

# Ammonia Kills Spoilage Molds in Corn Purchased by U. S. Dept. of Agriculture for Official Use

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### Abstract

Corn containing 26% and 12% moisture were treated with ammonia at 2% and .5% of corn dry weight, respectively. Ammoniation at both concentrations eliminated external and infecting molds and yeasts and tended to reduce bacterial counts. Molds killed were species of *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, and *Rhizopus*. In feeding trials, mice showed no preference between tempered and 2% ammoniated corn that initially had a pungent odor, but mice consumed more control corn than corn ammoniated at .5%.

tion the treatment has never been popular because it is difficult to control ammonia generation, and sensitive citrus fruits show darkened skin and injured stem ends (7). Eno et al. (8) reduced populations of fungi, bacteria, and nematodes in Florida sand with anhydrous ammonia. Neal et al. (16) stated that  $NH_3$  rapidly killed sclerotia of *Phymatotrichum omnivorum*, McCallan and Setterstrom (15) demonstrated that  $NH_3$  is toxic to many fungi, and others reported similar results (3, 4, 9, 19, 23). Therefore, our research was to determine the feasibility of using ammonia as a fungicide on shelled corn.

### Materials and Methods

Nine kilograms of U.S. Grade 5 white corn (12% moisture) from the bootheel area of Missouri were sealed in a 38-liter, sterile, stainless steel drum. Corn was tempered to 26% moisture with sterile water at 26 C for 24 hr, and 2.0%  $NH_3$  (w/w) was added as ammonium hydroxide to bring final moisture content of grain to 30%. Ammoniated corn was held 14 days at the same temperature. Samples of original corn, corn after 24 hr of tempering, 1 hr after ammoniation, and 14 days after ammoniation were removed for microbiological examination. Analyses for total aerobic bacteria, molds and yeasts, and infecting or in-

### Introduction

Studies at the Northern Regional Research Laboratory are being conducted to destroy aflatoxin in naturally contaminated corn using aqueous or gaseous ammonia. Preservation and storage of high-moisture corn have concerned the farmer and elevator operator. Concern has intensified with advent of the picker-sheller and artificial drying facilities, which have stimulated harvesting high-moisture corn (20 to 35% moisture) to reduce field losses. Corn at these moistures is subject to mold spoilage and mycotoxin formation unless it is rapidly dried. (An estimated 8.8 billion liters of corn is lost in the United States every year (5).) Methods used to prevent mold growth include drying, low-temperature storage, reduced oxygen content of storage atmosphere, and applying organic acids (acetic, propionic, formic, and sorbic). However, the fungicidal properties of ammonia on corn have never been investigated.

Bottini (2) reported that ammonia gas prevented decay of citrus fruits. Since, there has been much research on fumigation of citrus fruits with ammonia to control such molds as *Penicillium funiculosum*, *P. italicum*, *P. digitatum*, *Rhizopus nigricans*, and *Diplodia natalensis* (6, 10, 11, 12, 14, 17, 18, 21, and 22). Despite effectiveness of ammonia fumiga-

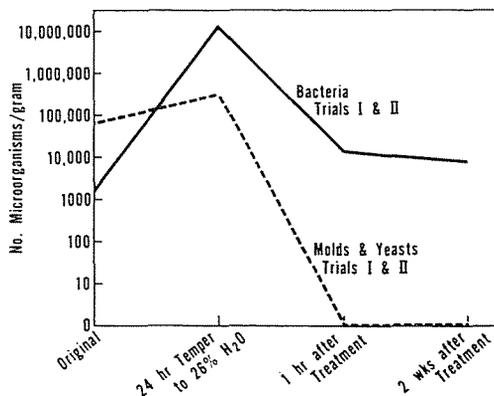


FIG. 1. The influence of applying 2% ammonia to high-moisture corn on microbial growth after 24 hr of tempering. Fungi (dotted line) were eliminated; bacteria (solid line) did not proliferate.

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ternal fungi were the procedures of Bothast et al. (1). In a second experiment, replicate treatments were made with 2.3 kg of original corn treated with .5%  $\text{NH}_3$  (w/w) as ammonium hydroxide and sampled after 1 hr and after 14 days. The first experiment was repeated on this smaller scale.

