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Aflatoxin in food and feed: occurrence, legislation and inactivation by physical methods

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Aflatoxins are secondary metabolites of the molds *Aspergillus flavus* and *Aspergillus parasiticus*. They are highly toxic, mutagenic, teratogenic and carcinogenic compounds found to contaminate a wide variety of important agricultural products such as peanuts, maize, rice and cottonseed. Aflatoxins are found in many countries, especially in tropical and subtropical regions where conditions of temperature and humidity are optimum for growth of the molds and for production of the toxin. Removal or inactivation of aflatoxin in food and feedstuffs is a major global concern. Aflatoxins can be detoxified or removed from contaminated foods and feeds by physical, chemical and biological methods. This article reviews some aspects of aflatoxins concerning their occurrence in food and feedstuffs, legislation and detoxification by physical methods. The physical methods include solvent extraction, adsorption, heat treatment and irradiation.

INTRODUCTION

The problem of food and feed contamination with aflatoxins is of current concern and has received a great deal of attention during the last three decades. The frequent incidence of these toxins in agricultural commodities has a potential negative impact on the economies of the affected regions, especially in the developing countries where harvest and post-harvest techniques adequate for the prevention of mold growth are seldom practised.

Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds that have been implicated as causative agents in human hepatic and extrahepatic carcinogenesis (Massey *et al.*, 1995). There is enough evidence from epidemiological studies regarding the hazardous nature of aflatoxins, as potent carcinogens, in connection with incidence of liver cancer in populations exposed to the toxin from contaminated foods (Shank *et al.*, 1972; Peers & Linsell, 1973; Dichter, 1984; Groopman *et al.*, 1988). Human exposure to aflatoxins can result directly from ingestion of contaminated foods, or indirectly from consumption of foods from animals previously exposed to aflatoxins in feeds. Moreover, aflatoxins have been associated with incidence of the disease kwashiorkor, a consequence of protein energy malnutrition, in children (Adhikari *et al.*, 1994).

Ingestion of aflatoxins leads to substantial loss of productivity and degradation of meat quality in farm animals consuming contaminated feeds (Bonomi *et al.*, 1993, 1994). In 1986 there was an estimated loss of about 140 million US dollars as a direct sequence of weight loss in broilers consuming low levels of mycotoxins (Palmgren & Hayes, 1987).

Aflatoxins are secondary metabolites produced by species of *Aspergillus*, specifically *Aspergillus flavus* and *Aspergillus parasiticus*, which are found worldwide in air and soil and are found to infest both living and dead plants and animals. The discovery of aflatoxins dates back to the year 1960 following the severe outbreak of the turkey 'X' disease, in the UK, which resulted in the deaths of more than 100 000 turkeys and other farm animals. The cause of the disease was attributed to a feed, containing Brazilian peanuts, which was heavily infested with *A. flavus*. Subsequent analysis of the feed using thin-layer chromatography (TLC) revealed that a series of fluorescent compounds, later termed aflatoxins, were responsible for the outbreak (Sargeant *et al.*, 1961; Bash & Rae, 1969; Davis & Diener, 1979). Hence, the name aflatoxin, an acronym, has been formed from the following combination: the first letter, 'A' for the genus *Aspergillus*, the next set of three letters, 'FLA', for the species *flavus*, and the noun 'TOXIN' meaning poison (Ellis *et al.*, 1991).

Efforts have been made to remove or detoxify aflatoxins in contaminated foods and feedstuff (Doyle *et al.*, 1982; Samarajeewa *et al.*, 1990; Beaver, 1991). This paper reviews some aspects of aflatoxins concerning their occurrence in food and feedstuffs, legislation and detoxification by physical methods.

OCCURRENCE

Many agricultural commodities in general, and peanuts in particular, are liable to infestation by aflatoxigenic molds, and thereby contamination with aflatoxins. In this context, aflatoxicosis is becoming a worldwide problem. Other important crops such as maize, rice and cottonseed have also been found to be infested by aflatoxigenic molds in a number of different countries (Table 1). The incidence of aflatoxins in foods and feeds is relatively high in tropical and subtropical regions (Table 2), where the warm and humid weather provides optimal conditions for the growth of the molds. The growth of *A. flavus* and production of aflatoxin in natural substrates are influenced by a number of factors, including the type of substrate, fungal species, moisture content of the substrate, presence of minerals, relative humidity of the surroundings, temperature and physical damage of kernels (Viquez *et al.*, 1994). Production of aflatoxin B_1 (AFB1) by *A. flavus* and *A. parasiticus* is higher in rice than in peanut (Pinto *et al.*, 1991). The minimum temperature range for *A. parasiticus* growth is 6–8°C, the maximum is 44–46°C, and the optimum is 25–35°C (Diener *et al.*, 1982). *A. flavus* can produce aflatoxin at temperatures of 12–42°C, but the optimum is 28–30°C (Brackett, 1989). The minimum moisture content of peanuts necessary for *A. flavus* growth is 8–10% at about 82% relative humidity, and aflatoxin production on peanuts is optimum at 15–35% moisture content (Brackett, 1989). Immature, broken, undersized, loose-shelled, rancid and discolored peanut kernels are most likely to be contaminated, in comparison with mature sound kernels (Davis & Diener, 1979; Beaver, 1991; Chiou *et al.*, 1994; Rucker *et al.*, 1994). Physical damage of peanut shell facilitates its penetration by the molds and thereby contamination with aflatoxin (Porter *et al.*, 1986). The physical damage can also improve the hygroscopic characteristics of peanuts during storage, thus leading to higher incidence of the molds as a result of increased moisture content of the kernels (Chiou *et al.*, 1984). Exposure of peanut pods to drought stress also increases the risk of contamination (Sanders *et al.*, 1993).

Peanuts of all varieties in all geographical locations are liable to contamination with aflatoxins in the field before and after harvest, during curing and storage. Some documented cases of aflatoxin incidence in peanuts and peanut products are listed in Table 3. The reason why the incidence is more frequent in peanuts than in other agricultural commodities is not fully understood. However, it could be attributed to the fact that *A. flavus* dominates the mycoflora of peanut field soils, where the kernels develop and mature beneath the surface (Diener *et al.*, 1982).

INACTIVATION

With increasing knowledge and awareness of aflatoxin as a potent source of health hazards to both man and farm animals, a great deal of effort has been made to completely eliminate the toxin or reduce its content in foods and feedstuffs to significantly lower levels.

