Influence of Modified Atmosphere Storage on Aflatoxin Production in High Moisture Corn

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Samples of freshly harvested corn and remoistened corn were inoculated with Aspergillus flavus and stored for 4 weeks at about 27 C in air and three modified atmospheres. Aflatoxins and fat acidity were determined weekly. Corn stored in the modified atmospheres did not accumulate over 15 µg of aflatoxin B₄ per kg and 20 µg of total aflatoxins per kg. Corn from the high CO₂ treatment (61.7% CO₂, 8.7% O₂, and 29.6% N₂) was visibly molded at 4 weeks and had a higher fat acidity than the other treatments. In the N₂ (99.7% N₂ and 0.3% O₂) and controlled atmosphere (13.5% CO₂, 0.5% O₂, 84.8% N₂) treatments, a fermentation-like odor was detected. When the corn was removed from the modified atmospheres it deteriorated rapidly and was soon contaminated with aflatoxins.

Modified atmospheres have shown promise in controlling the Aspergillus flavus group and the subsequent aflatoxin production in stored peanuts. Sanders et al. (9) reported that little aflatoxin was found on inoculated peanuts maintained in an atmosphere of 60% CO₂, 20% O₂, and 20% N₂, as compared with 206 µg of aflatoxin per g of peanuts stored in normal air at 25 C and 90% relative humidity (RH). The high CO₂ atmosphere is similar to that recommended by Jay (4) for control of insects in stored products. Landers et al. (7) reported little aflatoxin production on peanuts with a 28.9% moisture content held in an atmosphere of 99% N₂ and 1% O₂. Pattee and Sessoms (8) reported that increases in fat acidity were highly correlated with visible A. flavus growth, and that fat acidity reached 60 mg of KOH per 100 g of peanut kernels before aflatoxins were detected. However, Landers et al. (7) found aflatoxins in peanuts with fat acidity lower than 60 mg of KOH per 100 g of kernels. Sauer and Christensen (10) observed increases in fat acidity in corn stored at moisture contents of 15.5 to 16.5% at 25 to 30 C.

Recent studies by Jay et al. (6) and by Jay and Pearman (5) demonstrated that modified atmospheres created by purging storage structures with CO₂ were effective in controlling insects in stored products. In laboratory studies Harein and Press (1) found that insects in stored peanuts could be controlled with N₂ if less than 1% O₂ was present. Controlled atmosphere (CA) generators have been used for many years for maintaining the quality of stored apples and for preserving alfalfa pellets. These generators consume either natural or bottled gas and remove the water vapor caused by combustion. The atmospheres produced usually contain 10 to 14% CO₂ and less than 1% O₂; the balance is principally N₂ and argon, and occasionally some carbon monoxide if a high fuel-to-air ratio prevails. In field tests with large CA generators, adult confused flour beetles, Tribolium confusum Jacquelin du Val, were controlled in 20,000 bu silos of wheat within 24 h (11).

Aflatoxins have been of prime concern in peanuts and may occur in several other crops including corn. Trenk and Hartman (12) demonstrated that aflatoxins were produced in corn at moisture levels above 17.5% when temperature was 24 C or more. They also found that remoistened corn was subject to more rapid deterioration and subsequent aflatoxin formation than freshly harvested corn. Hodges et al. (2) found that fast cooling rates in bins were more effective in maintaining quality than slow cooling rates. They showed that slow cooling with initial temperatures above 70 F permitted rapid growth of A. flavus, heating, and some aflatoxin contamination in corn.

Modified atmospheres to achieve control of both insects and the A. flavus group of fungi seems to be a relevant approach to these problems. Therefore, tests were designed to study the effects of high CO₂, high N₂, and an atmosphere from a commercially available CA.
generator on A. flavus infestation and aflatoxin production on high moisture corn.