Ammoniated corn was fed to mice in two 1-wk preference trials. Twelve mice (4 to 8 wk old, nine males and three females) were put in four cages (three mice per cage, same sex in each cage) and given a daily choice of 50 kernels of ammoniated corn from the first experiment and 50 kernels of untreated tempered

corn in separate containers. Number of kernels consumed per day from each container was recorded. Analysis of variance and chi square tests (20) determined treatment differences. The second trial was identical to the first except for the mice (4 to 8 wk old, six males and six females) and the feed (.5% ammoniated and original 12% moisture corn).

### Results

Effect of tempering and subsequent effect of 2.0%  $\text{NH}_3$  on the microflora of corn are in Fig. 1. The original corn contained  $2.0 \times 10^3$

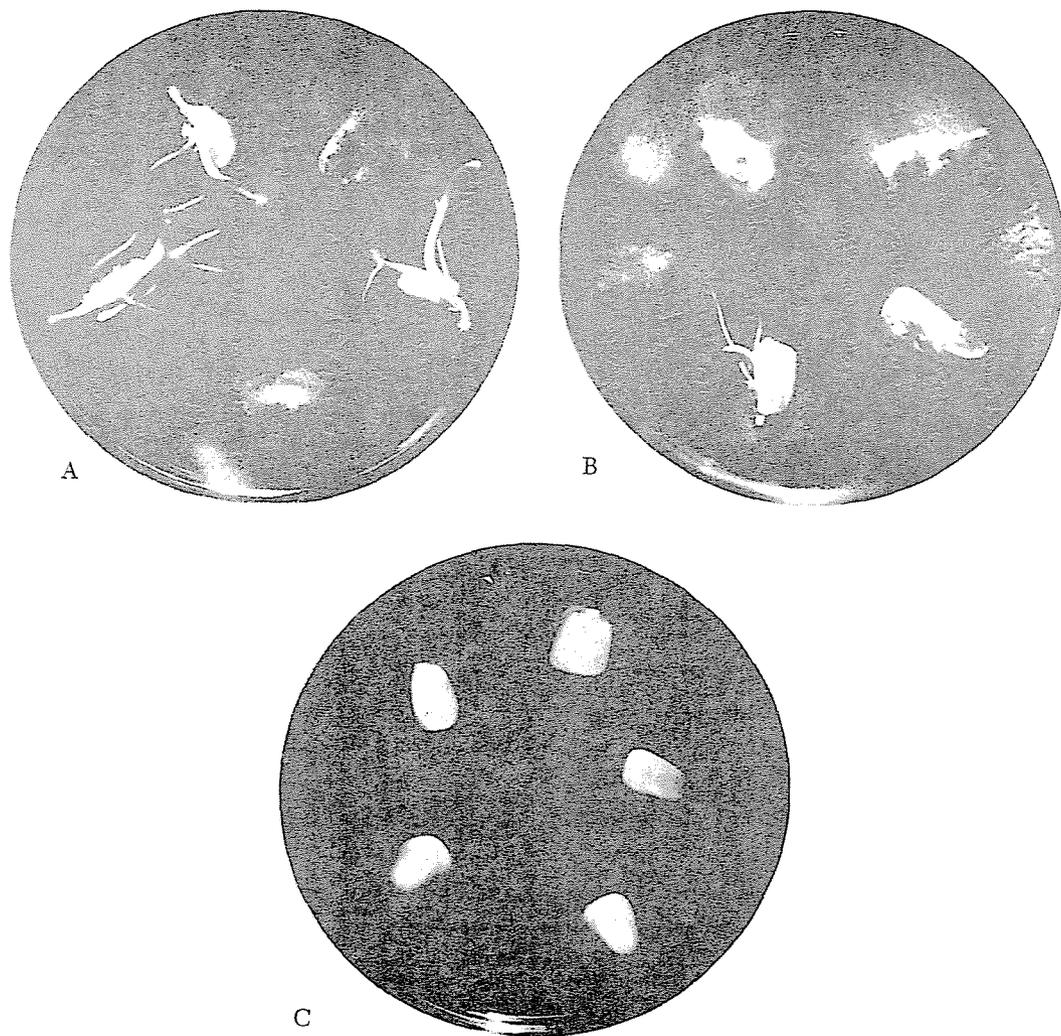


FIG. 2. Mold growth from surface-sterilized corn kernels after 5 to 7 days of incubation at 28 C. A, corn containing 12% moisture and not treated with ammonia; B, corn tempered to 26% moisture; and C, application of .5% ammonia which destroyed fungi and prevented germination. Same results with 2% ammonia.

bacteria per gram and  $7.9 \times 10^4$  molds and yeasts per gram. From tentative identification of each mold colony on dilution plates counted, species of *Fusarium* composed 75% of external mold population; the remainder was predominantly *Aspergillus flavus* and species of *Penicillium*. After 24 hr of tempering to 26% moisture, bacteria increased to  $1.4 \times 10^7$  per gram and molds and yeasts increased to  $5.1 \times 10^5$  per gram. But 1 hr after 2%  $\text{NH}_3$  was added, molds and yeasts were eliminated and bacterial counts reduced to  $1.8 \times 10^4$  per gram. After 2 wk of ammoniated storage, no viable molds and yeasts were evident, and bacterial counts were further reduced to  $9.7 \times 10^3$  per gram. Tentative identification indicated that species of *Bacillus* survived ammonia treatment.