Table 1. Occurrence of *Aspergillus* in some agricultural commodities

Commodity	Country	Species	Reference
Peanuts	Sudan	*A. flavus*	Elamin *et al.*, 1988
	Egypt	*A. flavus* + *A. niger*	Moubasher *et al.*, 1980
	S. Africa	*A. flavus* + *A. parasiticus*	Dutton & Westlake, 1985
Maize	India	*A. flavus*	Gaur & Siradhana, 1989
	China	*A. flavus*	Zhen-Zhen, 1989
	Uganda	*A. flavus* + *A. parasiticus*	Sebunya & Yourtee, 1990
	Nigeria	*A. flavus* + *A. parasiticus* + *A. niger*	Aja Nwachukwu & Emejuaiwe, 1994
	USA	*A. flavus*	Guo *et al.*, 1995
Wheat	China	*A. flavus*	Zhen-Zhen, 1989
	Russia	*A. flavus*	L'Vova *et al.*, 1993
Rice	China	*A. flavus*	Zhen-Zhen, 1989
	India	*A. flavus* + *A. parasiticus*	Jayaraman & Kalyanasundaram, 1990
Millet	India	*A. flavus* + *A. parasiticus*	Mishra & Daradhiyar, 1991
Soybean	Argentina	*A. flavus* + *A. parasiticus*	Pinto *et al.*, 1991
Sunflower oil	China	*A. flavus*	Zhen-Zhen, 1989
	Russia	*A. flavus*	L'Vova *et al.*, 1993
Coconut	India	*A. flavus*	Bilgrami & Choudhary, 1993
Pistachio nuts	USA	*A. niger* + *A. flavus* + *A. parasiticus*	Doster & Michailides, 1994
	Turkey	*A. flavus*	Heperkan *et al.*, 1994
Figs	Switzerland	*A. flavus* + *A. parasiticus*	Steiner *et al.*, 1988
Mustard seed	India	*A. flavus*	Sahay & Prasad, 1990

Pre-harvest contamination can be reduced by introduction of good crop husbandry and appropriate cultural practices that limit the growth of aflatoxigenic fungi. Post-harvest contamination can be minimized by application of proper curing, drying, sorting and storage procedures. However, the contamination is often unavoidable and still remains a serious problem associated with many important agricultural commodities, which emphasizes the need for a suitable process to inactivate the toxin. In addition to the capability of a

Table 2. Occurrence of aflatoxins in some foods and feed

Food	Country	Contaminated/ total examined	Aflatoxin	Concentration ($\mu g\ kg^{-1}$)	Reference
Rice	China	33/252	B1	5–50	Zhen-Zhen, 1989
	India	1/1	B1	20	Park & Njapau, 1989
Maize	Kenya	70/78	—[a]	30–920	Ellis *et al.*, 1991
	USA	2370/2633	—	10–700	Ellis *et al.*, 1991
	Mexico	86/96	—	2.5–30	Ellis *et al.*, 1991
	Brazil	40/328	B1	> 20	Sabino *et al.*, 1989*b*
	Denmark	6/197	Total[b]	5–174	Madsen & Rasmussen, 1988
	France	3/3	B1	20	Park & Njapau, 1989
	India	47%	B1	> 20	Kaushal, 1990
Sorghum	S. Africa	—	B1	0–25	Ruffell & Trinder, 1990
	India	—	B1	7–75	Usha *et al.*, 1994
Copra	Tonga island	1/2	B1	37	Lovelace & Aalbersberg, 1989
Millet	India	49/75	B1	17–2110	Mishra & Daradhiyar, 1991
Soybeans	USA	11/11	B1	< 20	Park & Njapau, 1989
		3/24	B1	< 6	Jacobsen *et al.*, 1995
	Argentina	9/94	B1	1–36	Pinto *et al.*, 1991
Oat, barely, and wheat	Sweden	20/116	B1	50–400	Pettersson *et al.*, 1989
Meat	Egypt	9/150	B1, B2	4–15, 2–25	Aziz & Youssef, 1991
Dried figs	UK	8/93	Total	10–40	Sharman *et al.*, 1991
	Sweden	16/27	—	5–67	Akerstrand & Möller, 1989
Potato	Fiji island	7/20	B1	6–12	Lovelace & Aalbersberg, 1989
Linseed	India	46/105	B1	120–810	Samarajeewa *et al.*, 1990
Bakery products	Spain	1/50	B1, G1	67, 46	Simón & Frenandez, 1984
Cottonseed	Argentina	5/5	B1	20–200	Park & Njapau, 1989
Sunflower	Spain	1/1	B1	20	Park & Njapau, 1989
Mustard seed	India	44/100	—	75	Sahay & Prasad, 1990
Milk	Sweden	19/267	M1	> 0.05	Pettersson *et al.*, 1989
	Spain	14/47	M1	20–100	Blanco *et al.*, 1988
Human milk	Abu Dhabi	443/445	M1	0.002–3.0	Saad *et al.*, 1995

[a] —, not mentioned.
[b] B1 + G1 + B2 + G2.

Table 3. Occurrence of aflatoxin in peanuts and peanut products

	Country	Contaminated/ total examined	Aflatoxin	Concentration ($\mu g\ kg^{-1}$)	Reference
Peanuts	Argentina	3/3	B1	20–200	Park & Njapau, 1989
	Senegal	72/72	B1	20–200	Park & Njapau, 1989
	Brazil	940/1044	—[a]	30–5000	Ellis *et al.*, 1991
		20/20	B1	80–6450	Fonseca *et al.*, 1989
		26/86	B1, G1	10–2000, 20–800	Araujo *et al.*, 1994
	Mexico	26/29	—	700	Park & Njapau, 1989
	USA	108/120	—	24	Park & Njapau, 1989
	Philippines	56/98	—	3–2888	Garcia, 1989
	Fiji island	11/22	B1	1–18	Lovelace & Aalbersberg, 1989
	India	10/20	B1	33–440	Singh *et al.*, 1982
Butter	China	1/2	B1	20	Zhen-Zhen, 1989
	UK	69/77	—	38–535	Ellis *et al.*, 1991
	USA	246/2510	Total[b]	20–100	Gagliardi *et al.*, 1991
Cake	Nigeria	28/32	B1	37–455	Akano & Atanda, 1990
	USA	223/401	Total	31–100	Applebaum *et al.*, 1982
Snacks	India	31%	B1	2–1500	Rati & Shantha, 1994

[a] —, not mentioned.
[b] B1 + G1 + B2 + G2.

process to degrade the toxin to safe levels, it should meet the following requirements (Jemmali, 1989; Ellis *et al.*, 1991; Park & Liang, 1993):

- It must not result in the formation of other toxic substances or leave any harmful residues that might diminish the overall safety of the treated product.
- The nutritional quality of the product should not be seriously suppressed.
- It should not adversely affect desirable physical and sensory properties of the product.
- It has to be economically feasible, and technically applicable.
- It must be capable of destroying the spores and mycelia of aflatoxigenic fungi, if they are present in the product, which might, under favourable conditions, proliferate and reproduce the toxin.

A number of methods have been investigated in connection with their ability to inactivate aflatoxins in contaminated food and feedstuffs. These methods aim at either removing the toxin from the food or destroying it in the food. They can be classified into chemical, biological and physical methods.

A large number of chemicals can react with aflatoxins and convert them to less toxic and mutagenic compounds. These chemicals include acids, bases, oxidizing agents, bisulphites and gases. However, most of the chemical processes that have been investigated are impractical (carried out under drastic conditions of temperature and pressure), unsafe (form toxic residues) and unfavourable (degrade the nutritional, sensory and functional properties of the product). Currently, ammoniation and treatment with sodium bisulphite are the major industrial processes widely used to inactivate peanut meal, maize and cottonseed destined for animal feeding.

Many microorganisms including bacteria and acid-producing molds can metabolize and inactivate aflatoxins, with *Flavobacterium aurantiacum* as the most active organism. It was postulated that the inactivation was a result of acid production and subsquent conversion of AFB1 to AFB2a, which is 1000 times less mutagenic than the parent toxin (Park & Liang, 1993).

Inactivation by physical methods involves extraction with solvents, adsorption, inactivation by heat and irradiation. The physical methods are discussed in detail in the following sections.