**MATERIALS AND METHODS**

Cylinders of gas were used to supply the desired atmospheres to 3.8-liter jars containing corn. The gas flowed from the cylinders through pressure regulators and micrometer regulating valves connected to flow meters, then through gas washing bottles containing glycerine-water mixtures calibrated to the initial RH of the inter-kernel atmosphere of the corn to temper the gas to the desired RH. The gas then passed through 7.6-m coils of copper tubing suspended in the 26.7 ± 1 C water bath connected to copper tubes (6.4 mm in outer diameter by 30 cm in length). These tubes entered through the lids, and the gas from these tubes diffused into the corn. The gas was exhausted to the atmosphere through an additional copper tube (6.4-mm outer diameter by 5.4 cm in length) mounted in the lid. This tube was also used to sample the gas composition in the jars. A bimetallic thermometer with a 22.9-cm stem and a humidity probe (Hygrodynamics, model 15-3001) were inserted through openings in the lid.

In the first series of tests two replicates were run, one in each water bath. In the first series, freshly harvested Iowa-grown shelled corn having an initial moisture content of 29.4% was used. Before exposure to the gases, the corn was inoculated using a power atomizer with a 150-ml spore suspension of A. flavus LK., isolate NRRL 5520 containing about 10⁸ spores/ml giving 780,000 spores/liter of corn. The jars were then filled with the corn, sealed with tape, and placed in the water baths. Initial gas flow into the jars was 150 ml/min and subsequent flow rate was reduced to 40 ml/min when the composition of the atmosphere in the jars approximated the atmospheres from the cylinders. In the first series the corn was exposed to four different atmospheres: (i) air with 0.03% CO₂, 21% O₂, and 78% N₂; (ii) the output product of a CA generator previously pumped into cylinders with about 13.5% CO₂, 0.5% O₂, and 84.8% N₂; (iii) 99.7% N₂ and 0.3% O₂; (iv) a blended atmosphere having a composition of about 61.7% CO₂, 8.7% O₂, and 29.6% N₂.

Two 0.4-liter samples were taken from each of four jars exposed to each of these four atmospheres after 1, 2, 3, and 4 weeks of exposure. One of the two 0.4-liter samples was oven dried at 65 C for 24 h. The duplicate sample was exposed to the atmosphere for 1 week in a room maintained at 26.7 ± 1 C and 60 ± 5% RH, then dried. The dried samples were held at 0 C until aflatoxin and fat acidity values were determined.

Three replicates were run in a similar manner in the second series of tests. However, there were no continuous exposures for the entire 4-week period without sampling. Corn used in the second series of tests was Georgia grown and had been in cold storage for about 1 year. It had an initial moisture content of 14.2% and was tempered to 19.6% by the addition of water and thorough mixing in a drum tumbler contained in a cold room maintained at 5.6 C. The corn was tumbled for 1 h a day for 1 week and then was inoculated in the same way as in the first series with A. flavus spores.

Moisture content of the corn in each container was determined initially and at the end of the 4-week exposures with a Motomoca model 919 moisture meter.

Temperature and RH in each jar were taken daily Monday through Friday during the 4-week incubation. Also, gas samples were taken daily from each container with a 10-ml gas-tight syringe and were injected into a Fisher-Hamilton model 29 gas partitioner for analysis. This partitioner was equipped with a 0.5-ml gas sample loop and a dual column, dual detector system for separating the individual components. Output from the partitioner was integrated with a Vidor 6300 digital integrator.

Aflatoxins from 50-g samples were extracted and determined using thin-layer chromatography according to the official AOAC method I (3). The fat acidity (mg of KOH required to neutralize free fatty acids from 100 g of corn) was determined using the official AOAC rapid method for corn (3).

**RESULTS**

Fat acidity and aflatoxin contents of corn after exposure to four different atmospheres for 1 to 4 weeks are given in Table 1. The fat acidity did not change greatly in corn exposed to CA or N₂ in either test. When subsamples from these treatments were exposed to laboratory atmosphere for 1 week, however, fat acidity increased in all cases. In the modified atmospheres fat acidity was highest in the CO₂ plus low O₂ treatment in both series; and increased with the length of exposures.

The corn in the freshly harvested series had little visible mold after 4 weeks in the CA or N₂ atmospheres. A pleasant aromatic odor was detected, suggesting that fermentation was in progress. Corn in the CO₂ plus low O₂ atmosphere was visibly molded in both series and had an unpleasant odor after 4 weeks. The corn exposed to the normal air was visibly moldy after 1 week. Fat acidity and aflatoxin values were not determined after the corn was badly deteriorated in the air treatment. Aflatoxin synthesis was slight in the modified atmospheres in both series. Substantial increases occurred, however, when exposed to air.

