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Fate of aflatoxin B_1 during the industrial production of edible defatted peanut protein flour from raw peanuts

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Peanut protein, isolated as a by-product from the peanut industry, is a cheap source of good-quality vegetable protein. Peanuts are high-risk agricultural commodities for natural contamination by aflatoxins. The present investigation highlights the fate of aflatoxin B_1 during the industrial processing of naturally contaminated raw peanuts to edible defatted peanut protein flour, meant for human consumption. Raw peanuts were separated, defatted and further processed to produce edible peanut protein flour. Samples taken at each stage were analysed for aflatoxin B_1. Shrivelled peanuts had the highest content of aflatoxin B_1 (126 ± 11 ppb), while the hand-picked selected peanuts had the lowest (9 ± 4·2 ppb). Defatted peanut protein flour had a higher level of aflatoxin B_1 (52 ± 6·9 ppb) than the hand-picked selected peanuts. The possible mechanism for accumulation of aflatoxin B_1 in the peanut protein flour, which is the final product of the industrial processing, is discussed.

INTRODUCTION

Cultivated peanut (*Arachis hypogaea*) is the most important oilseed crop in the developing world. India is, by far, the single most important peanut producer in the world with an estimated production, during the year 1989–1990, of about 16·7 million metric tonnes, accounting for 81% of the total oilseed production in the country (Chandhok *et al.*, 1990). Further, the peanut is an important source of high-quality cooking oil and a valuable source of vegetable protein for human and animal nutrition. The nutritive quality of peanut protein has been the subject of several studies in the past and has been periodically reviewed (Ayres & Davenport, 1977; Lusas, 1979; Natarajan, 1980; Rhee, 1985). A number of peanut protein ingredients have been developed for use in the food industry, with protein content ranging from 25 to 95%. These products include flakes, meals, flour, grits, protein concentrates, protein isolates and protein co-isolates (Natarajan, 1980; Rhee, 1985).

Peanuts are high-risk agricultural commodities for natural contamination by aflatoxins. Aflatoxins are a group of closely related heterocyclic compounds produced by *Aspergillus flavus* and *A. parasiticus*. Peanuts are susceptible to the attack of aflatoxin-producing fungus and subsequent elaboration of toxin under conducive conditions, during pre-harvest, post-harvest and storage stages (Mehan *et al.*, 1991).

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The presence of aflatoxin in raw peanuts and peanut products has been well documented (Mehan *et al.*, 1991; Jelinek *et al.*, 1989). Aflatoxin contamination of agricultural commodities has gained global significance as a result of its deleterious effect on human and animal health, and its importance in the international trade (Jelinek *et al.*, 1989; Anon, 1989; Ellis *et al.*, 1991; Sashidhar *et al.*, 1992).

For developing countries, peanut protein constitutes an enormous source of cheap food protein. Commercially, peanut protein and its isolates find wide application in the formulation of low-cost protein foods for infant and children feeding programmes in the areas where malnutrition is common. However, aflatoxin, being an adventitious toxic contaminant present in peanuts and peanut products, continues to be a serious problem to the food industry.

The present report investigates the fate of aflatoxin B_1 during the commercial processing of raw peanuts to edible defatted peanut protein flour which is used as an ingredient for food.

MATERIALS AND METHODS

Chemicals

All reagents and solvents used were of analytical grade. Aflatoxin B_1 standard was procured from Sigma Chemical Co., St Louis, USA. The purity and concentration of

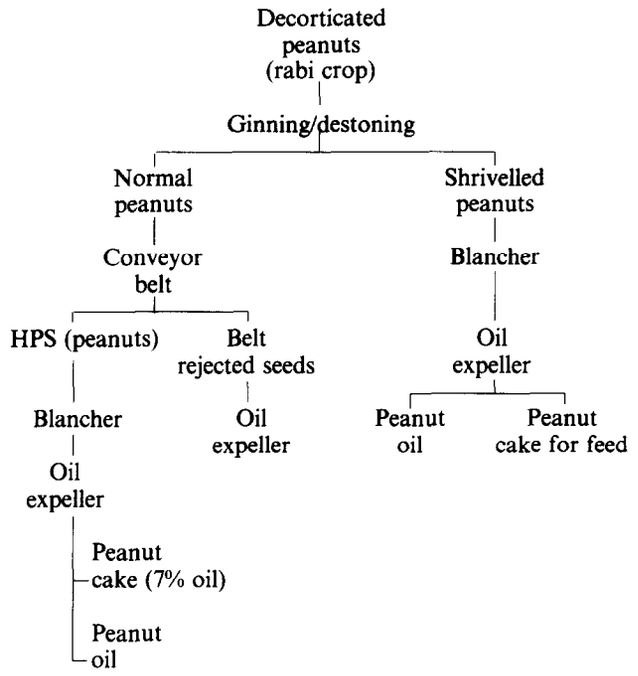

Fig. 1. Industrial production of edible defatted peanut protein flour: processing stage I. HPS, hand-picked selected.

aflatoxin B_1 were further determined by UV absorption at 360 nm in a Beckman DU-50 spectrophotometer. The aflatoxin B_1 reference standard was made up in benzene/acetonitrile (98 : 2) and stored in a refrigerator until further use at 4°C.