Extraction

Extraction with solvents has been used to remove aflatoxins from the oilseeds peanut and cottonseed (Dollear, 1969; Hron *et al.*, 1992, 1994). Materials treated in this way may only be suitable for animal feeding. The solvents used include 95% ethanol, 90% aqueous acetone, 80% isopropanol, hexane–methanol, methanol–water, acetonitrile–water, hexane–ethanol–water and acetone–hexane–water. The solvent:sample ratio was found to be crucial for recovery of the toxin (Cole & Dorner, 1994). Solvent extraction can remove all traces of aflatoxin from oilseed meals with no formation of toxic byproducts or reduction in protein content and quality. However, large-scale application of this technique is limited by high cost and problems related to disposal of the toxic extracts.

Adsorption

Some adsorbents can bind and thus remove aflatoxins from aqueous solutions. Bentonite clay adsorbed AFB1 from a Sörensen buffer solution (30°C, 5 days). Separation of the clay resulted in the removal of 94–100% of the toxin from the solution. Particle size reduction and pre-heating of the clay increased its ability to adsorb and retain the toxin (Masimango *et al.*, 1978). Also, bentonite removed 65–79% of AFM1 from milk (Doyle *et al.*, 1982). Hydrated sodium calcium aluminosilicate (HSCAS; NovaSil clay) was reported (Phillips *et al.*, 1988) to have a high affinity for AFB1. The HSCAS removed more than 80% of the toxin from solution. *In vivo* studies demonstrated the role of HSCAS in preventing the mutagenicity and toxicity of AFB1 (Phillips *et al.*, 1988).

Heat

Aflatoxins have high decomposition temperatures ranging from 237°C to 306°C. Solid AFB1 is quite stable to dry heating at temperatures below its thermal decomposition temperature of 267°C (Betina, 1989). The use of heat to inactivate aflatoxin in contaminated food has been attempted. Normal home cooking conditions such as boiling and frying (approx. 150°C) failed to destroy AFB1 and AFG1 in the solid state (Kamimura, 1989). Table 4 summarizes the results of some studies conducted in connection with the degradation of aflatoxin in foods by different heat treatments. Temperatures above 150°C were necessary to attain partial destruction of the toxin. The extent of the destruction achieved was very dependent on the initial level of contamination, heating temperature and time. Moreover, the type of food and aflatoxin also influenced the degree of inactivation achieved. Degradation of aflatoxin by heat is also governed by the moisture content, pH and ionic strength of the food. The moisture content is a critical factor; contaminated foods that contain more moisture can more easily be inactivated by heat. Mann *et al.* (1967) observed that heating a cottonseed meal containing 30% moisture at 100°C for 1 h degraded 74.8% of aflatoxins (B1 + B2) present in the meal, whereas only 32.7% of the toxins were destroyed after heating a similar meal containing 6.6% moisture, under the same conditions. The sensitivity of AFB1 to moist-heat was also demonstrated in irradiated foods. Nkama and Muller (1988) studied the effect of the moisture

content of a rice meal on destruction of AFB1 in the meal after exposure to a tungsten lamp light (intensity 43 mW cm^{-2}) at 36°C for 2 h. They observed that increasing the moisture content of the meal from 14.1% to 18.7% increased the rate of AFB1 destruction from 40% to 63%. It has been suggested that the presence of water helps in opening the lactone ring in AFB1 (by the addition of a water molecule to the ring) to form a terminal carboxylic acid. The terminal acid group thereafter undergoes heat-induced decarboxylation (Coomes *et al.*, 1966). The presence of ionic salts increased the extent of aflatoxin degradation by heat. Farah *et al.* (1983) cooked raw unshelled peanuts in a 5% NaCl solution in an autoclave at 116°C, 0.7 bar for 30 min. This treatment reduced the total content of aflatoxins (B1 + G1 + B2 + G2) by 80–100%. The removal of the toxins was attributed to the addition of NaCl, as compared to unsalted controls.

AFM1 is a monohydroxy derivative of AFB1 secreted in milk by the cow after ingestion of feeds containing AFB1. The effects of pasteurization and sterilization on reduction of AFM1 in milk were studied (Table 5). The percentage reduction in AFM1 levels reported by different researchers are conflicting. For example, pasteurization of milk at 62°C for 30 min was observed (Purchase *et al.*, 1972) to reduce the AFM1 content in milk by 32%, whereas a similar treatment did not reduce the toxin content, as concluded by Stoloff *et al.* (1975). The contradiction could be attributed to the following:

- AFM1 is not stable in milk; its concentration spontaneously declines with time. About 40% of AFM1 in naturally contaminated milk disappeared after 4 days and about 80% after 6 days storage at 0°C (McKinney *et al.*, 1973). Kiermeier and Mashaley (1977) reported a reduction ranging from 18.8% to 24.2% in AFM1 content in milk upon storage at 5°C for 1–3 days. Also, a 34% decrease in AFM1 content in milk over 2 weeks

Table 4. Destruction of aflatoxins in foods by different heat treatments

Treatment	Food	Aflatoxin	Initial level ($\mu g\ kg^{-1}$)	Destruction (%)	Contamination[a]	Reference
Roasting: 204°C	Peanuts	B1, G1	253, 186	52, 41	N	Waltking, 1971
Roasting: 204°C	Peanuts	B1, G1	346, 286	41, 51	N	Waltking, 1971
Roasting: 121°C, 30 min	Peanuts	B1, G1	2200, 4100	84, 85	A	Lee *et al.*, 1969
Roasting: 121°C, 30 min	Peanuts	B1, G1	90, 150	73, 76	A	Lee *et al.*, 1969
Roasting: 204°C, 5 min	Peanuts	B1, G1	90, 150	69, 64	A	Lee *et al.*, 1969
Roasting: 204°C, 5 min	Peanuts	B1, G1	2200, 4100	69, 64	A	Lee *et al.*, 1969
Roasting: 150°C, 30 min	Peanuts	B1	370	48	N	Pluyer *et al.*, 1987
Roasting: 150°C, 30 min	Peanuts	B1	317	47	A	Pluyer *et al.*, 1987
Autoclave: 116°C, 0.7 bar, 30 min, in 5% NaCl brine	Peanuts	Total[b]	5012–19992	80–100	A	Farah *et al.*, 1983
Heating: 200°C, 20 min	Olive oil	B1	100	25	A	Mahjoub & Bullerman, 1988
Heating: 250°C, 10 min	Olive oil	B1	100	65	A	Mahjoub & Bullerman, 1988
Extrusion: 105°C	Corn	Total	500	40–70	N	Hameed, 1994

[a] N, natural; A, artificial.
[b] B1 + G1 + B2 + G2.

Table 5. Reduction of AFM1 in milk by pasteurization and sterilization

Treatment	Contamination[a]	Reduction (%)	Reference
62°C, 30 min	N	32	Purchase *et al.*, 1972
62°C, 30 min	A	0	Stoloff *et al.*, 1975
62°C, 30 min	A	35	Kiermeier & Mashaley, 1977
71°C, 40 s	A	29	Kiermeier & Mashaley, 1977
71°C, 40 min	N	6–13	Kiermeier & Mashaley, 1977
72°C, 45 s	N	45	Purchase *et al.*, 1972
74°C, 30 min	A	40	Kiermeier & Mashaley, 1977
75°C, 40 s	N	12	Kiermeier & Mashaley, 1977
77°C, 16 s	N	0	Stoloff *et al.*, 1975
80°C, 45 s	N	64	Purchase *et al.*, 1972
120°C, 15 min	N	24	Kiermeier & Mashaley, 1977
120°C, 15 min	A	24	Kiermeier & Mashaley, 1977
Pasteurization	N	24	Gelosa & Buzzetti, 1994

[a] A, artificial; N, natural.

storage at 21°C was reported (Heimbecher *et al.*, 1988). The poor stability of AFM1 in milk probably leads to contradictory results when the concentration of AFM1 in milk is assayed by different individuals at different laboratories.

