Physical Properties and Aflatoxin Content of Individual Cateye Fluorescent Cottonseeds

S.P. KOLTUN, H.K. GARDNER, JR., F.G. DOLLEAR, and E.T. RAYNER, Southern Regional Research Center, New Orleans, Louisiana 70179

ABSTRACT

The density and aflatoxin content of individual cateye fluorescent cottonseeds have been investigated. In general, higher average levels of aflatoxin contamination were found among the lower density seeds. However, significant amounts of aflatoxins were detected in a few of the higher density seeds. Subjective evaluation of color and texture of dissected kernels indicated a predominance of poor quality seeds at the lower densities. Visually determined physical characteristics, however, were not indicative of the presence or absence of aflatoxins.

INTRODUCTION

The occurrence of aflatoxins in certain agricultural commodities has become well recognized and extensively publicized. The adverse nutritional and economic implications of the problem have prompted considerable research in the field, and government standards relating to the marketing and distribution of contaminated products have been formulated. In guidelines issued in 1969, for example, the Food and Drug Administration stipulated that agricultural products intended for use in animal feeds may not exceed a total aflatoxin concentration of 20 ppb (1). Therefore, much attention has been devoted to various methods of eliminating or lowering to acceptable levels the concentration of aflatoxins which sometimes occur in such commodities as cottonseed and peanuts or their respective meals. Primarily, these efforts have centered about chemical inactivation of the aflatoxins (2-5), removal of the aflatoxins by solvent extraction (6, 7), or by the physical separation of aflatoxin contaminated products from those free of contamination (8, 9). The physical separation of delintered, aflatoxin contaminated cottonseed from delintered uncontaminated seed by air classification was investigated intensively without definitive results. Because of these findings, the present study was undertaken to examine the relationship between density, aflatoxin content, and kernel appearance in individual cottonseeds. Cateye cottonseed were used in this study because of the greater probability of aflatoxin contamination occurring in such seed (10). The word cateye refers to the bright, greenish-yellow fluorescence which can be observed in the linters of certain cottonseeds when viewed under long-wave UV light. Such flurorescence has been reported (11) to be associated with growth on the fibers of the fungus *Aspergillus flavus*.

EXPERIMENTAL PROCEDURES

Materials

The cateye cottonseeds used in this study were obtained by examining and hand sorting, under long-wave UV light, 167 lb undelintered cottonseed containing an average of 3000 ppb total aflatoxins. The cateye seeds collected amounted to 0.20% by wt of the total seeds examined.

Dichloromethane (methylene chloride), bp 40-41 C, and N-hexane, spectral grade, density 0.6610 gm/ml, 25 C were obtained from Burdick & Jackson Laboratories, Inc., Muskegon, Mich.

[1]ARS, USDA.

Seed Density Determination

The density of the individual delintered cottonseeds was determined by Archimedes' principle of displacement. To calculate their densities, the seeds were weighed in two media, air and hexane, using the following procedure. With a Bausch and Lomb StereoZoom microscope (total magnification from 7-30 x), individual cateye cottonseeds were delintered carefully by cutting away the longer fibers with a single-edge razor blade; the remaining closely adhering fibers then were removed by mild abrasion with a pencil-shaped typewriter eraser. The sharpened eraser was a convenient means of contacting irregular surfaces of the seed, and the abrasion did not damage the seed coat. Seeds having cracked hulls or other penetrations were eliminated, since their displacement in hexane would not provide accurate values for density calculations.

The delintered seed was secured firmly inside a loop located at one end of a nickel-chromium wire (34 Brown and Sharpe gauge, 0.16 mm diameter) ca. 11 cm long. The other end of the wire was bent into a U-shape permitting the wire and seed to be suspended from the pan-bow hanger located at the top of a Mettler type H 15 standard anaalytical balance. Thus, the wt of the wire, previously determined (W_1), and the wt of the wire plus seed (W_2) were recorded. The wt of the seed in air (W_3) was obtained from the difference of W_1 and W_2. For determining the wt of the seed in hexane, a rectangular masonite platform 0.5 cm x 9 cm x 16 cm was supported at each corner 0.5 cm above the pan of the balance in such a manner as to clear the pan bow, allowing free movement of the pan. A 20 ml beaker of hexane of known density was placed on the platform, and the wire was positioned so that its loop was submerged ca. 1 cm below the level of the hexane and essentially in the center of the beaker. The wt of the submerged nickel-chromium wire (W_4) was recorded. The same procedure then was followed with the delintered seed positioned inside the wire loop to obtain the wt of the submerged wire and seed (W_5). Subtracting W_5-W_4 provided the wt of the delintered seed submerged in the hexane (W_6). The density of the delintered cottonseed (d) was then readily calculated using the formula:

