorial experiment, the aflatoxin content decreased sharply with increasing moisture content when 0.5% NH_3 was added to the corn (Table IV). Likewise, with the corn moisture held at 14%, progressive increases in the ammonia content gave successive decreases in the residual aflatoxin B_1 content. However, with all other conditions tried, the residual aflatoxin B_1 content leveled off at 10 μg/kg (SD = 3). A similar effect with essentially the same aflatoxin concentration (9 μg/kg) was observed in an earlier study when this lot of corn was treated with the same levels of NH_3 added as NH_4OH at corn moistures of 15 to 20% and held 2 days at 40 °C (Brekke et al., 1977a).

Reduction in Aflatoxin Content of Ammoniated Corn with Time. Ammoniation results in a rapid initial reduction in aflatoxin content but the rate falls off with time. For example, a quantity of corn was adjusted to 17.6% moisture content, then 1% NH_3 (dry corn basis) was added with recycle gas recirculation continued for 48 h after which the column contents were emptied and held at 25 °C. In 2 days the aflatoxin B_1 content fell from 1000 μg/kg initially to 32 μg/kg and in another 12 days the level fell to 6 μg/kg. These results are in agreement with those obtained when this same lot of corn was treated with NH_4OH (Brekke et al., 1977a).

Reduction in Aflatoxin Content among Various Corn Lots Given a Uniform Ammonia Treatment. Table V shows the reduction of aflatoxin levels of 14% moisture corn treated with 2% NH_3. Lots with an initial aflatoxin B_1 content of 200 μg/kg or less were reduced to a range of none detected (ND) to 5 μg/kg. Lots with higher aflatoxin contents proved a little more difficult to detoxify as indicated by the higher residual level and all the decontaminated lots except for no. 104 were at or below the residual aflatoxin B_1 limit we set. Ammoniation at a higher moisture content presumably would have lowered lot 104 to 10 μg/kg or less.

Table VI. Changes in Nitrogen Contents of Corn with Ammoniation Level[a]

added		found				
NH_3 (1)	N (2)	WE-NH_3[b] (3)	NH_3-N (4)	total N (5)	total N expected (6)	col. 6-5 (7)
0	0.0	0.0	0.0	1.28	1.28	0
0.5	0.41	0.24 ± 0.04	0.39 ± 0.08	1.80 ± 0.01	1.69	−0.11
1.0	0.82	0.62 ± 0.02	0.74 ± 0.05	2.27 ± 0.08	2.10	−0.17
1.5	1.23	0.87 ± 0.04	1.06 ± 0.03	2.46 ± 0.12	2.51	+0.05
2.0	1.64	1.20 ± 0.11	1.22 ± 0.04	2.70 ± 0.06	2.92	+0.22

[a] All values are reported as g/100 g of corn dry matter. Means and standard deviations for nitrogen analyses are from runs made with corn moisture levels of 14, 17, and 20%. [b] Determinations made on whole grain.

Treatment of Shelled vs. Cracked Corn. Brekke et al. (1977a) reported that one of the corn lots tested proved to be appreciably more resistant to detoxification than others of generally equal aflatoxin B_1 content when treated under similar conditions with NH_4OH. There was some indication that this same lot again resisted inactivation when subjected to gas-phase ammoniation (no. 104, Table V). When the corn was coarsely cracked, however, the residual aflatoxin B_1 content was reduced to an ND level by gaseous NH_3. The increased surface area and direct exposure of the more heavily contaminated kernel components such as the germ to the NH_3 gas presumably accounts for the better detoxification. While cracking the corn improved the degree of inactivation, cracking is not practical because of the increased and probably nonuniform bed resistance if fines and smaller pieces are not distributed uniformly throughout the stationary bed of corn. Such conditions could lead to gas channelling and nonuniform detoxification during large-scale ammoniation.

Related Process Information. The effect of temperature upon aflatoxin inactivation in corn treated with gaseous NH_3 was not studied but the effect is considerable for corn treated with NH_4OH (Brekke et al., 1977a). The effect is presumed to be comparable for both methods of NH_3 addition.