Surface-sterilized original, tempered, and treated corn samples plated on malt extract agar (3.0% malt extract and 1.5% agar) are shown in Fig. 2. Fungi that grew from these kernels are viable internal molds. The number and percent of molds infecting these samples are in Table 1. *A. flavus*, species of *Fusarium*, and *A. niger* infected 43.1, 23.5, and 19.0% of the original corn. However, during tempering the faster growing molds were favored; consequently, species of *Trichoderma* and *Rhizopus* were in a significant portion of kernels. At either 1 hr or 2 wk after ammonia treatment, no internal molds were viable. There was evidence of bacterial growth on a few ammoniated kernels, but zones on the periphery of kernels (Fig. 2C) are not bacterial growth but a precipitate in the medium. Perhaps ammonia diffused into the medium

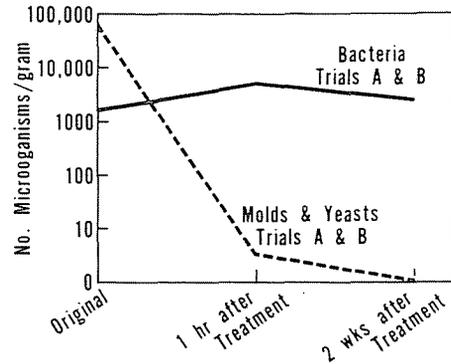


FIG. 3. The influence on microbial growth of treating 12%-moisture corn with .5% ammonia. Treatment eliminated fungi (dotted line) without stimulating bacteria (solid line).

and an ammonium salt formed. Also, agar in these plates became brown because of pigment diffusion from treated corn. However, this medium was still capable of supporting growth as demonstrated by *A. flavus* following inoculation. Such growth was expected since many molds, including most *Penicillia* and *Aspergilli*, can readily use ammonium salts as a nitrogen source.

The effect of .5%  $\text{NH}_3$  on external microflora of normal 12%-moisture corn is plotted in Fig. 3. This corn had the same flora as the original corn in the first experiment. However, in the second experiment the corn was not tempered. One hour after ammonia treatment, bacteria increased ( $2.0 \times 10^3$  to  $6.8 \times 10^3$  per gram) and molds and yeasts were nearly destroyed. Less than 10 molds per gram (species

TABLE 1. Effect of  $\text{NH}_3$  on internal molds of surface-sterilized whole corn.