- Differences in the initial level of contamination, and in the methods used to extract, clean-up and assay the toxin. Not all the methods are of equal accuracy, precision and sensitivity (Mehan *et al.*, 1985).
- Status of contamination, i.e. whether the treated milk was naturally or artificially contaminated. It is easier to inactivate aflatoxins in artificially contaminated substrates than in those naturally contaminated.

Rustom *et al.* (1993) studied the effects of pH (5.0, 8.0, 10.2), temperature (121°C, 130°C, 140°C) and heating time (5 s, 20 s, 15 min) on mutagenic activity (assayed by Ames test) of peanut beverages artificially contaminated with AFB1. Heat treatments at pH 8.0 were not effective in reducing the mutagenic activity. On the other hand, the treatments pH 10.2, 130°C, 20 s and pH 10.2, 121°C, 15 min reduced the mutagenic activity by 78% and 88%, respectively. The inactivation was attributed to partial conversion of AFB1 to AFD1 through hydrolysis of the lactone ring in AFB1, catalysed by NaOH used to adjust the pH. AFD1 is 450 times less mutagenic than AFB1. The treatments pH 5.0, 130°C, 20 s and pH 5.0, 121°C, 15 min reduced the mutagenic activity by 76% and 73%, respectively. The inactivation was attributed to partial hydration of AFB1, at the terminal furan ring, to AFB2a catalysed by HCl used to adjust the pH. AFB2a is 1000 times less mutagenic than AFB1. Changing the pH, from 8.0 to 5.0 or 10.2, without heating did not significantly reduce the mutagenic activity, thus emphasizing the synergistic role of pH and heat in reducing the mutagenic activity.

Microwave heating showed great potential for destruction of aflatoxin in contaminated peanuts depending on power level and treatment time. For instance, roasting an artificially contaminated peanut meal in a microwave at 6 kW for 4 min destroyed 95% of aflatoxin in the meal (Staron *et al.*, 1980). Similarly, microwave heating also reduced the level of aflatoxin in peanut kernels (Luter *et al.*, 1982). At lower energy (0.7 kW, 8.5 min), 48–61% of AFB1 was destroyed in artificially contaminated peanut kernels. However, the same treatment resulted in only 30–45% reduction of AFB1 in naturally contaminated kernels (Pluyer *et al.*, 1987).

Irradiation

Radiation is classified into two categories: ionizing and non-ionizing. In ionizing radiation (e.g. X-rays, gamma rays, ultraviolet rays) potential changes may occur in molecules of the irradiated object with little or virtually no temperature rise. These molecular changes might be quite harmful to living organisms exposed to large doses of ionizing radiation. On the other hand, non-ionizing radiation (e.g. radio waves, microwaves, infrared waves, visible light) in sufficient intensity leads to a rise in temperature, usually accompanied by molecular changes that are of no hazardous nature to man. The use of ionizing radiation to free foods from pathogenic microorganisms is among the methods applicable in food preservation (Kyzlink, 1990). Despite the debate on safety of irradiated foods in connection with human health, however, food irradiation is becoming a technique of potential application on a commercial scale to render food products sterile (Diehl, 1990).

Ultraviolet (UV) light

Aflatoxins are sensitive to UV radiation. AFB1 absorbs UV light at 222, 265 and 362 nm, with maximum absorption occurring at 362 nm, which may lead to the formation of up to 12 photodegradation products (Samarajeewa *et al.*, 1990). AFB1 and AFG1 underwent photochemically driven series of reactions when exposed to UV light (365 nm, 1 h) on silica gel TLC plates. The photodegradation products were less toxic, to chick embryos, than the parent toxins (Andrellos *et al.*, 1967). Treatment of peanut oil with UV light for 2 h destroyed 40–45% of aflatoxins initially present in the oil (Shantha & Murthy, 1977). Exposure of artificially contaminated milk to UV light inactivated 3.6–100% of AFM1 in the milk, depending on exposure time (2–60 min). Also, addition of hydrogen peroxide (1%) to the UV-irradiated milk (10 min) completely (100%) destroyed AFM1 (Yousef & Marth, 1985). Destruction of AFM1, in aqueous solution, by UV energy was of first-order irreversible reaction kinetics, and it was attributed to opening of the double bond in the terminal furan ring in AFM1 (Yousef & Marth, 1987). Also, UV irradiation (30 min) of dried figs artificially contaminated with AFB1 (250 μg kg^{-1}) reduced the toxin level by 45.7% (Altug *et al.*, 1990).

Gamma rays

The use of gamma radiation to inactivate aflatoxins was investigated. The toxicity of a peanut meal contaminated with AFB1 was reduced by 75% and 100% after irradiation with gamma rays at a dose of 1 and 10 kGy, respectively (Temcharoen & Thilly, 1982). However, doses higher than 10 kGy inhibited the seed germination, and increased the peroxide value of the oil in gamma-irradiated peanuts (Chiou *et al.*, 1990). The presence of water has an important role in the destruction of aflatoxin by gamma energy, since radiolysis of water leads to the formation of highly reactive free radicals. These radicals can readily attack AFB1, at the terminal furan ring, giving products of lower biological activity. The mutagenic activity of AFB1 in an aqueous

solution (5 μg ml^{-1} water) was reduced by 34%, 44%, 74% and 100% after exposure to gamma rays at 2.5, 5, 10 and 20 kGy, respectively (Van Dyck *et al.*, 1982). Also, a dose of 10 kGy completely (100%) inactivated AFB1, and destroyed 95% of AFG1 in a dimethylsulphoxide–water (1:9, v/v) solution (Mutluer & Erkoc, 1987). Addition of 1 ml of 5% hydrogen peroxide to an aqueous AFB1 solution (50 μg ml^{-1}) resulted in 37–100% degradation of the toxin at a lower dose (2 kGy). The final degradation products showed no biological activity in Ames mutagenicity test. The same treatment reduced the level of AFB1 in peanut kernels by 73–80% (Patel *et al.*, 1989).

Solar irradiation

Solar energy also destroyed aflatoxins in some foods (Table 6). Studies conducted in India revealed the efficacy of sunlight in degrading the toxin in different peanut products. It seems that UV rays from sunlight play an important role in the photodestruction of aflatoxin. Peanut protein can bind aflatoxins (Shantha & Murthy, 1980, 1981), and aflatoxins bound to the protein appear to be less susceptible to photodegradation than the free toxin. About 90% of AFB1 in artificially contaminated peanut flakes was destroyed by sunlight, whereas only 50% of the toxin was destroyed by the same treatment when it was present as a natural contaminant (Shantha *et al.*, 1986). Probably, aflatoxins were more bound to proteins in the naturally contaminated flakes than in the artificially contaminated flakes, which made them less liable to photodegradation by sunlight.

INTERNATIONAL LEGISLATION

The hazardous nature of aflatoxin to humans and animals has necessitated the need for establishment of control measures and tolerance levels by national and international authorities. Different countries have different regulations for aflatoxin. The general trend is that industrialized countries usually set lower tolerance levels than the developing countries, where most of the susceptible commodities are produced. For example, the tolerance level for aflatoxin in foods is 5 μg kg^{-1} in Sweden (Akerstrand & Möller, 1989) and 10 μg kg^{-1} in Japan (Aibara & Maeda, 1989), whereas it is 30 μg kg^{-1} in Brazil (Sabino *et al.*, 1989*a*). However, such lack of harmony may give rise to difficulties in the trade of some commodities.

