Distribution of Aflatoxins in Various Fractions Separated from Raw Peanuts and Defatted Peanut Meal[1]

K.R. NATARAJAN, K.C. RHEE, C.M. CATER, and K.F. MATTIL, Food Protein Research and Development Center, Oilseed Products Division, Texas A&M University, College Station, Texas 77843

ABSTRACT

The present investigation is the first definitive study of the distribution of aflatoxins in a wet-milling process of raw peanuts. The results show that the majority of the aflatoxins originally present in the peanuts remained in the solid fractions, particularly the protein fraction, during wet-milling. In the protein concentrate preparation, the concentrates carried 81-89% of the total toxin; crude oil, 5-8%; and whey fraction, 3-14%. In the case of protein isolate preparation, 51-56% of the total toxin remained with the isolates, 22-26% with the residue, 11-17% with the whey, and 7-8% with the crude oil. Distribution of aflatoxins in the preparation of protein isolates from defatted peanut meal showed that 55-65% of the total toxin originally present in the meal remained with the protein isolates, 20-28% with the residue, and 10-20% with the whey fraction. Changes in extraction pHs for the preparation of protein isolates either from raw peanuts or defatted meal did not alter the distribution pattern mentioned above. A new approach based upon charge-transfer (electron acceptor-donor) complex formation is suggested to shift this aflatoxin distribution from protein products to disposable whey or residue fraction during the processing of raw peanuts and defatted meal for protein products.

INTRODUCTION

The aflatoxins are a group of toxic metabolites produced by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus*. They originally were isolated from a sample of Brazilian peanut meal contaminated with *A. flavus* that caused poisoning among poultry (turkey X-disease) and other farm animals (1). They have since been shown to have carcinogenic activity to rats (2). A recent epidemiological study conducted in Thailand suggests an association between aflatoxin consumption and incident of primary liver cancer (3). Due to their toxicity and carcinogenicity, in some animals, aflatoxins are considered potential threats to food safety and public health in some parts of the world.

Since a number of agricultural commodities, such as rice, wheat, corn, barley, oats, soybeans, cottonseed, peanuts, copra, and milk sometimes are contaminated with aflatoxins, the fate of aflatoxins in the processing of some of these commodities for food uses has been studied by a number of workers (4-7). Schroeder, et al., (4) reported that ca. 60-80% of the toxins in contaminated rice was found in the combined bran and polish fraction after milling. Ruark and Watson (5) reported the fate of aflatoxins in the wet-milling of corn; Purchase, et al., (6) found that processing of contaminated milk into cottage cheese reduced its aflatoxin M content. Parker and Melnick (7) reported that conventional processing practices, either mechanical or solvent extraction, leave, in the defatted meal, the vast majority of any aflatoxin that may be present in the raw peanuts. As the defatted peanut meal is likely to be contaminated with aflatoxin, it is generally unfit for human consumption and is not allowed for use in the U.S. in animal feed unless the aflatoxin content is below the Food and Drug Administration (FDA) guideline of 20 ppb. However, it may be exported to various foreign countries with the aflatoxin content stated on the ocean freight bill, because several foreign countries allow different aflatoxin levels in feeds for different animal species depending upon their sensitivity to aflatoxin. It has been suggested that the contaminated meal be used as fertilizer (8), thus reducing the intrinsic economic value of the crop.

In view of the growing importance of vegetable proteins for the development of low cost protein foods and for the supplementation of low protein diets, several attempts have been made to recover protein and carbohydrate by-products from the defatted peanut meal and raw peanuts. Recently, Rhee, et al., (9) reported a new processing method for the simultaneous recovery of oil and protein products from raw peanuts based upon the principle of wet-milling. Since aflatoxin contamination in peanuts continues to be a serious problem for the food industry, there is a need for a definitive investigation of the fate of aflatoxin in the raw peanuts and defatted peanut meal undergoing processing for protein products. The present report

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TABLE I

Composition and Aflatoxin Content of Raw Peanuts

Constituent	Sample I	Sample II	Sample III
Moisture, %	7.7	8.6	7.1
Oil, % (as is basis)	42.6	42.2	43.2
Nitrogen, % (as is basis)	4.9	4.8	5.2
Aflatoxin B_1, ppb	41	129	725
Aflatoxin B_2, ppb	6	32	148
Amount of toxin, μg/100 g	4.7	16.1	87.3