$$d = W_3 / [(W_3 - W_6)/d_h]$$

where d = seed density, g/ml; W_3 = wt of delintered seed in air, g; W_6 = wt of delintered seed submerged in hexane, g; and d_h = density of hexane, g/ml.

Subjective Seed Evaluation

Prior to analysis for aflatoxin content, the individual seeds whose densities had been determined, were dissected and the kernels examined visually under a microscope. The overall appearance of the kernel, as related to color and texture, was recorded.

Aflatoxin Assay of Individual Kernels

The individual kernels were assayed for aflatoxin content using the method of Cucullu, et al., (12) for the determination of aflatoxin in individual peanuts and peanut sections with the exceptions discussed below. The extraction solvent was 85% acetone 15% water (v/v) with 8 ml glacial acetic acid/liter. Dichloromethane was used as the partitioning solvent and densitometric measurement of

TABLE I

Physical Properties and Aflatoxin Content of Cateye Cottonseed

	Seed no.	Delintered seed wt, mg	Delintered seed density, g/ml	Visual appearance of dissected kernel	Aflatoxin content of kernel, ppb			Group aflatoxin content average ppb
					B_1	B_2	Total	
	1	95.5	0.7842	Very poor[a]	ND[b]	ND	ND	
	2	85.0	0.8026	Poor[c]	164,600	43,550	208,150	
	3	71.3	0.8300	Very poor	ND	ND	ND	
A	4	79.7	0.8588	Very poor	596,300	104,430	700,730	287,193
	5	86.3	0.8639	Poor	206	93	299	
	6	60.2	0.8852	Fair[d]	909,370	191,800	1,101,170	
	7	94.2	0.8997	Good[e]	ND	ND	ND	
	8	85.2	0.9083	Poor	30,210	2,170	32,380	
	9	91.6	0.9096	Good	ND	ND	ND	
	10	102.2	0.9157	Poor	194,060	37,840	231,900	
	11	83.5	0.9177	Poor	ND	ND	ND	
	12	79.0	0.9250	Good	ND	ND	ND	
	13	116.0	0.9280	Good	204	51	255	278,614
B	14	91.9	0.9320	Good	92	41	133	
	15	103.8	0.9360	Poor	1,571,000	471,000	2,042,000	
	16	94.7	0.9432	Very poor	515,750	132,000	647,750	
	17	90.4	0.9446	Very poor	2,270	680	2,950	
	18	82.3	0.9449	Fair	297,000	89,000	386,000	
	19	95.4	0.9473	Good	ND	ND	ND	
	20	102.2	0.9596	Good	45,000	5,400	50,400	
	21	103.4	0.9655	Good	156	46	202	
	22	87.4	0.9732	Good	ND	ND	ND	
	23	89.1	0.9759	Good	11,300	1,070	12,370	
C	24	93.5	0.9811	Good	ND	ND	ND	148,822
	25	85.0	0.9861	Good	191	57	248	
	26	114.3	0.9879	Good	ND	ND	ND	
	27	95.4	0.9885	Good	ND	ND	ND	
	28	108.6	0.9890	Good	Trace	Trace	Trace	
	29	81.9	0.9915	Poor	1,096,000	329,000	1,425,000	
	30	105.7	1.0009	Good	11,800	3,280	15,080	
	31	86.8	1.0235	Good	ND	ND	ND	
	32	99.1	1.0237	Good	ND	ND	ND	
D	33	79.8	1.0363	Good	ND	ND	ND	2,171
	34	95.9	1.0365	Good	80	36	116	
	35	115.9	1.0385	Good	ND	ND	ND	
	36	98.8	1.0488	Good	ND	ND	ND	

[a]Dark brown coloration-major deterioration evident.
[b]ND = none detected.
[c]Brown coloration-soft flaky texture.
[d]Yellow to tan coloration-firm texture.
[e]Creamy white coloration-firm texture.

aflatoxins as described by Pons, et al., (13) was used.

