Solvent Extraction of Aflatoxins from Oilseed Meals

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Aflatoxin can be removed or significantly reduced in cottonseed and peanut meals by extracting with a tertiary solvent system of 54% acetone, 44% hexane, and 2% water (by weight) or a binary solvent system of 90% acetone and 10% water (by weight). The tertiary solvent system simultaneously removes oil and aflatoxin from prepressed cake containing 12 to 15% oil, resulting in residual lipids content of approximately 1% and aflatoxin levels of less than 40 p.p.b. The binary solvent system has reduced the aflatoxin content of prepressed cottonseed and peanut meals to less than 10 p.p.b. in small scale batch extractions and less than 40 p.p.b. in continuous pilot plant extractions. Both solvent systems offer definite economically feasible methods for reducing the aflatoxin in cottonseed and peanuts to a level of 30 p.p.b. (μg. per kg.) or below.

The presence of molds in feed materials has been a problem facing producers, processors, manufacturers, and users of their products for many years. The occurrence of the Turkey X disease in England in 1960, attributed to toxic metabolites (termed aflatoxins) found in an extracted peanut meal used in poultry rations, emphasized the danger which can result from infestation of feeds by molds (Allcroft and Carnaghan, 1963; Sargeant and Carnaghan, 1963; Sargeant *et al.*, 1963).

Research has been under way to investigate methods for removing or inactivating aflatoxins from lots or batches of contaminated commodities. For the most part, the work has been concerned with peanuts and cottonseed. These oilseeds, when contaminated with aflatoxins, can be extracted or crushed and processed to yield edible refined oils completely free from aflatoxin (Parker and Melnick, 1966). Petroleum-type solvents, such as hexane, commonly used to extract the oil from oilseed meats or prepressed cake, do not remove significant quantities of the aflatoxins (Feuell, 1966; Vorster, 1966). It was prepressed hexane-extracted peanut meal containing a high level of aflatoxin that caused the great loss of poultry in England in 1960 (Feuell, 1966).

Polar solvents, such as methanol and acetone, completely extract aflatoxins from substrates (Feuell, 1966; Pons and Goldblatt, 1965; Vorster, 1966). Some aflatoxins can be "carried" by water-soluble components of oilseed meals (Feuell, 1966). Based on the above findings, analytical procedures have been developed at this laboratory for determining the level of aflatoxin in oilseed products by thin-layer chromatography (TLC) procedures following extraction of the products with acetone and water, and acetone, hexane, and water (Goldblatt, 1965; Goldblatt and Robertson, 1965; Pons *et al.*, 1965; Pons and Goldblatt, 1966; Robertson *et al.*, 1965).

This paper reports the results of bench scale and pilot plant research with some modification of the two analytical solvent systems to remove aflatoxins from cottonseed and peanut meals. The two solvent systems studied were: a tertiary solvent consisting of 54% acetone, 44% hexane, and 2% water (by weight) for simultaneously extracting oil and aflatoxin from prepressed peanut cake, and a binary solvent consisting of 90% acetone and 10% water (by weight) for extracting aflatoxin from pre-extracted peanut and cottonseed meals of low oil content. Pons and Eaves (1967) have reported another technique for extracting gossypol and aflatoxin from flaked cottonseed meats using 70% acetone (by volume) followed by conventional oil-extraction procedures.

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EXTRACTION OF AFLATOXIN AND OIL FROM PEANUT CAKE WITH TERTIARY SOLVENT SYSTEM

Material and Preparation. Several tons of Runner-type peanuts reported to be contaminated with 400 to 700 p.p.b. (μg. per kg.) of aflatoxins were obtained for this work.

The distribution of aflatoxin in contaminated peanuts is usually nonuniform (Cucullu *et al.*, 1966). Several peanuts in a thousand can contaminate an entire lot. Even these few peanuts may have varying levels of aflatoxin, presenting an extremely difficult sampling problem. To obtain the most representative sample, random and equal portions of each sack of peanuts as received were placed in 55-gallon drums and stored at 0° F. Prior to each pilot plant run, several drums were removed from storage, equilibrated to ambient conditions, and thoroughly mixed. However, even after what would normally be considered more than adequate mixing, the variation in assays for aflatoxin was often in excess of 100 p.p.b.

The peanuts were cracked through corrugated rolls spaced 0.035 inch apart, preheated to 180° F. in a French 5-high cooker, adjusted to about 14% moisture, and cooked for 30 minutes at 220° F. The cooked meats were then hydraulically pressed for 15 minutes under 5400-p.s.i.g. ram pressure. The pressed oil assays ranged from 24 to 50 p.p.b. of aflatoxin. The pressed cakes were fragmented, cracked, and flaked to a thickness of approximately 0.010 inch. Lipid content of the prepress cakes ranged from 12 to 15%.