TABLE II

Composition and Aflatoxin Content of Peanut Meal

Constituent	Meal I	Meal II	Meal III
Moisture, %	10.0	11.5	9.2
Oil, % (as is basis)	0.3	1.2	0.7
Nitrogen, % (as is basis)	9.8	8.7	9.6
Aflatoxin B_1, ppb	136	363	1290
Aflatoxin B_2, ppb	36	95	247
Amount of toxin, μg/100 g	17.2	45.8	147.7

TABLE III

Distribution of Aflatoxins among Various Fractions of Protein Concentrate Process

Sample	Total aflatoxin content, μg[a]		
	Concentrate	Oil	Whey
I	4.1 (89)[b]	0.4 (8)[b]	0.1 (3)[b]
II	12.9 (81)	1.2 (7)	2.4 (14)
III	76.4 (88)	4.5 (5)	6.4 (7)

[a]Calculated from the wt of material recovered.

[b]The numbers in parenthesis indicate the percentage of toxin present in starting material which was found in the fraction.

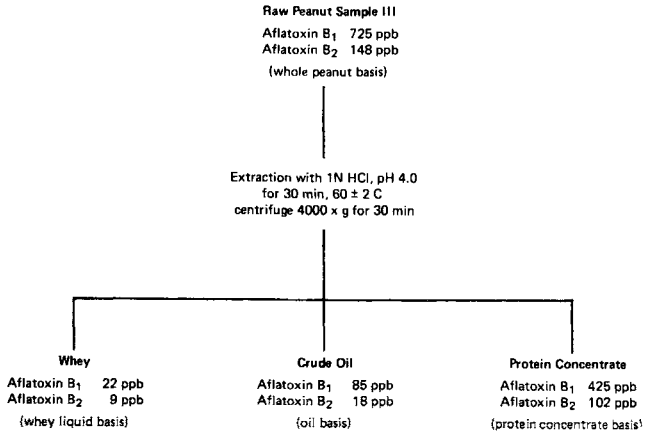

FIG. 1. Distribution of aflatoxins among the various fractions in the preparation of protein concentrates from raw peanuts.

describes the distribution of aflatoxins in the various products obtained from the above mentioned wet-milling process and from the defatted peanut meal in the preparation of protein isolates.

EXPERIMENTAL PROCEDURES

Materials and Methods

Peanut splits of Spanish type were used in all experiments. Split peanuts were blanched mechanically without heat to remove most of the skins and germs. The blanched kernels then were ground with an Urschel mill (Comitrol 3600) equipped with a medium head. Three peanut samples differing in their aflatoxin content were used. The composition and aflatoxin contents of these samples are given in Table I. No aflatoxin G_1 and G_2 were detected either in the raw peanuts or in the meal.

The peanut meal was produced by extraction with commercial hexane. The composition and aflatoxin content of three types of meal samples used in the experiments are given in Table II. Meal III was prepared from raw peanut sample III. Meal I and II were of different sources.

Both oil and protein concentrates or isolates were extracted simulatneously from raw peanuts by procedures described by Rhee, et al. (9,10).

Protein isolates from defatted meal were prepared as follows: peanut meal was suspended in deionized water to give 10% solids, and its pH was adjusted to the desired value (8.0, 9.0, or 10.0) with 1N NaOH solution. After 30 min extraction with continuous stirring at room temperature, the dispersion was centrifuged at 4000 x g for 30 min. Over 90% of the protein in the meal was extracted into the aqueous phase. The aqueous phase was removed, and its pH was adjusted to 4.5 with 1N HCl to precipitate the protein. A second centrifugation at 4000 x g resulted in the separation of protein isolates.

