Effect of Heat on Aflatoxins in Oilseed Meals

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A cottonseed meal containing aflatoxins was heated at atmospheric pressure in an effort to reduce the levels of these mycotoxins as measured by thin-layer chromatography. Temperature, period of heating, and moisture content of the meal were varied in these experiments. Heating at 60° and 80° C. did not lead to marked reductions in aflatoxin levels. More definite reductions were obtained at 100° C., greater decreases taking place with increasing periods of heating and increasing moisture contents. The lowest level of aflatoxins B_1 plus B_2 achieved practically was about 44 p.p.b. obtained by heating 120 minutes at 100° C. with a moisture content of 20%. This represented about 80% reduction in the 214 p.p.b. aflatoxins present in the unheated cottonseed meal. About 34% reduction in aflatoxins (111 to 73 p.p.b.) was obtained when a contaminated peanut meal was heated in a similar fashion.

In recent years, toxic metabolic products from certain strains of the mold *Aspergillus flavus* have been found in some agricultural commodities (Sargeant *et al.*, 1963). These products, termed aflatoxins, have been characterized chemically (Asao *et al.*, 1963; Dorp *et al.*, 1963), and feeding experiments have revealed that they have deleterious effects on a number of laboratory and farm animals (Allcroft and Carnaghan, 1962, 1963; Ashley *et al.*, 1964; Barnes and Butler, 1964; Dickens and Jones, 1964; Iongh *et al.*, 1965).

Aflatoxins have been detected in peanuts and cottonseed, and in the meals produced from these commodities (Prickett and Salmon, 1964; Whitten, 1966). Aflatoxin-contaminated stocks of peanuts can be crushed to yield edible oil because the oil refining process completely removes the aflatoxins from the finished product (Parker and Melnick, 1966). The residual meal, however, retains part of the aflatoxins and cannot be used for animal feeds. It has been recommended that this material be diverted to use as fertilizer (Banes, 1966). This lowers the economic value of the meal, and results in losses of valuable animal nutrients. Hence a program of research has been initiated to devise practical procedures to inactivate or remove the aflatoxins present in contaminated oilseed meals. Part of this program comprises the treatment of contaminated meals with various reagents at elevated temperatures and moisture contents, and the interpretation of these experiments requires knowledge of the effects of the parameters temperature, time of heating, and moisture content upon the meal aflatoxins in the absence of added reagents. Heat treatments have proved effective in the elimination of antinutritional or toxic effects in various legumes (Liener, 1962), but it has been stated that heat treatments, both dry and wet, were ineffective in eliminating aflatoxins from peanuts and peanut meal (Carnaghan, 1964, Pomeranz, 1964; UNICEF, 1963). More recent reports, however, reveal that autoclaving wet toxic peanut meals at 15 p.s i. (120° C.) caused a progressive reduction in toxicity with increasing time of autoclaving, and the reduction in toxicity was paralleled by decreases in aflatoxin contents as determined by thin-layer chromatography (Coomes *et al.*, 1966; Feuell, 1966).

The present investigation was undertaken to determine the effects of heat treatments on the aflatoxins present in a contaminated cottonseed meal.

EXPERIMENTAL

The samples of meal were heated under atmospheric pressure in a bench-scale reactor which had been designed and constructed at this laboratory (Eaves *et al.*, 1956). This reactor consists of a jacketed 3-liter stainless steel vessel fitted with a gasketed cover and an agitator designed to produce efficient shearing and kneading action in the charge of meal. The cover is provided with a thermometer well and ports for adding reagents and inserting a reflux condenser. Constant temperatures are maintained by control of the flow of hot water or steam through the jacket.

The various samples were assayed for aflatoxins using procedures developed at this laboratory (Pons *et al.*, 1966; Pons and Goldblatt, 1965). Briefly outlined, the procedure comprises extraction of the aflatoxins from the test material with 70% (v./v.) aqueous acetone, purification by lead acetate precipitations, partitioning the aflatoxins into chloroform, and thin-layer chromatographic separation of the aflatoxins on silica gel. Known amounts of aflatoxins are chromatographed on the plate with the test samples, and the spots visualized by their fluorescence under ultraviolet illumination. The amounts of aflatoxins in the test spots are estimated by visual comparison with the spots containing known amounts. Because of the difficulty of estimating small differences in fluorescent intensity with the

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eye, this assay is accurate to no more than about ±20%.

The cottonseed meal, CM-204, was a selected prepressed solvent-extracted sample and contained 144 p.p.b. aflatoxin B_1 and 70 p.p.b. aflatoxin B_2. No G aflatoxins could be detected. It is not known how this meal became contaminated with aflatoxins. Other analytical data on the meal include (moisture-free basis): 7.06% nitrogen, 15.2% crude fiber, 0.85% lipides, 0.03% free gossypol, and 0.94% total gossypol. The available lysine content was 3.42 grams per 16 grams of nitrogen. This meal had been ground so that about 89% would pass a 20-mesh screen, and it was used for the heat treatments without further grinding.