RESULTS AND DISCUSSION

Data pertaining to physical characteristics and aflatoxin content of the individual seeds are shown in Table I. For comparison, the seeds are listed in order of increasing densities and are subdivided into four groups which are categorized as follows: group A, densities of less than 0.9; group B, densities of 0.9 to less than 0.95; group C, densities of 0.95 to less than 1.0; and group D, densities of 1.0 or greater.

Average aflatoxin levels shown for each group indicated a progressive increase in aflatoxin contamination with decreasing seed density. In addition, the average incidence of aflatoxin contamination was higher among the lower density seed (63% of groups A and B) than among the higher density seed (47% of groups C and D). Further, 78% of the total aflatoxin contamination observed occurred in the lower density seed of groups A and B with only 22% in the higher density seeds of groups C and D. Significant levels of contamination, however, occurred in some of the higher density seeds, as indicated by seeds 29 and 30. These seeds, with densities in the immediate range of 1.0 suggest that aflatoxin contamination can occur with little or no effect upon seed density.

Data on the visual evaluation of dissected cottonseed kernels (Table I) indicate a preponderance of poorer quality seeds at the lower densities. In groups A and B, for example, 6 of the 19 seeds had very poor appearance. Conversely, in the higher density groups C and D, all of the seeds with the exception of 29 were rated as good.

As anticipated, the color, texture, or general appearance of the dissected kernels was in no way indicative of aflatoxin content. Seeds 1 and 3, which were listed as very poor, showed no detectable levels of aflatoxins. In direct contrast, seeds 20, 23, and 30, which were rated as good in appearance, contained significantly high levels of aflatoxins.

Thus, in the specimens of cateye cottonseed examined, aflatoxin contamination was more common among the seeds of lower density. However, a few higher density seeds were contaminated to significant levels. No correlation between physical appearance of individual kernels and aflatoxin content could be demonstrated.

ACKNOWLEDGMENT

The density data of the hexane was supplied by Burdick & Jackson Laboratories, Inc., Muskegon, Michigan.

REFERENCES

1. Duggan, R.E., FDA Papers, pp. 13-18, April 1970.
2. Dollear, F.G., in "Aflatoxin: Scientific Background, Control and Implications," Edited by L.A. Goldblatt, Academic Press, New York, N.Y., 1969, p. 378.

3. Gardner, H.K., Jr., S.P. Koltun, F.G. Dollear, and E.T. Rayner, JAOCS 48:70 (1971).
4. Mann, G.E., L.P. Codifer, Jr., H.K. Gardner, Jr., S.P. Koltun, and F.G. Dollear, Ibid 47:173 (1970).
5. Gardner, H.K., Jr., S.P. Koltun, and H.L.E. Vix, J. Agr. Food Chem. 16:990 (1968).
6. Rayner, E.T., and F.G. Dollear, JAOCS 45:622 (1968).
7. Rayner, E.T., F.G. Dollear, and L.P. Codifer, Jr., Ibid. 47:26 (1970).
8. Whitten, M.E., Cotton Gin Oil Mill Press 67:7 (1966).
9. Ashworth, L.J., Jr., J.L. McMeans, J.L. Pyle, C.M. Brown, J.W. Osgood, and R.E. Ponton, Phytophathology 58:102 (1968).
10. Marsh, P.B., M.E. Simpson, R.J. Ferretti, T.C. Campbell, and J. Donoso, J. Agr. Food Chem. 17:462 (1969).
11. Marsh, P.B., K. Bollenbacher, J.P. San Antonio, and G.V. Merola, Text. Res. J. 25:1007 (1955).
12. Cucullu, A.F., L.S. Lee, R.Y. Mayne and L.A. Goldblatt, JAOCS 43:89 (1966).
13. Pons, W.A., Jr., A.F. Cucullu, A.O. Franz, and L.A. Goldblatt, Ibid 45:694 (1968).

[Received December 19, 1973]