Lancaster et al. (1975) reported on the varying and irregular temperature change observed throughout a stationary bed of corn during an ammoniation run. For the conditions outlined in Table IV, and with the NH_3 added at a uniform hourly rate, maximum temperature observed in the corn bed was 29 °C when 0.5% NH_3 was added, 34 °C for 1%, and 35–36 °C for 1.5 and 2.0% NH_3, regardless of the initial corn moisture level (room temperature was 25 °C). The observed maximum temperature always occurred in the lower quarter or half of the bed.

After a period of total absorption, NH_3 concentration in the recycle gas at the column outlet climbed rapidly while NH_3 was being added, usually reached a maximum shortly after all NH_3 had been added, and then fell about as rapidly as it rose (Figure 2). With other variables held constant, the maximum gas concentration increased as NH_3 addition level increased and decreased as corn moisture content increased. For example, for 14% moisture corn, the values were 5, 12, 15, and 19 vol % when 0.5, 1.0, 1.5, and 2.0% NH_3, respectively, were added. For 1% NH_3 addition, the values were 12, 2.8, and 2.2% at corn moistures of 14, 17, and 20%, respectively. Variables such as rate of NH_3 addition and recycle gas flow rate also had some influence.

NH_3 concentration in gas entering the corn bed was about 8.4 vol % above that in gas leaving the bed. The explosive range for ammonia in air is 15–28 vol % (Manufacturing Chemists' Association, 1972), so measures should be taken not to exceed the lower explosive limit.

Increasing the corn moisture increased the stickiness of the ammoniated corn. Increasing the NH_3 addition level had a similar but lesser effect. Similarly, some stickiness or caking of the corn was noted previously when corn was treated with NH_4OH (Brekke et al., 1977a).

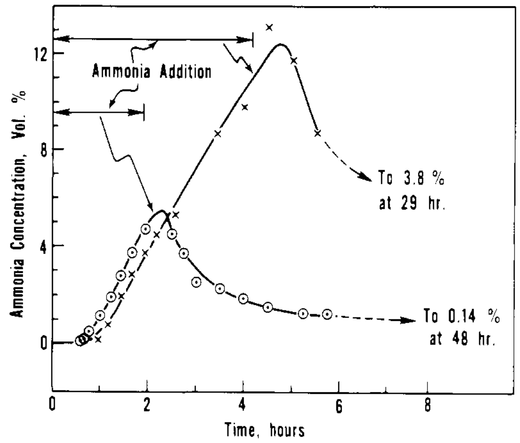

Figure 2. Ammonia concentration in recycle gas at column outlet: (—o—) 1% NH_3 added with recycle gas flow rate of 0.78 m^3 gas min^{-1} (m^3 corn)$^{-1}$; (—×—) 2% NH_3 added with recycle gas flow rate of 0.56 m^3 gas min^{-1} (m^3)$^{-1}$ corn. Corn moisture was 14.2%.

A slight amount of condensate formed in the recycle vapor line for some runs. The effect was greatest at the highest corn moisture and ammoniation levels. In most runs the condensate reevaporated before the recycle period ended.

Caking of the corn and condensation of moisture as NH_4OH were two problems encountered in the field trial (Brekke et al., 1976).

Nitrogen Contents of Ammoniated Corn. As more ammonia was added to the corn, increases occurred in WE-NH_3, NH_3-N, and total nitrogen contents of the corn (Table VI). Corn moisture content, however, had no effect on any of these forms of nitrogen in the ammoniated corn. Amount of NH_3 recovered as WE-NH_3 approximated 50–60% of that added. The difference between NH_3 added and WE-NH_3 is taken as a combination of fixed ammonia and ammonia lost in sample collection and preparation. NH_3-N content in neutralized samples of the corn rose in successive increments of 0.39, 0.35, 0.32, and 0.16% as against addition increments of 0.41%. No explanation is apparent for these decreases although normal precautions were taken to minimize NH_3 losses in sample collection and preparation. However, differences between total N expected and the amount found are within the limits of experimental error. Possibly some of the added nitrogen was no longer present in the ammoniacal form.