The first legislative act was undertaken in 1965 by the Food and Drug Administration (FDA) of the USA, which proposed a tolerance level of 30 μg kg^{-1} of total aflatoxins (B1 + G1 + B2 + G2). With increasing awareness of aflatoxins as potent toxic substances, the proposed level was lowered to 20 μg kg^{-1} in 1969 (FDA, 1977). Since then, the tolerance levels have been revised and re-evaluated occasionally. The current tolerance levels established by the FDA for food and feeds are listed in Table 7.

In 1973, the European Economic Community (EEC) established legislation on maximum permitted levels of AFB1 in different types of feedstuffs (EEC, 1974). The legislation has been frequently amended since then. However, the main framework of the legislation remains more or less the same. Table 8 lists the maximum allowable levels of AFB1 in animal feeds as established by the EEC. The legislation has been in action since 30 November 1991 (EEC, 1991).

Contamination of agricultural commodities with aflatoxin, and hence establishment of regulations, has been a major concern to the Joint FAO/WHO Expert Committee. The Committee recommends that the presence of aflatoxin in food should be limited to 'irreducible levels'. An irreducible level is defined as: 'the concentration of a substance that can not be eliminated from a food without involving the discarding of that food altogether, or severely compromising the ultimate availability of major food supplies' (FAO/WHO, 1987). The current regulations for aflatoxin established by the Joint FAO/WHO Committee is given in Table 9.

The Dutch authorities undertook a worldwide enquiry, during 1986 and 1987, in which 66 countries were requested to report on their regulations and tolerance limits for mycotoxins in food and feeds. Of the 66 countries, 50 had enacted or proposed legislation for aflatoxins in foods, 35 for aflatoxins in feedstuffs, and 14 for aflatoxin M1 in milk and dairy products. An

Table 6. Destruction of aflatoxins in foods by solar energy

Exposure time	Country	Food	Destruction (%)	Reference
6 h	India	Peanut cake[a]	50	Shantha & Murthy, 1981
6 h	India	Casein[a]	83	Shantha & Murthy, 1981
10 h	Nigeria	Maize, millet[b]	30, 16	Okonkwo & Nwokolo, 1978
14 h	India	Peanut flakes[a]	90	Shantha *et al.*, 1986
14 h	India	Peanut flakes[b]	50	Shantha *et al.*, 1986
15 min	India	Peanut oil[b]	99	Shantha & Murthy, 1977
10, 40 min	USA	Olive oil[a]	55, 95	Mahjoub & Bullerman, 1988

[a] Artificially contaminated.
[b] Naturally contaminated.

Table 7. FDA tolerance levels for total aflatoxin

Item	Tolerance level ($\mu g\ kg^{-1}$)
Food for human consumption	20
Feed for beef cattle and poultry	300
Feed for swine	200
Feed for breeding livestock	100
Feed for dairy cattle	20
Milk	0.5

Source: Park & Njapau (1989); Park & Liang (1993).

Table 8. Maximum permitted level of aflatoxin B1 in different animal feeds established by the EEC[a]

Feed	Level ($\mu g\ kg^{-1}$)[b]
Straight feeds[c]	
Peanut, copra, palm kernels, cotton seed, babassu, maize and products derived therefrom	20
Compound feeds[d]	
Complete feeds	
Feeds for cattle, sheep and goat (except dairy cattle, calves and lambs)	50
Feeds for pigs and poultry (except young animals)	20
Other complete feeds	10
Complementary feeds	
Feeds for cattle, sheep and goats (with exception of dairy animals)	50
Feeds for pigs and poultry (with exception of young animals)	30
Other complementary feeds	5

[a] EEC (1991).
[b] Based on feed moisture content of 12%.
[c] Feed composed of a single item.
[d] Feed composed of a mixture of items.

Table 9. Aflatoxin legislations set by the Joint FAO/WHO Expert Committee[a]

Aflatoxin	Tolerance level ($\mu g\ kg^{-1}$)	Food/feed
B1	5	Feed for dairy cattle
M1	0.05	Milk
B1 + G1 + B2 + G2	15	Raw peanut for human consumption
B1 + G1 + B2 + G2	10	Processed peanut for human consumption

[a]FAO/WHO (1990, 1992).

extensive overview of aflatoxin regulations in these countries is provided by Van Egmond (1989), later updated by Stoloff *et al.* (1991). The study revealed that, in most of the countries surveyed, the rationales for establishment of tolerance limits and regulations for aflatoxins were based on vague unsupported statements of carcinogenic risk for humans due to exposure to aflatoxins.

CONCLUSIONS

No doubt consideration of preventive measures aimed at reducing infestation of agricultural commodities with aflatoxgenic molds is the best way to control contamination with aflatoxin. In addition to application of proper cultural, storage and segregation practices, the development of crop species with high resistance to aflatoxigenic molds is of great potential in this context. Establishment of a unique detoxification method for all foods and feedstuffs in not possible. The efficiency of a method to inactivate aflatoxins depends, to a great extent, on the nature of the food, its moisture content, type of aflatoxin, level of contamination and degree of association of aflatoxins with the food constituents.

REFERENCES

Adhikari, M., Ramjee, G. & Berjak, P. (1994). Aflatoxin, kwashiorkor, and morbidity. *Nat. Toxins*, **2**, 1–3.

Aibara, K. & Maeda, K. (1989). The regulation for aflatoxin in Japan and the current situation of aflatoxin contamination of food imported from throughout the world. In *Mycotoxins and Phycotoxins*, eds S. Natori, K. Hashimoto and Y. Ueno. Elsevier, Amsterdam, pp. 243–248.

Aja Nwachukwu, J. & Emejuaiwe, S. O. (1994). Aflatoxin-producing fungi associated with Nigerian maize. *Environ. Toxicol. Water Qual.*, **9**, 17–23.

Akano, D. A. & Atanda, O. O. (1990). The present level of aflatoxin in Nigerian groundnut cake (Kulikuli). *Lett. Appl. Microbiol.*, **10**, 187–189.

Akerstrand, K. & Möller, T. (1989). Examination of dried figs. *Vår Föda*, **41**, 308–317.

Altug, T., Yousef, A. E. & Marth, E. H. (1990). Degradation of aflatoxin B1 in dried figs by sodium bisulfite with or without heat, ultraviolet energy or hydrogen peroxide. *J. Food Protect.*, **53**, 581–582.

Andrellos, P. J., Beckwith, A. C. & Eppley, R. M. (1967). Photochemical changes of aflatoxin B1. *J. Assoc. Off. Anal. Chem.*, **50**, 346–350.

Applebaum, R. S., Brackett, R. E., Wiseman, D. W. & Marth, E. H. (1982). Aflatoxin: toxicity to dairy cattle and occurrence in milk and milk products—a review. *J. Food Protect.*, **45**, 752–777.

Araujo, A. C. P., Telles, D. L., Gracas Almeida, M. & Nascimento, E. S. (1994). Determination of aflatoxins in peanuts marketed in Recife PE. *Brazil. Alimentaria*, **249**, 35–37.

Aziz, N. H. & Youssef, Y. A. (1991). Occurrence of aflatoxins and aflatoxin-producing moulds in fresh and processed meat in Egypt. *Food Addit. Contam.*, **8**, 321–331.

Bash G., & Rae, I. D. (1969). The structure and chemistry of aflatoxins. In *Aflatoxin*, ed. L. A. Goldblatt. Academic Press, New York, pp. 55–75.