Raw peanuts, meal, and various fractions were assayed for aflatoxin by the method of Pons, et al. (11). Briefly, the procedure involved extraction of the aflatoxin from the samples with 85% v/v aqueous acetone, purification by precipitation with lead acetate, partitioning of aflatoxins into chloroform, purification of the extract on an acidic alumina (Brockman activity 2, 80-200 mesh) column, separation of aflatoxins on thin layer chromatographic (TLC) plates coated with Adsorbosil-1 (Applied Science Laboratories, State College, Pa.), and visual evaluation of the intensity of fluorescence of test spots viewed under long wave UV light. Moisture content of the fractions was taken into consideration to make up 85% acetone for extraction. In some cases, whey liquid was freeze-dried and aflatoxin content determined in the whey solid for comparison with the whey liquid. Unless otherwise specified, the data presented represent the average of three replicate analyses.

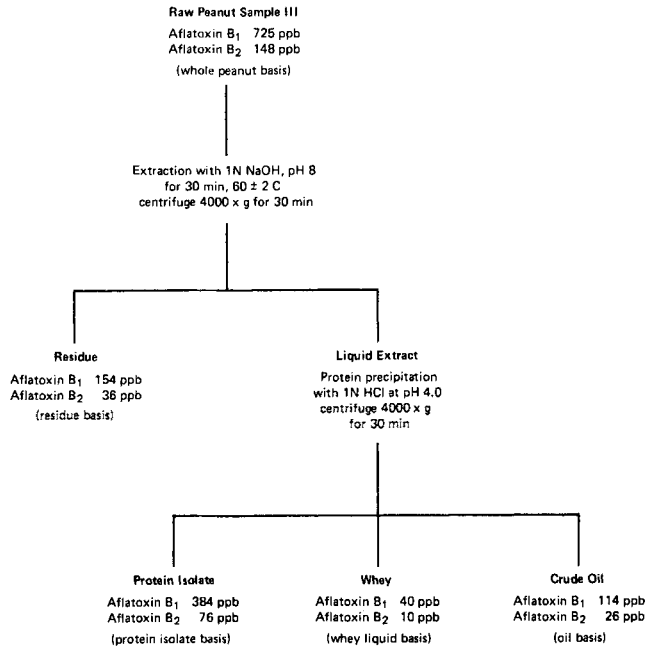

FIG. 2. Distribution of aflatoxins among the various fractions in the preparation of protein isolates from raw peanuts.

RESULTS AND DISCUSSION

Fate of Aflatoxin in Protein Concentrate Procedure

Distribution of aflatoxins among the various products from sample III in wet-processing for the recovery of oil and protein concentrates is given in Figure 1. Most of the aflatoxin remained in the protein concentrates. Crude oil and whey liquid contained ca. 5 and 7%, respectively, of the total toxin present in the sample.

Table III presents the results obtained with the three samples. The initial toxin levels of the 3 batches of raw peanuts were 4.7, 16.1, and 87.3 μg/100 g sample, respectively. The protein concentrates from these samples carried 4.1, 12.9, and 76.4 μg toxin, respectively. It is clear from these data that 81-89% of the total toxin in the raw peanuts remains with the protein concentrates, 5-8% with the crude oil and 3-14% with the whey fraction.

Fate of Aflatoxin in Protein Isolate Procedure

Distribution of aflatoxins among the various products obtained from sample III in wet-processing for the recovery of oil and protein isolates is given in Figure 2. Results obtained with various samples (Table IV) show that 51-56% of the total toxin remains with the protein isolates, 7-8% with the oil, 11-17% with the whey, and 22-26% with the residue. It is clear from these data that the major portion of the aflatoxins in the raw peanuts is found in the solid fractions which are composed of the protein isolates and residue. Similar results were obtained with protein isolates made at pHs 9 and 10. A comparable situation was found in lipid-protein complex or lypro process (12) and van der Berg's process (13). Smith (12) reported that much of the toxin originally present in the nut was found in the lypro. van der Berg (13) reported that ca. half of the original toxin in the nuts was present in the protein isolates made from raw peanuts. One very important observation in the isolate procedure is that, when aflatoxin containing peanuts were dispersed in water and the pH adjusted to the desired values, there was a significant reduction in detectable aflatoxin content in the dispersion of ca. 40%. However, when

TABLE IV

Distribution of Aflatoxin among Various Fractions of Protein Isolate Process

Sample	Total aflatoxin content, μg[a]			
	Isolate	Oil	Whey	Residue
I	2.6 (56)[b]	0.3 (7)[b]	0.7 (14)[b]	1.1 (24)[b]
II	8.5 (53)	1.1 (7)	1.8 (11)	4.2 (26)
III	44.8 (51)	6.7 (8)	15.1 (17)	18.9 (22)

[a]Calculated from the wt of material recovered.