Table 2 presents the original gas compositions and the mean gas concentration in the effluent during the tests. The CO₂ rose above 13% and the O₂ concentration dropped below 10% in both of the air treatments in the first series due to the high rate of respiration and germination of the moist corn and microbial respiration. In the first series, corn exposed to gas from the CA generator or the N₂ exhibited less than 1% rise in CO₂ during the exposure period, whereas the
Table 1. Fat acidity and aflatoxin content of freshly harvested and tempered high moisture corn inoculated with Aspergillus flavus exposed to modified atmospheres

| Weeks exposed | Atmosphere description | CA generator | Nitrogen | CO₂ plus low O₂ | Air |
|---------------|------------------------|--------------|----------|-----------------|-----|
|               |                        | Fat acidity* | Aflatoxins (μg/kg) | Fat acidity* | Aflatoxins (μg/kg) | Fat acidity* | Aflatoxins (μg/kg) | Fat acidity* | Aflatoxins (μg/kg) |
| First series<sup>b</sup> | 1 | 11 | <2 | 12 | <2 | 18 | <2 | 44 | <2 |
|               | 2 | 16 | <2 | 12 | <2 | 24 | <2 | 61 | 296 |
|               | 3 | 13 | <2 | 12 | <2 | 44 | 20 | 68 | — |
|               | 4 | 16<sup>*</sup> | <2<sup>*</sup> | 18 | <2 | 56 | <2<sup>*</sup> | 68<sup>*</sup> | — |
|               | 1 + 1 Air<sup>d</sup> | 61 | 160 | 79 | 150 | 47 | <2 | 51 | 180 |
|               | 2 + 1 Air<sup>d</sup> | 80 | 70 | 71 | 150 | 46 | 9 | 68 | — |
|               | 3 + 1 Air<sup>d</sup> | 50 | 150 | 54 | 150 | 50 | 5 | 63 | — |
|               | 4 + 1 Air<sup>d</sup> | 42<sup>*</sup> | <2<sup>*</sup> | 40 | 150 | 45 | 5 | — | — |
| Second series<sup>c</sup> | 1 | 23 | 10 | 20 | 3 | 20 | <2<sup>*</sup> | 43 | 400 |
|               | 2 | 28 | <2 | 26 | <2<sup>*</sup> | 24 | 6 | 80 | >1021 |
|               | 3 | 29 | <2 | 25 | <2<sup>*</sup> | 42 | <2<sup>*</sup> | 98 | >1021 |
|               | 4 | 44 | <2<sup>*</sup> | 32 | <2<sup>*</sup> | 52 | <2<sup>*</sup> | — | — |
|               | 1 + 1 Air<sup>d</sup> | 83 | 540 | 80 | 450 | 55 | 296<sup>+</sup> | 95 | >1021 |
|               | 2 + 1 Air<sup>d</sup> | 80 | 430 | 83 | 650 | 61 | 296<sup>+</sup> | 108 | >1021 |
|               | 3 + 1 Air<sup>d</sup> | 90 | 286<sup>+</sup> | 82 | 650 | 64 | 296<sup>+</sup> | — | — |
|               | 4 + 1 Air<sup>d</sup> | 84 | 317 | 78 | 260 | 65 | 85 | — | — |

*Fat acidity is mg of KOH required to neutralize free fatty acids from 100 g of corn (dry basis). Values rounded to nearest whole number.

*Freshly harvested corn with 29.4% initial moisture inoculated with 780,000 spores of A. flavus per liter of corn. Values are means of two replications.

*Remoistened corn with 19.6% initial moisture inoculated with 780,000 spores of A. flavus per liter of corn. Values are means of three replications.

*Exposed to indicated atmosphere for stated length of time and then subsample exposed to the atmosphere for 1 week before sampling.

*Mean of four replications.

*No data taken due to deterioration of corn.