Beaver, R. W. (1991). Decontamination of mycotoxin-containing foods and feedstuffs. *Trends Food Sci. Technol.*, **7**, 170–173.

Betina, V. (1989). Mycotoxins: chemical, biological and environmental aspects. In *Bioactive Molecules*, ed. V. Betina. Elsevier Applied Science, London, pp. 114–150.

Bilgrami, K. S. & Choudhary, A. K. (1993). Impact of habitats on toxigenic potential of *Aspergillus flavus*. *J. Stored Prod. Res.*, **29**, 351–355.

Blanco, J. J., Dominguez, L., Gómez-Lucia, E., Garayzabal, J. F. F., Garcia, J. A. & Suarez, G. (1988). Presence of aflatoxin M1 in commercial ultra-high-temperature treated milk. *Appl. Environ. Microbiol.*, **54**, 1622–1623.

Bonomi, A., Quarantelli, A., Mazzali, I., Cabassi, E., Corradi, A., Ubaldi, A., Fusari, A. & Chizzolini, R. (1993). The effects of aflatoxin G1 contaminated rations on the productive efficiency and on the meat yield and quality in fattening pigs (experimental contribution). *Riv. Sci. Aliment.*, **22**, 351–377.

Bonomi, A., Quarantelli, A., Mazzali, I., Cabassi, E., Corradi, A., Lecce, R., Ubaldi, A., Fusari, A. & Chizzolini, A. (1994). Effects of aflatoxins B1 and G1 on productive efficiency, meat yield and quality in fattening pigs (experimental contribution). *Riv. Sci. Aliment.*, **23**, 251–277.

Brackett, R. E. (1989). Strategies for dealing with aflatoxins in peanuts. In *Trends in Food Product Development*, eds T. C. Yam & C. Tan. Singapore Institute of Food Science and Technology, Singapore, pp. 83–91.

Chiou, R. Y. Y., Koehler, P. E. & Beuchat, L. R. (1984). Hygroscopic characteristics of peanut components and their influence on growth and aflatoxin production by *Aspergillus parasiticus*. *J. Food Protect.*, **47**, 791–794.

Chiou, R. Y. Y., Lin, C. M. & Shyu, S. L. (1990). Property characterization of peanut kernels subjected to gamma irradiation and its effect on the outgrowth and aflatoxin production by *Aspergillus parasiticus*. *J. Food Sci.*, **55**, 210–213.

Chiou, R. Y. Y., Pei, Y. W. & Yue, H. Y. (1994). Color sorting of lightly roasted and deskinned peanut kernels to diminish aflatoxin contamination in commercial lots. *J. Agric. Food Chem.*, **42**, 2156–2160.

Cole, R. J. & Dorner, J. W. (1994). Extraction of aflatoxins from naturally contaminated peanuts with different solvents and solvent/peanut ratios. *J. AOAC Int.*, **77**, 1509–1511.

Coomes, T. J., Crowther, P. C., Feuell, A. J. & Francis, B. J. (1966). Experimental detoxification of groundnut meals containing aflatoxin. *Nature*, **209**, 406–407.

Davis, N. D. and Diener, U. L. (1979). Mycotoxins. In *Food and Beverage Mycology*, ed. L. R. Beuchat. AVI Publishing Company, West Port, CT, pp. 397–444.

Dichter, C. R. (1984). Risk estimates of liver cancer due to aflatoxin exposure from peanuts and peanut products. *Food Chem. Toxicol.*, **22**, 431–437.

Diehl, J. F. (1990). *Safety of Irradiated Foods*, ed. J. F. Diehl. Marcel Dekker, New York, pp. 145–179.

Diener, U. L., Pettit, R. E. & Cole, R. J. (1982). Aflatoxins and other mycotoxins in peanuts. In *Peanut Science and Technology*, eds H. E. Pattee & C. T. Young. American Peanut Research and Education Society, Yoakum, TX, pp. 486–519.

Dollear, F. G. (1969). Detoxification of aflatoxins in foods and feeds. In *Aflatoxin*, ed. L. A. Goldblatt. Academic Press, New York.

Doster, M. A. & Michailides, T. J. (1994). *Aspergillus* molds and aflatoxins in pistachio nuts in California. *Phytopathology*, **84**, 583–590.

Doyle, M. P., Applebaum, R. S., Brackett, R. E. & Marth, E. H. (1982). Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. *J. Food Protect.*, **45**, 964–971.

Dutton, M. F. & Westlake, K. (1985). Occurrence of mycotoxins in cereals and animal feedstuffs in Natal, South Africa. *J. Assoc. Off. Anal. Chem.*, **68**, 839–842.

EEC (1974). Council Directive 74/63/EEC on the fixing of maximum permitted levels for undesirable substances and products in feedingstuffs. *Off. J. Eur. Commun.*, No. L 38.

EEC (1991). EEC Council Directive 91/126/EEC. Amending the annexes to Council Directive 74/63/EEC on undesirable substances and products in animal nutrition. *Off. J. Eur. Commun.*, No. L 60.

Elamin, N. H. H., Abdel-Rahim, A. M. & Khalid, A. E. (1988). Aflatoxin contamination of groundnuts in Sudan. *Mycopathologia*, **104**, 25–31.

Ellis, W. O., Smith, J. P., Simpson, B. K. & Oldham, J. H. (1991). Aflatoxins in food: occurrence, biosynthesis, effects on organisms, detection, and methods of control. *Crit. Rev. Food Sci. Nutr.*, **30**, 403–439.

FAO/WHO (1987). The Joint FAO/WHO Expert Committee. Evaluation of certain food additives and contaminants. *WHO Tech. Rep. Ser.*, 759.

FAO/WHO (1990). FAO/WHO Standards Programme. Codex Alimentarius Commission, Alinorm 91/29.

FAO/WHO (1992). FAO/WHO Standards Programme. Codex Alimentarius Commission, Alinorm 93/12.

Farah, Z., Martins, M. J. R. & Bachmann, M. R. (1983). Removal of aflatoxin in raw unshelled peanuts by a traditional salt boiling process practised in Northeast of Brazil. *Lebensm. Wiss. Technol.*, **16**, 122–124.

FDA (1977). FDA proposed guidelines for aflatoxin. *Cereal Foods World*, **22**, 532–533, 541.

Fonseca, H., Nogueira, J. N. & Calori, M. A. (1989). Prevention of peanut contamination with aflatoxin after harvest and during storage. In *Trends in Food Product Development*, eds T. C. Yam & C. Tan. Singapore Institute of Food Science and Technology, Singapore, pp. 342–346.

Gagliardi, S. J., Cheatle, T. F., mooney, R. L., Llewellyn, M. G., O'Rear, C. E. & Llewellyn, G. C. (1991). The occurrence of aflatoxin in peanut butter from 1982 to 1989. *J. Food Protect.*, **54**, 627–631.

Garcia, V. V. (1989). An overview of peanut utilization in the Philippines. In *Trends in Food Product Development*, eds T. C. Yam & C. Tan. Singapore Institute of Food Science and Technology, Singapore, pp. 21–26.

Gaur, V. K. & Siradhana, B. S. (1989). Comparison of aflatoxin B1 formation by *Aspergillus flavus* on bread and uncooked grains of maize. *Indian Phytopathol.*, **42**, 589–590.

Gelosa, M. & Buzzetti, I. (1994). Effects of heat treatment of milk on the aflatoxin M1 content. *Riv. Sci. Aliment.*, **23**, 115–117.

