Effect of Storage Conditions on Increase in Aflatoxins in Cottonseed: A Small Lot Study[1]

MARION E. WHITTEN, Market Quality Research Division, ARS, USDA, Beltsville, Maryland 20705, and **LLOYD L. SMITH**, Transportation and Facilities Research Division, ARS, USDA, Stoneville, Mississippi 38776

ABSTRACT

Small lots of cottonseed with low aflatoxin contamination were stored at 15, 18 and 22% moisture at 80 and 85 F. Maximum aflatoxin content was reached within 30 days' storage. The range in moisture content was sufficient to cause a 10-fold increase in aflatoxin content between seed stored at 15.1 and 21.8% moisture. Aeration caused about a five-fold mean increase in aflatoxin content.

INTRODUCTION

A 3 year survey by the U.S. Department of Agriculture and industry showed that ca. 5% of the cottonseed contained 100 parts or more per billion of aflatoxins (1).

Aflatoxins are toxic compounds which generally are produced by certain strains of *Aspergillus flavus* under conditions favorable for growth (2). Schroeder and Hein found that production of aflatoxins on moistened cottonseed reached its peak at 78 F with nearly as much at 86 F and somewhat less at 68 F. At 60 F progressively less aflatoxins were produced than at higher temperatures (3). Ashworth and coworkers found that cottonseed, with 9-10% moisture and containing aflatoxins, could be stored for several months without increase in aflatoxin content (4).

Variability in Sampling for Aflatoxin Assay

Investigations at this laboratory and at the Southern Regional Laboratory and elsewhere indicate that, in both cottonseed and peanuts, aflatoxins are usually concentrated in relatively few kernels of a given lot (5 and W.A. Pons, unpublished communication). We found that, in a sample of cottonseed containing 8000 ppb aflatoxin B_1, only 18 out of 150 randomly selected seed contained aflatoxins. One cottonseed contained 600,000 ppb of aflatoxin B_1. In another lot of cottonseed that assayed 250 ppb aflatoxin B_1, more than 350 randomly selected seed were assayed before one seed was found that contained a measurable amount of aflatoxins. The difficulty in obtaining representative 1-2 lb samples of any lot of cottonseed is apparent. Sampling error is therefore very great and tends to weaken statistical conclusions.

Ten replications drawn from the lot of cottonseed used in this study averaged 30 ppb aflatoxins and ranged from 0 to 113 ppb. As expected, some 1-2 lb samples were free of aflatoxins.

EXPERIMENTAL PROCEDURES

Cottonseed of the 1969 crop (288 lb), with an average aflatoxin content of 30 ppb, was divided into three lots, and moisture was added until they contained ca. 15, 18 and 22%, respectively. After equilibration each lot was split in half, one portion to be aerated, the other with no aeration. Then each sublot was split for storage at two temperatures, 80 and 85 F.

Each of the resulting 12 sublots was placed in a straight-walled 3 gal container. The containers had wire screen floors to provide for air movement and were partitioned by wire screen into quadrants.

Six of the containers were stored at 80 F and six at 85 F. At the end of 30, 60, 90 and 120 days' storage, two samples of cottonseed were removed from one quadrant of each container and analyzed for aflatoxins (6), free fatty acids, oil and moisture (7). When cottonseed samples were removed from a quadrant, they were replaced with an equal amount of the original seed.

To prevent drying and to maintain desired moisture, humidified air was drawn through the aerated seed. The correct humidity was obtained by bubbling air through a saturated salt solution. In nonaerated lots, air inlets were sealed so that air could not move through the cottonseed

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TABLE I

Experimental Analysis of Variance (Common Logarithms Transformation)

Source	Degrees of freedom	Mean square	Variance ratio
Total	95		
Moisture content	2	8.65220	15.73[a]
Treatment (aeration)	1	12.41226	22.55[a]
Temperature, F	1	19.08723	34.68[a]
Days storage	3	1.02314	1.86 n.s.[b]
Moisture x aeration	2	9.56130	17.37[a]
Moisture x temperature	2	0.06258	0.11 n.s.
Moisture x days storage	6	0.49490	0.90 n.s.
Aeration x temperature	1	4.08740	7.43[a]
Aeration x days storage	3	0.83991	1.53 n.s.
Temperature x days storage	3	0.09060	0.16 n.s.
Moisture x aeration x temperature	2	1.88181	3.42[c]
Moisture x aeration x days	6	0.48108	0.87 n.s.
Moisture x temperature x days	6	0.72176	1.31 n.s.
Aeration x temperature x days	3	0.26577	0.48 n.s.
Moisture x aeration x temperature x days	6	0.78959	1.43 n.s.
Error	48	0.55031	

[a]Significant at the 1% level.

