Comparison of Controlled-Release Ammonia Solutions and Aqueous Ammonia for Preserving High-Moisture Maize

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ABSTRACT

Controlled-release ammonia solutions[3] were compared with aqueous ammonia as preservatives for high-moisture maize. The release solutions were composed of ammonia, urea, biuret, and urease enzyme. These components were combined in such a way that immediate, slow, and very slow ammonia release was possible. Freshly harvested, high-moisture (25%) yellow dent maize was treated in each of two trials. The total weight of ammonia was 0.2% of the total weight of maize in trial A and 0.8% in trial B. An initial free ammonia concentration near 0.16% (weight of NH_3 per weight of maize) was necessary to prevent microbial deterioration at the beginning of storage. Consequently, the controlled-release ammonia solutions applied in trial A were ineffective. However, at the higher treatment level of trial B, these solutions controlled microbial deterioration three times longer than did the aqueous ammonia solution, from which all ammonia was available immediately.

Investigations have proved that ammonia kills molds in high-moisture maize (Bothast et al 1973) and is effective as a microbial inhibitor to extend the time for drying high-moisture maize at ambient temperatures (Nofsinger et al 1977, 1979). This experiment was designed to compare ammonia in an immediately available form with ammonia released at controlled rates for effectiveness as a one-dose, long-term preservative.

The controlled-release ammonia solutions depend upon a combination of chemical and biochemical mechanisms. Urease hydrolyzes urea to ammonia and carbon dioxide. In the absence of urease, urea hydrolysis is negligible at ambient temperatures. Urease is inhibited by ammonia, and when the ammonia concentration rises to a given level, hydrolysis of urea ceases. When the ammonia concentration decreases due to loss or fixation, additional urea is hydrolyzed by the enzyme. Biuret slowly hydrolyzes to urea, ammonia, and CO_2. The urea produced by this chemical process is then susceptible to enzymatic hydrolysis to produce additional ammonia and CO_2. By varying the concentration of ammonia, urea, urease enzyme, and biuret, the rate of release can be controlled to provide a continuous fungistatic concentration of ammonia of the treated maize.

MATERIALS AND METHODS

Three solutions of controlled-release ammonia[3] and one of aqueous ammonia (Table I) were tested as preservatives for high-moisture (25%), freshly harvested, yellow dent maize in two separate trials. Maize used in these experiments was representative of 4,500 bu of hybrid varieties delivered to our bin site for other experiments. In trial A, the total weight of NH_3 in each solution was 2% of the corn weight. Each solution (Table II) was added to 300 g of high-moisture maize in a 1,000-ml Erlenmeyer flask. A stainless-steel aeration apparatus (Nofsinger et al 1980) was placed in the opening of the flask and connected to an air manifold, which supplied highly humidified air at a rate of 10 ml/min. All tests were performed in a room having a constant temperature of 28°C. Microbial activity was monitored by measuring respiratory CO_2 in the headspace of each flask by gas chromatography (Ramstack et al 1979). A daily visual check for mold development was also made. When storage was terminated, maize samples were removed from the aeration flasks and assayed for aerobic bacteria and molds according to the procedures outlined by Bothast et al (1974).

Trial B used ammonia at 0.8%, by weight, of maize weight for solutions 2 and 3 (of which 0.2%, maize weight, was immediately released) and aqueous ammonia at 0.2 and 0.8% of maize weight (Table II) for solution 4. The same procedures for evaluating efficacy were followed as for trial A. Rate of ammonia release was not determined in either trial because precise measurements could not be made with the gas chromatograph. Duplicates of all solutions were tested in both trials.

RESULTS AND DISCUSSION

Trial A demonstrated that a critical amount of ammonia must be present initially for the solution to be effective as a preservative. Both the maize treated with solution 1 (Table I), which had no immediate-release ammonia, and the untreated, aerated control became visibly moldy after three days of storage (Table III). The small amounts of immediate-release ammonia in solutions 2 and 3 delayed any visible microbiological activity until the fifth storage day (Table III). Similarly, the nonaerated control sample did not show visible microbial deterioration until the fifth day. Perhaps the high CO_2 concentration in the headspace of this nonaerated flask

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[3]The controlled-release ammonia solutions were donated by Union Oil Company of California, Research Department, Brea, CA 92621.

TABLE I
Composition of Ammonia Solutions

Solution	Composition[a] Ammonia (%)	Urea (%)	Urease Enzyme (ppm)	Biuret (%)	Total NH_3 in Solution (%)	Ammonia Release[a] (%) Immediate	Slow	Very Slow
1	···	40	500	···	21	0	20	1
2[b]	10	40	500	2	31	6	23	2
3[b]	10	40	500	···	30	6	24	0
4[b]	(aqueous)	···	···	···	30	30	0	0

[a] Compositions and release percentages were provided by the Union Oil Company of California.
[b] A strong ammonia odor was present in the original solution.

was responsible for the slight improvement in storage. Nevertheless, high levels of CO_2 (Fig. 1) after two days in flasks containing maize treated with solutions 1–3 emphasized that low ammonia levels (0.04%, maize weight) were ineffective as long-term preservatives. However, solution 4 contained ammonia (0.2%, maize weight) in an immediately available form and was effective for 15 days.