Groopman, J. D., Cain, L. G. & Kensler, T. W. (1988). Aflatoxin exposure in human populations: measurements and relationship to cancer. *Crit. Rev. Toxicol.*, **19**, 113–146.

Guo, B. Z., Russin, J. S., Cleveland, T. E., Brown, R. L. & Widstrom, N. W. (1995). Wax and cutin layers in maize kernels associated with resistance to aflatoxin production by *Aspergillus flavus*. *J. Food Protect.*, **58**, 296–300.

Hameed, H. G. (1994). Extrusion and chemical treatments for destruction of aflatoxin in naturally-contaminated corn. *Dissert. Abstr. Int.*, B.54.11.5607, Order No. DA9410659.

Heimbecher, S. K., Jorgensen, K. V. & Price, R. L. (1988). Interactive effects of duration of storage and addition of formaldehyde on levels of aflatoxin M1 in milk. *J. Assoc. Off. Anal. Chem.*, **71**, 285–287.

Heperkan, D., Aran, N. & Ayfer, M. (1994). Mycoflora and aflatoxin contamination in shelled pistachio nuts. *J. Sci. Food Agric.*, **66**, 273–278.

Hron, R. J., Abraham, G., Kuk, M. S. & Fisher, G. S. (1992). Ethanol extraction of oil, gossypol and aflatoxin from cottonseed. *J. Am. Oil Chem. Soc.*, **71**, 417–421.

Hron, R. J., Kuk, M. S., Abraham, G. & Wan, R. J. (1994). Acidic ethanol extraction of cottonseed. *J. Am. Oil Chem. Soc.*, **69**, 951–952.

Jacobsen, B. J., Harlin, K. S., Swanson, S. P., Lambert, R. J., Beasley, V. R., Sinclair, J. B. & Wei, L. S. (1995). Occurrence of fungi and mycotoxins associated with field mold damaged soybeans in the midwest. *Plant Dis.*, **79**, 86–89.

Jayaraman, P. & Kalyanasundaram, I. (1990). Natural

occurrence of toxigenic fungi and mycotoxins in rice bran. *Mycopathologia*, **110**, 81–85.

Jemmali, M. (1989). Safety evaluation of mycotoxin-decontaminated feedstuffs. In *Mycotoxins and Phycotoxins*, eds S. Natori, K. Hashimoto & Y. Ueno. Elsevier Science Publishers, Amsterdam, pp. 233–241.

Kamimura, H. (1989). Removal of mycotoxins during food processing. In *Mycotoxins and Phycotoxins*, eds S. Natori, K. Hashimoto & Y. Ueno. Elsevier Science Publishers, Amsterdam, pp. 169–176.

Kaushal, K. S. (1990). Incidence of mycotoxins in maize grains in Bihar State. *India. Food Addit. Contam.*, **7**, 55–61.

Kiermeier, F. & Mashaley, R. (1977). Influence of raw milk processing om the aflatoxin M1 content of milk products. *Z. Lebensm. Unters. Forsch.*, **164**, 183–187.

Kyzlink, V. (1990). Principles of food preservation. *Dev. Food Sci.*, **22**, 337–355.

Lee, L. S., Cucullu, A. F., Franz, A. O. & Pons, W. A. (1969). Destruction of aflatoxins in peanuts during dry and oil roasting. *J. Agric. Food Chem.*, **17**, 451–453.

Lovelace, C. E. A. & Aalbersberg, W. G. L. (1989). Aflatoxin levels in foodstuffs in Fiji and Tonga island. *Plant Food Human Nutr.*, **39**, 393–399.

Luter, L., Wyslouzil, W. & Kashyap, S. C. (1982). The destruction of aflatoxins in peanuts by microwave roasting. *Can. Inst. Food Sci. Technol. J.*, **15**, 236–238.

L'Vova, L. S., Orlova, N. Y., Bystryakova, Z. K., Omel'chenko, M. D. & Remele, V. V. (1993). Occurrence of toxigenic fungi and mycotoxins in various crop grains. *Appl. Biochem. Microbiol.*, **29**, 53–60.

Madsen, B. & Rasmussen, G. (1988). Aflatoxin B1, B2, G1 and G2 in maize, rice, millet, buckwheat, lentils, beans and similar commodities in 1987–1988. Publikation No. 200, Levnedsmiddelstyrelsen, Soborg, Denmark.

Mahjoub, A. & Bullerman, L. B. (1988). Effect of storage time, sunlight, temperature, and frying on stability of aflatoxin B1 in olive oil. *Lebensm. Wiss. Technol.*, **21**, 29–32.

Mann, G. E., Codifer, L. P. & Dollear, F. G. (1967). Effect of heat on aflatoxin in oilseed meals. *J. Agric. Food Sci.*, **15**, 1090–1092.

Masimango, N., Remacle, J. & Ramaut, J. L. (1978). The role of adsorption in the elimination of aflatoxin B1 from contaminated media. *Eur. J. Appl. Microbiol. Biotechnol.*, **6**, 101–105.

Massey, T. E., Stewart, R. K., Daniels, J. M. & Ling, L. (1995). Biochemical and molecular aspects of mammalian susceptibility to aflatoxin B1 carcinogenicity. *Proceed. Soc. Exp. Biol. Med.*, **208**, 213–227.

McKinney, J. D., Cavanagh, G. C., Bell, J. T., Hoverslund, A. S., Nelson, D. M., Pearson, J. & Selkrik, R. J. (1973). Effects of ammoniation of aflatoxins in rations fed lactating cows. *J. Am. Oil Chem. Soc.*, **50**, 78–84.

Mehan, V. K., Bhavanishankar, T. N. & Bedi, J. S. (1985). Comparison of different methods for extraction and estimation of aflatoxin B1 in groundnut. *J. Food Sci. Technol.*, **22**, 123–125.

Mishra, N. K. & Daradhiyar, S. K. (1991). Mold flora and aflatoxin contamination of stored and cooked samples of pearl millet in the Baharia tribal belt of Santhal Pargana. *Bihar, India. Appl. Environ. Microbiol.*, **57**, 1223–1226.

Moubasher, A. H., Abdelhafex, S. I. I., Elhissy, F. T. & Hassan, S. M. K. (1980). Effect of temperature and moisture content on Egyptian peanut seed-borne fungi. *Mycopathologia*, **70**, 49–54.

Mutluer, B. & Erkoc, F. U. (1987). Effects of gamma irradiation on aflatoxins. *Z. Lebensm. Unters. Forsch.*, **164**, 398–401.

Nkama, I. & Muller, H. G. (1988). The effect of moisture content and light intensity on the photo-degradation of aflatoxin B1 in contaminated rice. *J. Cereal Sci.*, **8**, 269–274.

Okonkwo, P. O. & Nwokolo, C. (1978). Aflatoxin B1: simple procedures to reduce levels in tropical foods. *Nutr. Rep. Int.*, **17**, 387–395.

Palmgren, M. S. & Hayes, A.W. (1987). Aflatoxins in foods. In *Mycotoxins in Food*, ed. P. Krogh. Academic Press, London, pp. 65–96.

Park, D. L. & Liang, B. (1993). Perspectives on aflatoxin control for human food and animal feed. *Trends Food Sci. Technol.*, **4**, 334–342.

Park, D. L. & Njapau, H. (1989). Contamination issues and padding. *J. Am. Oil Chem. Soc.*, **66**, 1402–1405.

Patel, U. D., Govindarajan, P. & Dave, P. J. (1989). Inactivation of aflatoxin B1 by using the synergistic effect of hydrogen peroxide and gamma radiation. *Appl. Environ. Microbiol.*, **55**, 465–467.