Solvent Extraction. The extraction equipment consisted of a flake feeder and a horizontal, screw-type extractor discharging into an inclined drag conveyor. Ex-

traction took place in the horizontal extractor and in the lower third of the drag conveyor, which were filled with the tertiary solvent. Fresh solvent was fed into the drag conveyor at a point where the miscella begins to drain from the marc.

Product miscella was removed from the input end of the extractor into which the flakes were fed.

A solvent-flake ratio of 2 to 1 and extraction time of 70 minutes were maintained throughout the runs. Extraction temperature ranged from 98° to 110° F. The marc produced in the first run was desolventized under vacuum in a Patterson-Kelly tumble dryer (Table I). The marcs from runs 2 and 3 were desolventized in continuous Schnecken-type dryers. Pertinent processing conditions are outlined in Table I.

Analytical data on the flaked press cakes and the extracted meals are presented in Table II.

Moisture, lipids, and total nitrogen were determined by appropriate methods (American Oil Chemists' Society, 1960); epsilon amino free lysine was determined by the procedure of Rao *et al.* (1963); aflatoxins, by the method of Pons and Goldblatt (1965).

Results and Discussion. Because of difficulty in obtaining representative samples of the whole peanuts, the flaked cakes were selected as controls. Assays of the flaked press cakes were compared with those of the extracted meal products to determine the degree of aflatoxin reduction achieved. In the first run, aflatoxin B_1 content was reduced from 160 p.p.b. in the flaked cake feed to about 2 p.p.b. in the extracted and desolventized meal. Although this result was encouraging, it was not considered representative, since equilibrium processing conditions were not reached because of the short duration of this run. In runs 2 and 3, total aflatoxin (B_1, B_2, G_1, and G_2) was considered. These runs were longer and equilibrium conditions were approached, as confirmed by assays of consecutive meal samples. Although aflatoxin was considerably reduced (85 and 93%), the aflatoxin remaining in the extracted and desolventized meals was slightly in excess of the level (30 μg. per kg.) recommended in August 1966 by the Protein Advisory Group sponsored by the Food and Agriculture Organization, World Health Organization, and the United Nations Children's Fund (FAO/WHO/UNICEF Protein Advisory Group, 1967).

EXTRACTION OF AFLATOXIN FROM PEANUT AND COTTONSEED MEALS WITH BINARY SOLVENT SYSTEM

Analytical methodology research has shown that acetone-water (70:30, v./v.) is an effective solvent for aflatoxins in peanut and cottonseed meals. However, for the practical application of this aqueous polar solvent on a pilot plant basis, a 90-to-10 weight ratio of acetone to water facilitated processing and minimized solubilization of some of the meal components.

Batch Extraction. MATERIALS. The peanut and cottonseed meals used were produced by a prepress solvent-extraction process. The peanut meal assayed 113 p.p.b. of total aflatoxins and contained 0.70% lipids, 7.2% moisture, 9.11% nitrogen, and 2.78 grams epsilon amino free lysine per 16 grams of N, and had 82.44% nitrogen solubility. The cottonseed meal assayed 180 p.p.b. of total aflatoxins and contained 0.70% lipids, 7.0% moisture, 6.56% nitrogen, and 2.74 grams of epsilon amino free lysine per 16 grams of N, and had 71.80% nitrogen solubility.

EQUIPMENT AND PROCEDURE. In these small scale experiments, two insulated extractors were used: a glass column 3.16 inches in i.d. × 25 inches high fitted with a 20-mesh stainless steel screen bottom, with a capacity of approximately 3 pounds of meal, and a sheet metal extractor 8 inches square and 23 inches high, with a removable stainless steel filter screen and a capacity of approximately 15 pounds. Both extractors were equipped with valves to control the effluent drainage rates.

In each case the extractor was charged simultaneously with meal and solvent to minimize entrapment of air. This also permitted the addition of the solvent at a faster rate, which reduced the solvent temperature drop throughout the meal bed. After the initial charge to the glass column extractor, the solvent was added continuously at a

Table I. Processing Conditions for Continuous AHW[a] Extraction of Prepressed Peanut Cake

Run No.	Flake Thickness, Inch	Solvent-Meal Ratio	Solvent Feed, Temp., ° F.	Miscella Temp., ° F.	Extraction Time, Min.	Drying Time, Min.	Meal Temp., ° F.
1	0.014	2:1	110	98–110	69	60	...
2	0.009	2:1	108	108	72	60	205
3	0.010	2:1	105	106	72	60	205

[a] Solvent composition, wt. %: acetone 54, hexane 44, water 2.