Sampling

In-line sample lots of peanuts and peanut protein isolates were drawn randomly during the in-process manufacture, as recommended by FAO (Anon., 1988). These lots were pooled and sub-sampled, by systematic random sampling, so as to draw five representative samples of 100 g each from the pooled sub-samples, which were further used for aflatoxin analysis. The samples selected for aflatoxin analysis included ones from various stages of the processing, from raw peanut to defatted peanut protein flour. The sequence of processing includes (1) decortication (raw peanuts), (2) destoned seeds, (3) shrivelled seeds, (4) belt-rejected seeds, (5) hand-picked selected (HPS) seeds and (6) defatted peanut protein flour.

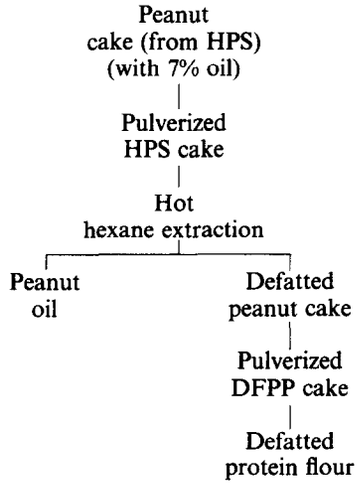

Fig. 2. Industrial production of edible defatted peanut protein flour: Processing stage II. DFPP, defatted peanut protein cake.

Analysis of aflatoxin B_1 and proximate principles in peanuts and defatted protein flour

Aflatoxin B_1 was quantified by a TLC–fluorodensitometric method (Egan, 1982), on a Bio-Med laser scanning ID densitometer. The Contamination Bureau (CB) method (Egan, 1982) was adopted for extraction and purification of aflatoxin B_1 from peanut and defatted peanut protein flour. The moisture, protein and oil contents of peanut samples and defatted protein flour were analysed by AOAC methods (Williams, 1984).

Industrial production of defatted peanut protein flour

Figures 1 and 2 give the flow sheets for the industrial processing of the raw peanut to defatted peanut protein flour. Briefly, the peanuts from the Rabi crop (post-rainy season crop) were decorticated. The decorticated seeds were further destoned and ginned, to yield normal peanut seeds and shrivelled seeds. Normal peanuts were further segregated into lots consisting of hand-picked selected (HPS) and rejects (small-size seeds, visually contaminated seeds, shrivelled seeds, etc.), while they were moving on the conveyor belt. HPS seeds were blanched, before the extraction of oil by an oil expeller. The temperature during the extraction process was 60°C. This process yielded crude peanut oil and HPS cake (with 7% oil). Refined peanut oil was obtained by further refinement after treatment with alkali (NaOH) and activated charcoal. Similarly, the oil was extracted from belt-rejected peanuts and shrivelled seeds. The HPS cake so obtained was further subjected to solvent extraction (hot hexane treatment) to yield peanut oil and defatted HPS cake. Edible defatted peanut protein flour was further processed from defatted HPS cake, after pulverizing and vacuum-drying (Fig. 2).

RESULTS

Table 1 gives the gross composition and aflatoxin B_1 content in the decorticated peanuts, which were used in the production of defatted peanut protein flour. Figures 1 and 2 give the detailed flow sheets of the

Table 1. Gross composition of decorticated peanuts used in the production of peanut protein isolates

Component	Average (%)
Moisture	6·0
Protein	25·6
Oil	48·0
Aflatoxin B_1[a]	56·8 ± 10·33

[a] Mean ± SD (n = 5); concn of toxin ($\mu g\ kg^{-1}$, i.e. ppb).

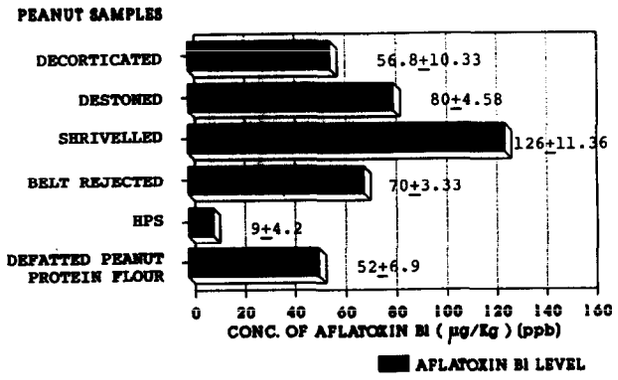

Fig. 3. Aflatoxin levels in peanuts during processing. The values are means ± S.D.