[b]The numbers in parenthesis indicate the percentage of toxin present in starting material which was found in the fraction.

TABLE V

Distribution of Aflatoxins among Various Fractions of Protein Isolate Process from Peanut Meal

Meal	Total aflatoxin content, μg		
	Isolate	Whey	Residue
I	9.5 (55)[b]	2.3 (18)[b]	4.8 (28)[b]
II	26.6 (58)	9.2 (20)	9.2 (20)
III	96.7 (65)	14.8 (10)	35.6 (24)

[a]Calculated from the wt of material recovered.

[b]The numbers in parenthesis indicate the percentage of toxin present in starting material which was found in the fraction.

the pH of the dispersion was readjusted to 4.0 to precipitate proteins, the total detectable aflatoxin content again increased to the original value.

Fate of Aflatoxin in Protein Isolate Preparation from Peanut Meal

Our results indicate that during the solvent extraction of raw peanuts 1-3% of the original toxin goes with the oil, with the meal retaining 97-99% of the original toxin. Our results confirm those results reported earlier by Parker and Melnick (7,14), who found only a trace of aflatoxin in the oil with the solvent extracted meal still retaining 99% of the original toxin.

It can be seen from the data that the aflatoxin content of crude oil from the wet-process is two-three times higher than that in solvent or mechanically extracted oils. Parker and Melnick (7) established quite conclusively that conventional processing and refining of peanut oil, deliberately prepared to contain high levels of aflatoxin, remove essentially all of the toxin. Since crude oil obtained in the wet-processing is subject to further alkaline refining and bleaching, aflatoxin in the crude oil does not appear to pose a serious health problem.

Figure 3 shows the distribution of aflatoxins among the various fractions during the isolation of protein from defatted peanut meal. The initial toxin levels of the three batches of meal were 17.2, 45.8, and 147.7 μg/100 g meal, respectively. The protein isolates prepared from these samples carried 9.5, 26.6, and 96.7 μg original toxin (Table V), while the toxin level in the whey and the residues of the 3 samples in order were 2.3, 9.2, and 14.8 μg and 4.8, 9.2, and 35.6 μg. On a percentage basis, the results presented in Table V show that 55-65% of the total toxin goes with the protein isolates, 20-28% with the residues, and 10-20% with the whey fraction. While this work was being completed, Basappa, et al., (15) reported the distribution of aflatoxin in the various fractions separated from peanut cake flour which contained much lower concentrations of toxin than the samples used in the present experiments. It was reported that, using an extraction pH of 9.5 and protein precipitation pH of 4.8, 50-60% of the total toxin remained with the protein fraction, 30% with the residue, and 10-18% with the supernatant. Our results showed a higher level of toxin in the protein isolates. We observed that the