Table II. Meal Analyses in Continuous AHW[a] Extraction of Prepressed Peanut Cake

Run No.	Lipids, %	Moisture, %	Aflatoxins, P.P.B. B_1	B_2	G_1	G_2	Total
Flaked press cake 1	14.74	9.92	160	..	...	...	...
Extracted meal 1	0.63	1.00	2	..	...	...	...
Flaked press cake 2	15.20	9.61	300	..	...	...	...
Extracted meal 2	1.35	5.2	19	10	2	T[b]	31+
Flaked press cake 3	12.81	8.84	114	69	24	ND[c]	207
Extracted meal 3	0.56	2.9	38	T[b]	ND[c]	ND[c]	38+

[a] Solvent composition, wt. %: acetone 54, hexane 44, water 2.
[b] Trace quantities.
[c] None detected.

Table III. Processing Conditions for Batch Aqueous Acetone Extraction of Prepressed Solvent-Extracted Peanut Meal

Run No.	Solvent Feed Temp., ° F.	Miscella Temp., ° F.	Mean Extraction Temp., ° F.	Extraction Time, Min.	Mass Velocity, Lb./Sq. Ft.-Hr.	Solvent in Marc, %
1	54	55	55	50	354	29
2	90	88	89	51	347	32
3	100	95	98	61	288	32
4	107	102	105	73	237	34
5	112	105	109	52	325	33
6	122	116	119	40	380	34

Solvent concentration, wt. %: acetone 90, water 10. Solvent-meal ratio per pass, 1.5 to 1. No. of passes or equivalent, 6.

rate equal to the effluent discharge. The total quantity of solvent added was nine times the weight of the meal, and was the equivalent of six passes, or exchanges, of solvent. The initial charge of solvent to the metal extractor was allowed to remain in contact with the meal for about 30 minutes before drainage. Five subsequent passes of heated solvent were added, allowing 10 minutes' residence time for each pass. The solvent level in each extractor was maintained approximately 1 inch above the meal bed. The extracted meal products from both extractors were air-desolventized at ambient temperatures and then thoroughly mixed prior to sampling. The conditions for extraction of aflatoxin from peanut meal are outlined in Table III and from cottonseed meal in Table IV.

RESULTS AND DISCUSSION. The aflatoxin assays of the peanut and cottonseed feed meals and the solvent-extracted meals are set forth in Table V. Acetone-water mixtures containing more than 10% of water removed excessive amounts of water-soluble meal constituents, and led to processing difficulties.

The effect of extraction temperature is illustrated by Figure 1, which shows that an almost straight-line relationship exists between extraction temperature and aflatoxin removal from peanut meal when the same solvent-meal ratio is used.

Compared with the effect of temperature, time of extraction had little influence on the removal of aflatoxin from peanut meal (Tables III and V). For example, in run 6, an extraction time of 40 minutes with an extraction temperature of 119° F. removed most aflatoxin (93%). By comparison, in run 4, an extraction time of 73 minutes and an extraction temperature of 105° F. resulted in only 85% aflatoxin removal.

Solvent temperature and extraction time had a similar effect on the extraction of aflatoxin from cottonseed meal. As indicated in Tables IV and V, an increase in solvent temperature resulted in a higher percentage reduction of aflatoxin (from 77 to 94% in runs 2 and 3) even though the extraction time was reduced.

Continuous Extraction. MATERIAL, EQUIPMENT, AND PROCEDURE. Prepressed solvent-extracted peanut meal assaying 256 p.p.b. of total aflatoxins was used for these studies. This meal contained 1.3% lipids, 6.0% moisture,

Table IV. Processing Conditions for Batch Aqueous Acetone Extraction of Prepressed Solvent-Extracted Cottonseed Meal

Run No.	Solvent Concn., % Acetone	Solvent Feed Temp., ° F.	Extraction Time, Min.	Mass Velocity, Lb./Sq. Ft.-Hr.	Solvent in Marc, %
1	93.7	63	90	365	..
2	90	100–75	135	230	..
3	90	114	60	543	35

Solvent-meal ratio per pass, 1.5 to 1, No. of passes, 6.