processes involved in the production of defatted peanut protein flour from raw peanuts procured in the Rabi (post-rainy) season. Other important products obtained during the process are refined peanut oil and peanut cakes for animal feed purposes. Figure 3 gives a profile of the aflatoxin B_1 levels at various stages of peanut protein processing. It was observed that the shrivelled peanuts had the highest content of aflatoxin B_1, while the HPS seeds had the lowest. HPS seeds constituted approximately 65% of the load, while shrivelled peanuts were 10% per load. Further, the aflatoxin B_1 concentration (52 ± 6·9 ppb) in the defatted peanut protein flour was higher than the permissible limit (30 ppb), even though the HPS seeds had 9 ± 4·2 ppb, which is well within the permissible limit according to the Government of India legislation (Trivedi, 1986). It was observed that the process of hand-picking and selection of contaminated seeds had drastically reduced the aflatoxin B_1 level in HPS seeds. The protein content of the defatted peanut protein flour was 90%.

DISCUSSION

Peanuts are an important food and cash crop in India and other developing countries of the world. Peanut protein, isolated as a by-product from the peanut industry, is a cheap source of good-quality protein. Peanut protein has been used to improve the protein content and quality of cereal-based foods, for human consumption in India and other developing countries. These formulations have been successfully used to counter the problem of malnutrition faced by developing countries of the world, under various social welfare programmes sponsored both by the Government and by non-Governmental agencies (Natarajan, 1980; Rhee, 1985; Kadam & Chavan, 1991).

However, the food industry processing the peanut protein to various formulations used for the supplementation programmes has been confronted with the problem of aflatoxin contamination. The present investigation was undertaken to study the systematic distributions of aflatoxin during the processing and production of naturally contaminated raw peanuts to defatted peanut protein flour. The results indicate that the aflatoxin B_1, originally present in the raw peanuts, was not totally lost in the processing of the defatted peanut protein flour, which is the final product of the industrial process. It is interesting to note that the HPS peanuts had very low toxin content as compared with the defatted protein flour (Fig. 3). During the processing (Fig. 2), defatted peanut protein flours were obtained from the HPS cake after solvent extraction (treatment with hot hexane). The higher level of aflatoxin B_1, observed in the defatted peanut protein flour can be attributed to the binding of aflatoxin B_1 (being highly hydrophobic) to the hydrophobic amino acid residues present in the peanut proteins, as the toxin is insoluble in hexane. Possibly the presence of hexane enhances the interactions between the toxin and the hydrophobic residues of the protein which are likely to be exposed during solvent extraction. Aflatoxin B_1 may have a greater affinity for protein fractions than hexane. This can be explained on the basis of the charge-transfer interaction between aflatoxin and π-electrons donors in amino acids such as tyrosine, phenylalanine, histidine and tryptophan present in the protein (Rayner & Dollear, 1968; Lilley, 1985). Further, the reduction in weight of HPS cake due to extraction of oil (with 7% oil) by solvent to defatted protein flour would contribute a marginal increase in aflatoxin B_1 content in the defatted peanut protein flour.

Peanut protein has the potential to meet the growing need for cheap and commercially viable food protein for various supplementation programmes undertaken in developing countries. In India, high-protein supplements based on peanut protein have been formulated for mass feeding programmes to counter undernourishment among pre-school children under the Indian Multipurpose Foods (IMPF) scheme; they are based on peanut protein and bengal gram (*Cicer arietnum*) (Prassannappa *et al.*, 1972). Miltone, a commercial product based on blending equal parts of peanut protein isolate and milk powder, was developed in India for feeding children, pregnant mothers and nursing mothers under social welfare projects (Chandrashekhara, 1991). The potential use of peanut protein flour in developing composite formulations based on cereals and legumes on a commercial scale for preparation of traditional Indian foods such as roti, chapati and bhakri (Indian bread) has been explored (Kadam & Chavan, 1991). In view of the present finding, it is important that the quality of the peanut protein used in these commercial preparations be free of aflatoxin contamination. The food safety aspect is of utmost importance in these preparations, as they enter the most susceptible group of the human population, who may thus be directly exposed to the deleterious effects of dietary aflatoxin.

Keeping in view the vast potential of peanut protein as a source of cheap protein for the developing countries, it is important to ensure toxin-free products for various commercial exploitations of peanut by-products by the peanut industry. Methods for detoxification based on chemicals such as hypochlorite, hydrogen peroxide and alkali treatment may result in unfavourable changes in

the protein which affect the protein quality. Simple methods, based on solvent treatment of isolated protein (physical processing), may be used for removing the toxin present in the peanut protein flour before marketing the products (Rhee, 1985; Rayner & Dollear, 1968; Rhee *et al.*, 1977).

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