Aflatoxin Inactivation in Pilot Experiment. A corn sample obtained on the fifth day by multiple vertical probes of one-half the bin assayed 10 μg of aflatoxin B_1/kg of corn; a similar sample taken 30 days later assayed 8 μg/kg. The initial B_1 content was 90 μg/kg. A composite sample taken from each half when the bin was emptied

Table VII. Analysis of Corn Before and After Ammoniation in Bin[a]

constituent	untreated corn	ammoniated corn[b] first half	ammoniated corn[b] second half
moisture	11.0	10.4	10.3
total nitrogen	1.67	1.95	1.92
ammoniacal nitrogen	0.00	0.37	0.35
water-extracted ammonia			
whole grain	0.00	0.32	0.22
ground grain		0.43	0.32
reducing sugars	0.28	0.08	0.08
soluble solids	6.0	7.4	7.4
crude fat	4.3	4.3	4.2
fat composition[c]			
palmitic	14.8	14.8	14.8
stearic	1.3	1.4	1.8
oleic	31.1	30.0	32.3
linoleic	52.8	42.4	44.1
linolenic	0.1	0.1	0.4
not eluted	0.0	11.3	6.6

[a] All results reported on % dry basis, except moisture is on wet basis. [b] After 7 months of storage. [c] As % of total fatty acids. Palmitic acid content of ammoniated corn samples was normalized to 14.8%. Not eluted values represent portion of sample not eluted from chromatographic column during analysis.

had no detectable level of aflatoxins B_1, B_2, G_1, or G_2. As shown above and in a previous study (Brekke et al., 1977a), the corn's low-moisture content (11%) accounted for the relatively slow rate of inactivation but in this case did not prevent the aflatoxin content from being reduced to a ND level.

NH_3 Distribution in Corn in Bin. Mean value for WE-NH_3 content of the 17 samples in the perforated plastic bottles removed after 5 days was 0.69% d.b. (RSD = 2.4%), and mean value for the NH_3-N content was 0.92% d.b. (RSD = 3.7%). These results demonstrate that good NH_3 distribution occurred throughout the bin in spite of the 9% of BCFM present.

From the NH_3-N values, which other unreported studies have shown change very little over a 5-day period, the amount of NH_3 absorbed by the corn was calculated as 1.1 g/100 g corn dry matter.

Changes in Chemical Composition of Corn from Bin. Ammoniation increased the nitrogen content of the ground samples by 0.25–0.28 percentage points (Table VII), which could be the equivalent of an additional 1.4–1.6% protein (N × 5.7) if the corn were fed to ruminants. Both the whole grain and ground samples contained appreciable amounts of WE-NH_3, with a larger amount being extracted from the ground corn.

In a related study, Johanning et al. (1978) reported that during in vitro rumen-inoculated incubations, ammonia was released at a slower and more desirable rate from ammoniated corn than from an isonitrogenous mixture of unammoniated corn plus ammonium bicarbonate. They also reported that the starch component in ammoniated corn was more completely degraded by amyloglucosidase and thus was made more available in related biological reactions than the starch in unammoniated corn.

The ammoniated corn showed an increase in NH_3-N and soluble solids contents. A reduction occurred in amount of reducing sugars (reported as glucose) and linoleic acid content of the fat extractable by petroleum ether. The chemical changes are in agreement with those observed in a field trial gas-phase ammoniation of corn (Brekke et al., 1976) and in corn treated with NH_4OH (Brekke et al., 1977a) or with high-moisture corn treated with both NH_4OH and gaseous NH_3 (Peplinski et al., 1978). Black et al. (1978) have observed that air (oxygen) in sufficient quantity must be present for a reduction to occur in linoleic acid content of the ammoniated corn.

Table VIII. Comparative Effectiveness of Gaseous and Aqueous Ammonia for Inactivation of Aflatoxin in Corn at 25 °C

NH_3 added[a]	NH_3 form	corn moisture, % w.b.	reaction time, days	residual aflatoxin B_1[b] µg/kg
0.5	gas	16.7	14	23
	aqueous	17.5	14	30[c]
1.0	gas	19.7	14	8
	aqueous	20.0	8	7
1.5	gas	16.7	15	8
	aqueous	17.5	8	15
2.0	gas	16.7	15	11
	aqueous	17.5	8	11

[a] Gram per 100 g of corn dry matter. [b] Initial aflatoxin B_1 content = 1000 µg/kg. [c] Extrapolated from 43 µg/kg at 8 days based upon Figure 1 (Brekke et al., 1977a).