[b]n.s. = Not significant.

[c]Significant at the 5% level.

TABLE II

Mean Aflatoxin Content (ppb) and Mean Logarithm of Stored Cottonseed[a]

Source moisture,	Aerated	Nonaerated	80 F	85 F	Aerated, 80 F	Nonaerated, 80 F	Aerated, 85 F	Nonaerated, 85 F	Moisture
15.1	2221 2.083c	323 1.990c	139 1.612d	2406 2.461c	199 1.648def	78 1.576ef	4244 2.518bc	569 2.404bcd	1272 2.037c
18.7	787 2.696b	989 2.613b	319 2.238c	1457 3.070ab	397 2.441bcd	241 2.035cde	1177 2.951b	1737 3.190b	888 2.654b
21.8	17194 4.061a	3109 2.079c	10433 2.573bc	9870 3.567a	16124 4.032a	4742 1.114f	18263 4.089a	1476 3.044b	10151 3.070a
Mean	6734 2.947a	1474 2.227b	3630 2.141b	4578 3.033a	5574 2.707b	1687 1.575c	7895 3.186a	1261 2.880ab	

[a]Comparable means followed by all different lower case letters indicate significance at the 5% level. Upper figure refers to mean aflatoxin content and lower figure pertains to mean logarithm.

by convection. (Vented lids were used on nonaerated as well as aerated containers.)

RESULTS

A factorial analysis involving 96 observations was used for this experiment. Aflatoxin values (not shown) were converted to common logarithms, which minimize large differences due to sampling error, for statistical treatment. Moisture content, aeration and temperatures are significant at the 0.01 probability level (Table I). Interactions between moisture content and aeration and between aeration and temperatures were significant at the 0.01 probability level. The third-order interaction between moisture content, aeration and temperature was significant at the 0.05 probability level. Effects involving days of storage were not significant.

Mean logarithmic values for all factors studied, except days of storage, are shown in Table II. The Duncan Multiple Range test (8) was applied to sets of comparable means. Aeration, temperature and moisture during storage resulted in significant differences in aflatoxin content of the cottonseed. Only at 21.8% moisture, however, was the difference significant for aeration for a given moisture at all three moisture levels. Storage at 85 F resulted in a significant increase in aflatoxin over that at 80 F. The really large amounts of aflatoxin were almost entirely associated with 21.8% moisture, although even at this moisture variation was considerable.

Table II also presents the parts per billion aflatoxin means. The variation shown and discrepancies point out the improvement in the data that was obtained by transformation to logarithms.

DISCUSSION

A gradual increase of aflatoxin was expected to reach a maximum at midpoint of the experiment, but instead the maximum was reached before the first series of samples was even tested. Hence no conclusions may be drawn with respect to days of storage.

Aflatoxin contents differed widely between duplicate samples. Conversion of results to logarithms allowed a more reasonable analysis.

The range in cottonseed moisture content was sufficient to cause a 10-fold mean increase in the mean aflatoxin content between the seed stored at 15.1 and 21.8% moisture. Aeration caused about a five-fold mean increase of aflatoxin content.

The differences tabulated in Table II do not indicate a significant difference between aeration and nonaeration as it affects moisture and oil contents of cottonseed over a period of 30 through 130 days.

Oil content was included as one of the indices that might interact with the growth of *Aspergillus flavus*. The results indicate a slight but consistent increase in the reduction of oil content with increase in moisture content during storage.

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REFERENCES

1. Whitten, M.E., JAOCS 46:39 (1969).
2. Diener, U.L., and N.D. Davis, "Aflatoxin," Edited by L.A. Goldblatt, Academic Press, New York, 1969, p. 25.
3. Schroeder, H.A., and H.L. Hein, Appl. Microbiol. 15:441 (1967).
4. Ashworth, L.J., Jr., J.L. McMeans, B.R. Houston, M.E. Whitten and C.M. Brown, JAOCS 48:129 (1971).
5. Whitten, M.E., "Cotton Gin and Oil Mill Press," December 17, 1966, p. 12.
6. Velasco, J., J. Ass. Off. Anal. Chem. 53(3):611 (1970).
7. "Official and Tentative Methods of the American Oil Chemists' Society," Third edition, AOCS, Champaign, Ill., 1964 (revised to 1971).
8. Mean Separation ARS-20-3, ARS, USDA, May 1957.

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