To obtain the specified moisture content, a weighed amount of the meal (moisture content 6.6%, as-is basis) was mixed with the calculated quantity of water to yield 500 grams of hydrated material. The mixture was blended for 10 minutes in a Model C-10 Hobart mixer equipped with a stainless steel bowl and beater, and then transferred to the bench-scale reactor. Under constant agitation, the temperature of the mixture was elevated to the specified value and maintained at this value for the designated time. After heating, the mixture was spread in a glass tray and allowed to air-dry at ambient temperatures for at least 24 hours before assaying for aflatoxins.

RESULTS AND DISCUSSION

The effects of treatments at 60° and 80° C. upon the aflatoxins of cottonseed meal CM-204 are presented in Table I. These data were obtained following a Latin square arrangement designed to survey the effects of the three variables temperature, moisture content, and time of heating with the minimal number of experiments. The data reveal that the 60° C. heat treatments were ineffective in reducing the aflatoxin B_1 content. Aflatoxin B_2 was reduced about 50%, but the total levels of aflatoxins were still unsatisfactorily high. Elevating the temperature of the treatments to 80° C. appeared to effect a more definite lowering of the B_1 level, particularly at the higher moisture contents.

The effects of heating at 100° C. for various periods and moisture contents are given in Table II. The 100° C. treatments were more effective than the two lower temperatures of Table I, and, in general, more reduction in aflatoxins was achieved by longer periods of heating for a given moisture content. Elevation of the meal moisture content from 6.6 to 15.0 and 20.0% also led to generally decreased aflatoxin levels, particularly after the longer heating periods. The greatest reduction was achieved at 30% moisture, but treatments at this relatively high moisture level were very difficult because the meal tended to form a tough, plastic mass which nearly stalled the agitator in the bench-scale reactor. Further increases in moisture content to destroy more aflatoxins were impractical because of the formation of this plastic mass, and increasing the temperature and the time of heating also appeared undesirable because of excessive darkening of the meal. Hence the lowest level of aflatoxins obtained under conditions deemed practical was approximately 43 p.p.b. (15.0% moisture, 150 minutes at 100° C.) to 44 p.p.b. (20% moisture, 120 and 150 minutes at 100° C.).

Table I. Heat Treatments of Cottonseed Meal CM-204[a]

Temp., ° C.	Time, Min.	Moisture Content, %	Aflatoxins in Heated Products, P.P.B.[b]		
			B_1	B_2	Total
60	30	6.6	144	27	171
60	60	15.0	144	27	171
60	90	20.0	144	20	164
80	30	20.0	108	27	135
80	60	6.6	135	27	162
80	90	15.0	108	20	128

[a] Cottonseed meal CM-204 contained 144 p.p.b. aflatoxin B_1 and 70 p.p.b. aflatoxin B_2, total 214 p.p.b. No aflatoxin G was detected in this meal or in any of its heated products.
[b] Aflatoxin contents expressed as p.p.b. or μg. per kg.

Table II. 100° C. Heat Treatments of Cottonseed Meal CM-204

Moisture Content, %	Time, Min.	Aflatoxins in Heated Products, P.P.B.		
		B_1	B_2	Total
6.6	30	145	36	181
6.6	60	108	36	144
6.6	90	108	36	144
6.6	120	108	Trace[a]	108+
6.6	150	108	Trace	108+
15.0	30	108	36	144
15.0	60	81	36	117
15.0	90	54	22	76
15.0	120	65	29	94
15.0	150	29	14	43
20.0	30	81	27	108
20.0	60	65	27	92
20.0	90	54	27	81
20.0	120	33	11	44
20.0	150	33	11	44
30.0	60	54	Trace	54+
30.0	150	31	Trace	31+

[a] Trace means less than 10 p.p.b. aflatoxin.

The destruction of aflatoxins achieved probably will be reflected in reduced toxicity of the treated meal, since previous work (Coomes *et al.*, 1966; Newberne, 1965) has indicated a reliable correlation between the toxic properties of peanut products and their aflatoxin contents as determined by thin-layer chromatography. Furthermore, there is evidence that this correlation is also valid for cottonseed products (Loosmore *et al.*, 1964). However, the U.S. Food and Drug Administration has not indicated that it will find an aflatoxin level of 44 p.p.b. acceptable. Therefore, the heat treatments described here apparently failed to achieve the desired degree of aflatoxin destruction.

Results similar to those found with cottonseed meal were obtained by heating a selected peanut meal contaminated with aflatoxins (total 111 p.p.b.). A 2-hour heat treatment at 100° C. in the presence of 30% moisture yielded a product containing a total of 73 p.p.b. aflatoxins. This reduction of about 34% was less than the reduction noted for the contaminated cottonseed meal under similar experimental conditions.

Efforts are being continued to develop a practical method for lowering the aflatoxin levels to acceptable values in con-

taminated cottonseed meals and in other oilseed products containing these mycotoxins.

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