No significant changes occurred in the contents of starch, ash, crude fiber, or nonreducing sugars.

Ammoniation changed the corn's color from natural white to a light tan.

At the low-moisture content at which this corn was ammoniated, no caking problem developed as it did in the field trial with 17.5% moisture corn, and the low-moisture corn flowed freely upon removal from the bin.

Feeding Results. When ammoniated, undeodorized corn from the bin was fed to swine, good performance was obtained when the rations contained ground corn but not when the grain was fed in whole kernel form (Jensen et al., 1977). In a related study, rainbow trout grew satisfactorily on a diet containing corn treated with aqueous NH_3 to inactivate aflatoxin (Brekke et al., 1977b).

Inactivation by Aqueous or Gaseous Ammonia. Comparative Effectiveness. Several selected laboratory-scale experiments made with NH_3 levels and corn moistures in the range that might be used in a commercial operation show that at 25 °C, the two forms of NH_3 gave essentially comparable levels of detoxification (Table VIII). Thus, the choice as to type of NH_3 to apply depends in part upon material handling problems. Gaseous application requires an essentially gas-tight system, recycle blower and piping, and NH_3 vaporizer, and the corn must be moisture conditioned before NH_3 is added. Also, heat input from the blower can help elevate or maintain the corn temperature, a beneficial factor because the reaction is quite temperature sensitive (Brekke et al., 1977a).

Relatively simple equipment can be used with NH_4OH. The solution can be sprayed onto the corn as it moves through a screw conveyor. Because of run-off a maximum of only about 1% NH_3 can be applied in one pass and addition of larger amounts will require two or more passes. Corn freshly wetted with NH_4OH will rapidly lose NH_3 upon exposure to air but holding the wetted corn in a closed container for 0.5–4 h will bring the NH_3 losses down to or near practical levels during transfer of the corn. Use of a closed holding tank after each pass is recommended before more NH_3 is added or the corn transferred. Such a tank can be designed for continuous feed and withdrawal of the corn.

Use of NH_4OH also adds moisture to the corn, and thus the moisture conditioning and ammoniation steps can be combined provided they give the desired final moisture content. Ammoniation at temperatures above ambient would require some means for heating the corn.

CONCLUSION

Gaseous NH_3 effectively detoxified aflatoxin B_1 in corn to low residual levels when the proper conditions were used. The data demonstrate the need for adequate and uniform moisture conditioning of the corn for uniform detoxification. The data also indicate there is a degree of flexibility among various factors such as recycle gas flow rate and recirculation time to achieve uniform NH_3 distribution throughout the stationary corn bed and between corn moisture and NH_3 addition level to achieve detoxification.

Information gained from these experiments and prior NH_4OH experiments (Brekke et al., 1977a) was used to select conditions subsequently employed for a field trial (Brekke et al., 1976) wherein gaseous ammoniation of a 1000-bu lot of corn reduced its aflatoxin B_1 content from 750 to 7 μg/kg.

The information developed parallels that reported by Masri et al. (1969) for inactivation of aflatoxin in oilseed meals by ammoniation at ambient conditions.

Approval by the FDA of ammoniated corn and the specific ammoniation process is required before the USDA can give its recommendation. Feeding and laboratory tests are underway to obtain such approval for the gas-phase ammoniation process and product.

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LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", 11th ed, Sec. 26.015-26.020. Washington, D.C., 1970; *J. Assoc. Off. Anal. Chem.* **55**, 426 (1972).

Beckwith, A. C., Vesonder, R. F., Ciegler, A., *Adv. Chem. Ser.* **No. 149**, 58–67 (1976).

Black, L. T., Spencer, G. F., Brekke, O. L., *J. Am. Oil Chem. Soc.* **55**, 526 (1978).

Brekke, O. L., Peplinski, A. J., Lancaster, E. B., *Trans. ASAE* **20**, 1160 (1977a).