Microorganism	Original		Corn tempered to 26% $\text{H}_2\text{O}$				12%-moisture corn after .5% ammonia treatment	
			After 24 hr		After 2% ammonia treatment			
	(no.) <sup>a</sup>	(%) <sup>b</sup>	(no.)	(%)	1 hr (no. and %)	2 wk (no. and %)	1 hr (no. and %)	2 wk (no. and %)
<i>Aspergillus flavus</i>	66	43.1	30	19.7	0	0	0	0
<i>Aspergillus niger</i>	29	19.0	7	4.6	0	0	0	0
<i>Fusarium</i>	36	23.5	51	33.6	0	0	8 (100)	0
<i>Penicillium</i>	15	9.8	8	5.3	0	0	0	0
<i>Trichoderma</i>	4	2.6	23	15.1	0	0	0	0
<i>Rhizopus</i>			28	18.4	0	0	0	0
Other	3	2.0	5	3.3	0	0	0	0
Total	153	100	152	100	0	0	8 (100)	0

<sup>a</sup> Number of kernels from which mold grew (100 kernels were examined).

<sup>b</sup> Per cent of total mold population.

TABLE 2. Mouse preference for ammoniated corn.

Experiment	Treatment	
No. 1, corn tempered to 26% moisture	2.0% NH <sub>3</sub> added	Tempered
	Kernels remaining	477
	Consumed	923
	Total	1,400
	Percent consumed	65.9
No. 2, corn with 12% moisture	0.5% NH <sub>3</sub> added	None
	Kernels remaining	640
	Consumed	960
	Total	1,600
	Percent consumed	60.0
		878
		1,400
		62.7
		550
		1,050
		1,600
		65.6

of *Fusarium* only) survived ammonia treatment and after a 2-wk storage with ammonia, they were also eliminated. Bacterial counts ( $4.2 \times 10^8$ ) remained about the same after 2 wk storage. The effect of .5% NH<sub>3</sub> on internal molds of surface-sterilized corn is in Table 1. As with external molds, a few species of *Fusarium* initially withstood ammonia treatment. However, after a 2-wk storage they also were destroyed.

Preliminary observations of ammoniated corn stored 2 to 3 mo indicated that bacterial flora did not change with .5% NH<sub>3</sub> treatment and the 2% treatment destroyed bacteria during storage. Apparently, ammonia will not create a health hazard by allowing pathogenic organisms to propagate.

Based on analysis of variance and chi square tests for Experiment 1, there was no evidence of treatment differences (Table 2); e.g., the 2% ammoniated and tempered corn were consumed equally well by mice. However, results were extremely inconsistent among four cages. In Experiment 2, differences among cages were more consistent. Effect of treatment was not significant by analysis of variance, but on the chi square test untreated control corn was consumed slightly better than .5% ammoniated corn.

### Discussion

Our findings indicate that ammonia has excellent fungicidal properties as low as .5% of the dry weight of corn. Importance to the farmer or elevator operator has yet to be assessed. Considering similarity of ammonia to urea, now used as a protein supplement for ruminants, prospects for satisfactory or beneficial feeding of ammoniated grain are encouraging. However, its utility depends on ad-

verse effect from discoloration of grain, residual ammonia odor, or chemical changes in corn constituents.

Urea has a protein equivalent for the ruminant animal of 6.25 times the nitrogen content and is rapidly decomposed to ammonia and carbon dioxide by rumen microorganisms. Ammonia in corn should be equally useful as a protein supplement. Huber and Santana (13) reported that heifers ate more ammoniated corn silage than control silage and that lactating cows showed higher milk yields on ammonia- and urea-treated silages than on negative control rations with no added nitrogen. No significant differences in production were noted for cows fed control, urea-treated, or ammoniated silages at equal dietary nitrogen. Since ammoniated corn has some residual ammonia odor, acceptability of treated corn must be determined.

Ammonia, being volatile, would be expected to permeate rapidly stored corn, killing all internal fungi, and eliminating hot spots. Ammonia's diffusibility should make application technically simple.

Along with large-scale treatment and feeding studies, further laboratory experiments to determine separate and combined effects of moisture, temperature, and ammonia content would be useful. Microbial and biological effects of ammonia for longer times than are included in this study need to be investigated. The ultimate test would be to treat and feed high-moisture corn from the field.

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