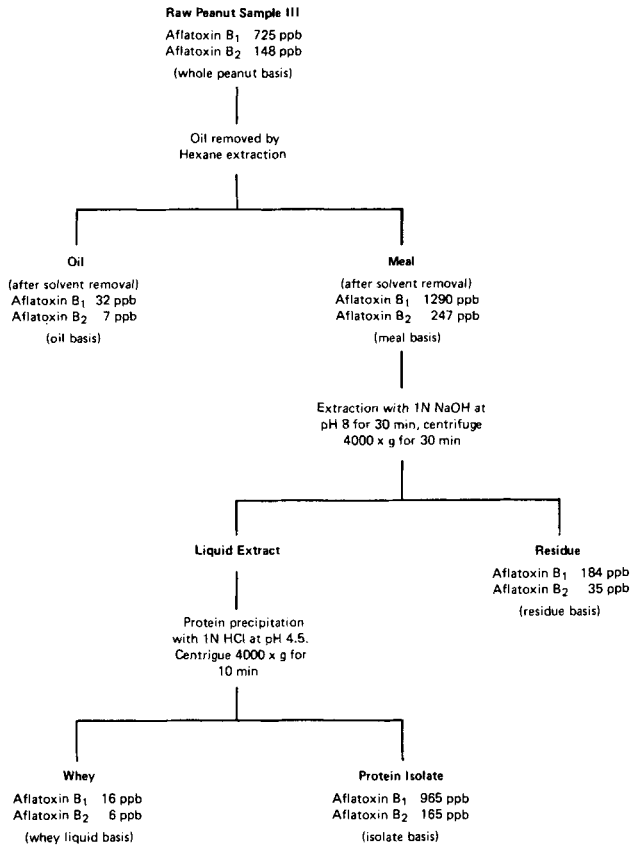

FIG. 3. Distribution of aflatoxins among the various fractions in the preparation of protein isolates from defatted peanut meal.

amount of toxin in the protein isolates prepared at pHs 9 and 10 were not different from the isolates prepared at pH 8. The protein isolates prepared from raw peanuts contained 4-9% less than the amount going with the protein isolates from defatted meal. As in protein isolates from raw peanuts, the aflatoxin content in the alkaline dispersion was reduced significantly and increased to the original value after acidification. This may be due to opening of the lactone ring in the aflatoxin molecule under alkaline conditions (16) to give a salt of substituted o-Coumaric acid which is nonfluorescent (7) and to the inability of chloroform to extract the toxin under alkaline conditions (17). The fact that the lactone ring is reformed under acidic conditions suggests that the sodium derivative can be reconverted to the original lactone and, thus, can be potentially as toxin as the aflatoxins (18). In the light of these findings, it is suggested that caution should be exercised in interpreting the results of alkaline treatment for detoxication of aflatoxins.

The results presented in this report indicate that the protein isolates prepared from either raw peanuts or defatted peanut meal contained over 50% of the toxin originally present in the starting materials. Therefore, the toxin levels in the protein isolates were still much higher than the level of aflatoxin permissable in food products (19). It is essential that the significance of the concentration of the toxin in the protein products be recognized and efforts be made to reduce the aflatoxin content below this level. The FDA of the U.S. Department of Health, Education, and Welfare advised that a guidelines of 20 ppb would be used in routine regulatory actions (19). The guideline can be changed by FDA administrative decisions (20).

From the results presented in Tables IV and V, it is obvious that the amount of toxin associated with the protein fraction is ca. 15-20% higher than the amount associated with the residue and the supernatant together. This suggests

that the toxin may have a greater affinity for the protein fraction than the other two fractions. This can be explained on the basis of the charge-transfer interaction between aflatoxin and π-electron donors in macromolecules (21,22). Noh and Chu (23) reported evidence for the electron accepting properties of aflatoxin B_1 based upon its ability to interact with electron donors. Electron donating amino acids, such as histidine, pheylalanine, tyrosine, and tryptophan, in the peanut proteins and other electron donating moleculares in the raw peanuts or meal may form charge-transfer complexes with aflatoxins and bind the toxin firmly.

It would be reasonable to speculate that, by using certain electron donating organic or inorganic compounds which would not only enhance the electron acceptability for aflatoxins but also form charge-transfer (electron donor-acceptor) complexes more readily than the electron donating amino acids in the proteins, it may be possible to prevent the toxin from going with the protein fraction. Mann, et al., (24) found lower aflatoxin levels when contaminated peanut meal was treated with organic nitrogen compounds which are good electron donors as compared with inorganic acids and bases. According to this work, treatments with ethylenediamine and methylamine yielded products in which no aflatoxins could be detected. It is probable that these reagents may have formed complexes with aflatoxins which were not extractable by either acetone or chloroform. However, more research is needed on the interaction between aflatoxins and electron donor compounds and the distribution of aflatoxins in the presence of electron donating compounds during processing of raw peanuts and peanut meal for protein products. Since considerable amounts of toxin are retained in the residue and whey fractions in the isolate procedures, it is also important to investigate what causes these fractions to retain the toxin.

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