CO₂ concentration increased 3.6% in containers of corn exposed to the CO₂ plus low O₂ mixture, and the O₂ concentration dropped 4.5%. In the second series (Table 2), the corn had a lower initial moisture content and corn exposed to air had a CO₂ increase of 3.7%, while the O₂ concentration dropped from 21 to 17.6%. Carbon dioxide increase in corn exposed to gas from a CA generator or to N₂ was slight, whereas no CO₂ increase was observed in corn exposed to CO₂ plus low O₂. An O₂ reduction of less than 1% occurred in the CO₂ plus low O₂ in the first series in which the RH was about the same as that in containers exposed to air. The increases in RH and moisture were due to respiration. In the first series when corn was exposed to air, the moisture content rose from 29.4 to over 50%, whereas moisture in corn exposed to either CA or N₂ rose only 3.4 to 4.2%. The moisture content of corn exposed to CO₂ plus low O₂ rose to 49.1%, or 19.7% during the exposure. Mean temperature was highest in corn exposed to air in the first series and ranged to over 30 C. Mean temperature of corn in this series exposed to other gases was about the same as that of the waterbaths (26.7 ± 1 C) with the exception of the exposure to the CO₂ plus low O₂ mixture in which the mean was 27.6 C, and the highest temperature recorded was 28.3 C. In the second series the mean temperature of corn exposed to air was
TABLE 2. Original cylinder composition and mean composition during tests of modified atmospheres used to treat containers of high moisture corn

| Atmosphere description | Original composition | Mean composition during tests |
|------------------------|----------------------|------------------------------|
|                        | CO₂ (%) | O₂ (%) | N₂ (%) | CO₂ (%) | O₂ (%) | N₂ (%) |
| First series<sup>c</sup> |         |         |        |         |         |        |
| Air                    | 0.03    | 21.0   | 78.0   | 13.4    | 9.8     | 75.9    |
| Air<sup>a</sup>        | 0.03    | 21.0   | 78.0   | 16.5    | 7.0     | 75.6    |
| CA generator           | 13.60   | 0.6    | 84.9   | 14.3    | 0.5     | 84.3    |
| CA generator<sup>a</sup> | 13.60  | 0.7    | 84.8   | 14.2    | 0.5     | 84.4    |
| Nitrogen               | 0.00    | 0.3    | 99.7   | 0.7     | 0.3     | 99.0    |
| CO₂ plus low O₂        | 61.70   | 8.7    | 29.6   | 65.3    | 4.2     | 30.2    |
| Second series<sup>d</sup> |       |        |        |         |         |        |
| Air                    | 0.03    | 21.0   | 78.0   | 3.7     | 17.6    | 77.8    |
| CA generator           | 13.40   | 0.7    | 84.7   | 14.0    | 0.6     | 84.5    |
| Nitrogen               | 0.00    | 0.3    | 99.7   | 0.4     | 0.4     | 99.2    |
| CO₂ plus low O₂        | 61.90   | 8.4    | 29.7   | 61.9    | 7.5     | 30.6    |

* Balance of air and CA generator samples is argon and rare gases.
* Means calculated from 20 air samples from each container of each replication of each series; means from the first series are from two replications; means from the second series are from three replications.
* Freshly harvested corn with 29.4% initial moisture.
* Remoistened corn with 19.6% initial moisture.
* Corn held for 4-week period and sampled at the end of the 4-week exposure.

1 C or more higher than the other exposures. Temperatures in all exposures except air did not rise above 27.2 C.

DISCUSSION

None of the modified atmospheres tested allowed high levels of aflatoxin contamination. Subsamples of corn from all treatments exposed to normal atmosphere for 1 week, however, were generally contaminated with aflatoxins. Freshly harvested corn exposed to CO₂ plus low O₂, and the 4-week treatments with CA were not contaminated with aflatoxin upon exposure to air. Aflatoxins were formed in the corresponding treatments in the remoistened corn, this possibly reflects microbial activity or physiological differences between freshly harvested and remoistened corn.

Appearance of remoistened corn did not seem to change much with exposure to CA or N₂ for 4 weeks. Freshly harvested corn in these treatments had undergone some fermentation but still appeared sound. The use of N₂ or CA treatments may produce corn desirable for feed. Further studies on the quality factors are needed to find if CA treatment would be useful as a means of holding corn temporarily before drying. If the gases from CA were mixed with enough O₂ to block anaerobic fermentation, the fermentation problem might be eliminated and a residue-free, economical storage system could then be adopted. Experiments are planned using higher levels of O₂.

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