As soon as deterioration was visible, all samples in trial A were removed from the air manifold and assayed for microbial counts. Generally, both mold and bacteria counts increased 100-fold during deterioration (Table III). The most common molds present on the maize were species of *Absidia, Penicillium,* and *Rhizopus.*

Only solutions 2–4 were used in trial B. Solution 1 was omitted because no initial ammonia was released, and the controls were omitted because the original maize was uniform and the two trials were conducted on consecutive days. All solutions caused slight browning of the maize. In trial B, the higher concentration of NH_3 in solution 4 (0.8%, maize weight) was effective for 38 days. The CO_2 levels in the headspace gas (Fig. 1) increased steadily from day 17 until day 39, when the samples were removed. Mold and bacteria counts were 1.33×10^7/g and 1.34×10^8/g, respectively (Table III). After 40 days, replicate flasks containing maize treated with solutions 2 and 3 were removed from the manifold to determine the microflora present. Bacterial counts were lower than on the original maize and no molds were detected. The remaining flasks of maize treated with solutions 2 and 3 showed an increase in the CO_2 level on the 88th day and were removed from the manifold on the 96th day, when visible deterioration was evident. Analyses showed that bacteria were responsible for the activity; no molds were detected. Previous studies have demonstrated that increases in respiratory CO_2 parallel or precede slightly visible microbial deterioration (Bothast et al 1981).

Because urea hydrolysis occurs when the ammonia level decreases, causing a subsequent increase in the CO_2 level, we were able to speculate when the ammonia releases took place (Fig. 1) in trial B. Solutions 2 and 3 in trial B apparently began urea hydrolysis at approximately day 14 and again at day 31 (resulting in CO_2 increase), with the ammonia concentration increasing to inhibit the urease enzyme at approximately days 19 and 36 (resulting in CO_2 decrease). At day 88, the CO_2 level began to increase, possibly from the bacterial activity seen at day 96. In comparison, the maize treated with the 0.2% aqueous ammonia solution used in trial A gave evidence of microbial growth by day 15, and the maize treated with the 0.8% aqueous ammonia solution used in trial B evidenced microbial growth by day 39. The controlled-release ammonia solutions with NH_3 at 0.8% of maize weight (containing at least 0.16% initial ammonia concentration) are therefore more effective than a like quantity of aqueous ammonia as a one-dose, long-term preservative agent.

Furthermore, the controlled-release concept may offer yet

TABLE II
Ammonia Solutions Added to Maize

Trial	Solution[a]	Total NH_3 Added (percent of maize, w/w)	NH_3 Released Immediately (Theoretical) (percent of maize, w/w)
A	1	0.2	0.00
	2	0.2	0.04
	3	0.2	0.04
	4	0.2	0.20
B	2	0.8	0.16
	3	0.8	0.16
	4	0.8	0.80

[a] Solutions are described in Table I.

TABLE III
Microbial Counts

Treatment	Storage Time (days)[a]	Microbial Counts[b] Molds	Bacteria
Trial A			
Controls			
Air	3	1.50×10^7	3.33×10^7
No air	5	5.00×10^5	1.73×10^7
Solutions[c]			
1	3	1.40×10^7	1.16×10^8
2	5	1.78×10^6	2.02×10^8
3	5	2.40×10^6	2.91×10^8
4	15	1.30×10^5	4.43×10^8
Trial B			
Solutions			
2	40	ND[d]	1.38×10^4
	96	ND	1.83×10^4
3	40	ND	1.25×10^5
	96	ND	1.87×10^5
4	39	1.33×10^7	1.34×10^8

[a] Trial A, to visible deterioration; trial B, to removal from manifold.
[b] Mean microbial counts of the maize as received from the field: molds, 8.50×10^4/g; bacteria, 2.9×10^6/g.
[c] Solutions are described in Table I.
[d] No mold detected.

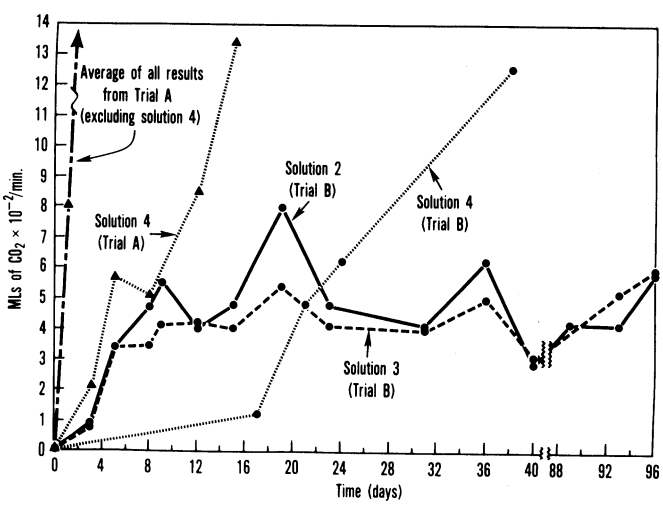

Fig. 1. CO_2 production from high-moisture maize treated with ammonia solutions. Solutions 1–3, controlled release; solution 4, immediate release.

another alternative for using ammonia as a mycostatic agent during low-temperature drying of high-moisture maize. In contrast to the intermittent application of ammonia gas during the "trickle ammonia process" described by Nofsinger et al (1977, 1979) and Bothast and Anderson (1979), these controlled-release solutions should require only a single application.

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