Table V. Aflatoxin Assays for Batch Aqueous Acetone Extraction of Prepressed Solvent-Extracted Peanut and Cottonseed Meals

Meal	Run No.	Aflatoxins, P.P.B.[a] B_1	B_2	G_1	Total
Peanut	Unextracted	68	34	11	113
	1	34	17	7	58
	2	12	17	ND[b]	29
	3	17	12	ND[b]	29
	4	10	7	ND[b]	17
	5	7	8	ND[b]	15
	6	4	4	T[c]	8+
Cottonseed	Unextracted	144	36	ND[b]	180
	1	87	22	ND[b]	109
	2	28	11	ND[b]	39
	3	7	4	ND[b]	11

[a] No aflatoxin G_2 detected in any unextracted or extracted meals.
[b] None detected.
[c] Trace quantities.

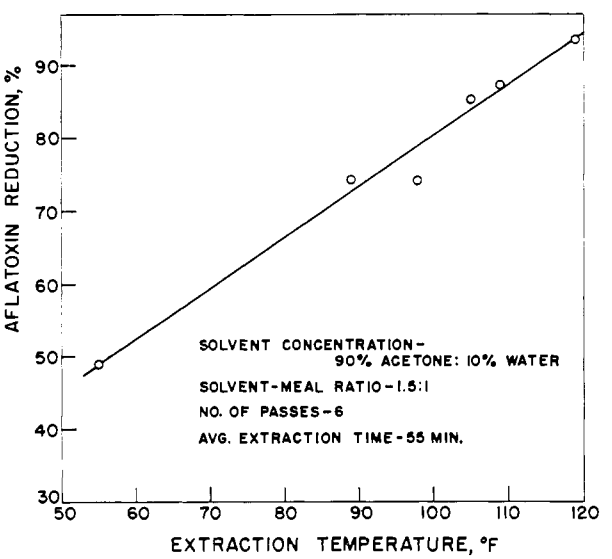

Figure 1. Aflatoxin reduction *vs.* extraction temperature

Table VI. Processing Conditions for Continuous Aqueous Acetone Extraction Prepressed Solvent-Extracted Peanut Meal

Run No.	Solvent-Meal Ratio	Solvent Feed Temp., ° F.	Miscella Temp., ° F.	Extraction Time, Min.	Solvent in Marc, %	Drying Time, Min.	Meal Temp., ° F.
1	1.3:1	105	118	70	...	60	212
2	1.5:1	111	118	77	40+	60	212
3	1.6:1	116	120	82	40+	60	203

Table VII. Meal Analyses in Continuous Aqueous Acetone Extraction of Prepressed Solvent-Extracted Peanut Meal

		Screen Analysis, %				Aflatoxins, P.P.B.[a]			
Run No.	Meal	On 20-mesh	On 60-mesh	On 100-mesh	Through 100-mesh	B_1	B_2	G_1	Total
	As received	51	38	6	5	142	57	57	256
1	Extracted	..	..	..	..	26	17	20	63
	Comminuted	8	72	10	10	...	..	..	...
2	Extracted	..	..	..	..	17	11	11	39
	Comminuted	22	64	8	6	...	..	..	...
3	Extracted	..	..	..	..	20	14	T[b]	34+

[a] No aflatoxin G_2 detected.
[b] Trace quantities.

8.21% nitrogen, 2.85 grams of epsilon amino free lysine per 16 grams of N, and had 82.58% nitrogen solubility. The continuous, immersion-type extractor was employed because of the simplicity of scaling up the process for commercial operations.

In three runs, meal particle size, solvent-meal ratio, and extraction time and temperature were varied. The conditions for each run are shown in Tables VI and VII.

RESULTS AND DISCUSSION. In the initial run, the peanut meal was extracted as received. By TLC assay, 75% of the total aflatoxin was extracted. In runs 2 and 3, the peanut meal as received was passed through flaking rolls with roll clearances of 0.018 and 0.021 inch, respectively, to make the material more accessible to the solvent.

The comminuted meal in run 2 was excessively fine, which resulted in reduced solvent percolation through the meal and reduced solvent drainage from the marc, as shown by solvent level fluctuations within the extractor and excessive solvent in the discharged marc. In spite of these problems, aflatoxin was reduced by 85% compared with 75% in run 1.

The peanut meal in run 3 had a particle size distribution ranging between that used in runs 1 and 2. The solvent feed rate and temperature were increased 6.5% and 5° F., respectively, over conditions for run 2. These changes resulted in a slight increase in aflatoxin removal, 88% compared with 85% for run 2. Solvent percolation through the meal bed and drainage of solvent from the marc were significantly improved.

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