Brekke, O. L., Peplinski, A. J., Nofsinger, G. W., Conway, H. F., Montgomery, R. R., Silman, R. W., Sohns, V. E., Bagley, E. B., Paper No. 76-3526 presented at the meeting of the American Society of Agricultural Engineers, Chicago, Ill., Dec 1976.

Brekke, O. L., Sinnhuber, R. O., Peplinski, A. J., Wales, J. H., Putnam, G. B., Lee, D. J., *Appl. Environ. Microbiol.* **34**(1), 34 (1977b).

Ciegler, A., *Lloydia* **38**, 21 (1975).

Detroy, R. W., Lillehoj, E. B., Ciegler, A., "Microbial Toxins, Vol. VI, Fungal Toxins", Ciegler, A., Kadis, S., Ajl, S. J., Ed, Academic Press, New York, N.Y., 1971.

Dollear, F. G., "Aflatoxin-Scientific Background, Control, and Implications", Goldblatt, L. A., Ed., Academic Press, New York, N.Y., 1969, pp 378–383.

Gardner, H. K., Jr., Koltun, S. P., Dollear, F. G., Rayner, E. T., *J. Am. Oil Chem. Soc.* **48**, 70 (1971).

Goldblatt, L. A., *J. Am. Oil Chem. Soc.* **48**, 605 (1971).

Goldblatt, L. A., "Aflatoxin-Scientific Background, Control and Implications", Goldblatt, L. A., Ed., Academic Press, New York, N.Y., 1969.

Jensen, A. H., Brekke, O. L., Frank, G. R., Peplinski, A. J., *J. Anim. Sci.* **45**(1), 8 (1977).

Johanning, G. L., Muhrer, M. E., Bagley, E. B., Brekke, O. L., University of Missouri, Columbia, and Northern Regional Research Center, SEA, U.S. Department of Agriculture, Peoria, Ill., 1978.

Lancaster, E. B., Hall, G. E., Brekke, O. L. *Trans. ASAE* **17**, 331 (1974).

Lancaster, E. B., Stringfellow, A. C., Brekke, O. L., *Trans. ASAE* **18**, 1158 (1975).

McKinney, J. D., Cavanagh, G. C., Bell, J. T., Hoversland, A. S., Nelson, D. M., Pearson, J., Selkirk, R. J., *J. Am. Oil Chem. Soc.* **50**, 79 (1973).

Mann, G. E., Gardner, H. K., Jr., Booth, A. N., Gumbmann, M. R., *J. Agric. Food Chem.* **19**, 1155 (1971).

Mann, G. E., Codifer, L. P., Jr., Gardner, H. K., Jr., Koltun, S. P., Dollear, F. G., *J. Am. Oil Chem. Soc.* **47**, 173 (1970).

Manufacturing Chemists' Association, "Guide for Safety in the Chemical Laboratory", 2nd ed, Van Nostrand Reinhold Co., New York, N.Y., 1972, p 183.

Masri, M. S., Vix, H. L. E., Goldblatt, L. A., U.S. Patent 3 429 709 (1969).

Peplinski, A. J., Brekke, O. L., Bothast, R. J., Black, L. T., to be published by *Trans. ASAE.*

Rodricks, J. V., *Proc. Am. Peanut Res. Educ. Assoc.* **7**(1), 47 (1975).

Shotwell, O. L., Kwolek, W. F., Goulden, M. L., Jackson, L. K., Hesseltine, C. W., *Cereal Chem.* **52**, 373 (1975a).

Shotwell, O. L., Shannon, G. M., Hesseltine, C. W., *Cereal Chem.* **52**, 381 (1975b).

Uhl, D. E., Lancaster, E. B., Vojnovich, C., *Anal. Chem.*, **43**, 990 (1971).

U.S. Department of Agriculture, Service and Regulatory Announcement No. 147, Air-Oven (103 °C) Method for Corn and Beans, Washington, D.C., 1959.

U.S. Department of Agriculture, Consumer and Marketing Service, Grain Division, Official Grain Standards of the United States, Washington, D.C., revised Feb 1970.

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