Peers, F. G. & Linsell, C. A. (1973). Dietary aflatoxins and liver cancer—a population based study in Kenya. *Br. J. Cancer*, **27**, 473–484.

Pettersson, H., Holmberg, T., Larsson, K. & Kaspersson, A. (1989). Aflatoxins in acid-treated grain in Sweden and occurrence of aflatoxin M1 in milk. *J. Sci. Food Agric.*, **48**, 411–420.

Phillips, T. D., Kubena, L. F., Harvey, R. B., Taylor, D. S. & Heidelbaugh, N. D. (1988). Hydrated sodium calcium alominosilicate: a high affinity sorbent for aflatoxin. *Poult. Sci.*, **67**, 243–247.

Pinto, V. E. F., Vaamonde, G., Brizzio, S. B. & Apro, N. (1991). Aflatoxin production in soybean varieties grown in Argentina. *J. Food Protect.*, **54**, 542–545.

Pluyer, H. R., Ahmed, E. M. & Wei, C. I. (1987). Destruction of aflatoxin on peanuts by oven- and microwave-roasting. *J. Food Protect.*, **50**, 504–508.

Porter, D. M., Wright, F. S. & Steele, J. L. (1986). Relationship to microscopic shell damage to colonization of peanut by *Aspergillus flavus*. *Oléagneux*, **41**, 23–28.

Purchase, I. F. H., Steyn, M., Rinsma, R. & Tusin, R. C. (1972). Reduction of the aflatoxin M content of milk by processing. *Food Cosmet. Toxicol.*, **10**, 383–387.

Rati, E. R. & Shantha, T. (1994). Incidence of aflatoxin in groundnut-based snack products. *J. Food Sci. Technol.*, **31**, 327–329.

Rucker, K. S., Kvien, C. K., Calhoun, K., Henning, R. J., Koehler, P. E., Ghate, S. R. & Holbrook, C. C. (1994). Sorting peanuts by pod density to improve quality and kernel maturity distribution and to reduce aflatoxin. *Peanut Sci.*, **21**, 147–152.

Ruffell, P. L. & Trinder, D. W. (1990). A mini-column screening test for aflatoxins in sorghum malt. *J. Inst. Brew.*, **96**, 7–10.

Rustom, I. Y. S., López-Leiva, M. H. & Nair, B. M. (1993). Effect of pH and heat treatment on the mutagenic activity of peanut beverage contaminated with aflatoxin B1. *Food Chem.*, **46**, 37–42.

Saad, A. M., Abdelgadir, A. M. & Moss, M. O. (1995). Exposure of infants to aflatoxin M1 from mothers' breast milk in Abu Dhabi, UAE. *Food Addit. Contam.*, **12**, 255–261.

Sabino, M., Zorzetto, M. A. P., Pedroso, M. O. & Milanez, T. V. (1989). Incidence of aflatoxins in peanuts and peanut products consumed in Sao Paulo, between 1980 and 1987. *Rev. Inst. Adolfo Lutz*, **49**, 41–44.

Sabino, M., Prado, G., Inomata, E. I., Oilveira-Pedroso, M.-de and Valeiro-Garcia, R. (1989*b*). Natural occurrence of aflatoxins and zearalenone in maize in Brazil. *Food Addit. Contam.*, **6**, 327–331.

Sahay, S. S. & Prasad, T. (1990). The occurrence of aflatoxins in mustard and mustard products. *Food Addit. Contam.*, **7**, 509–513.

Samarajeewa, U., Sen, A. C., Cohen, M. D. & Wei, C. I. (1990). Detoxification of aflatoxins in foods and feeds by physical and chemical methods. *J. Food Protect.*, **53**, 489–501.

Sanders, T. H., Cole, R. J., Blankenship, P. D. & Dorner, J. W. (1993). Aflatoxin contamination of peanuts from plants drought stressed in pod or root zones. *Peanut Sci.*, **20**, 5–8.

Sargeant, K., Sheridan, A., O'Kelly, J. & Carnaghan, R. B. A. (1961). Toxicity associated with certain samples of groundnuts. *Nature*, **192**, 1095–1097.

Sebunya, T. K. & Yourtee, D. M. (1990). Aflatoxigenic aspergilli in foods and feeds in Uganda. *J. Food Qual.*, **13**, 97–107.

Shank, R. C., Wogan, G. N., Gibson, J. B. & Nondasuta, A. (1972). Dietary aflatoxins and human liver cancer II. Aflatoxins in market foods and foodstuffs of Thailand and Hong Kong. *Food Cosmet. Toxicol.*, **10**, 61–69.

Shantha, T. & Murthy, V. S. (1977). Photo-destruction of aflatoxin in groundnut oil. *Indian J. Technol.*, **15**, 453–454.

Shantha, T. & Murthy, V. S. (1980). Storage of groundnut oil detoxified by exposure to sunlight. *Indian J. Technol.*, **18**, 346–347.

Shantha, T. & Murthy, V. S. (1981). Use of sunlight to partially detoxify groundnut cake flour and casein contaminated with aflatoxin B1. *J. Assoc. Off. Anal. Chem.*, **64**, 291–293.

Shantha, T., Murthy, V. S., Rat, E. R. & Prema, V. (1986). Detoxification of groundnut seeds by urea and sunlight. *J. Food Safety*, **7**, 225–231.

Sharman, M., Patey, A. L., Bloomfield, D. A. & Gilbert, J. (1991). Surveillance and control of aflatoxin contamination of dried figs and fig paste imported into the United Kingdom. *Food Addit. Contam.*, **8**, 299–304.

Simón, M. T. & Frenandez, G. S. (1984). Determination of aflatoxins in bread and bakery products. *J. Food Protect.*, **47**, 627–628.

Singh, T., Tyagi, R. P. S. & Varma, B. K. (1982). Study of the occurrence of aflatoxin B1 in food grains. *J. Food Sci. Technol.*, **19**, 35–37.

Staron, T., Thirouin, D., Perrin, L. & Frere, G. (1980). Microwave treatment of biological food product. *Ind. Aliment. Agric.*, **12**, 1305–1312.

Steiner, W. E., Rieker, R. H. & Battaglia, R. (1988). Aflatoxin contamination in dried figs: distribution and association with fluorescence. *J. Agric. Food Chem.*, **36**, 88–91.

Stoloff, L., Truckness, M., Hardin, N., Francis, O. J., Hayes, J. R., Polan, C. E. & Campbell, T. C. (1975). Stability of aflatoxin M1 in milk. *J. Dairy Sci.*, **58**, 1789–1793.

Stoloff, L., Van Egmond, H. P. & Park, D. L. (1991). Rationales for the establishment of limits and regulations for mycotoxins. *Food Addit. Contam.*, **8**, 213–222.

Temcharoen, P. & Thilly, W. G. (1982). Removal of aflatoxin B1 toxicity but not mutagenicity by 1 megarad gamma radiation of peanut meal. *J. Food Safety*, **4**, 199–205.

Usha, C. M., Patkar, K. L., Shetty, H. S., Kennedy, R. & Lacey, J. (1994). Mycoflora of developing sorghum grains with special reference to *Aspergillus flavus*. *Trop. Sci.*, **34**, 353–360.

Van Egmond, H. P. (1989). Current situation on regulations of mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis. *Food Addit. Contam.*, **6**, 139–188.

Van Dyck, P. J., Tobback, P., Feys, M. & Van De Voorde, H. (1982). Sensitivity of aflatoxin B1 to ionizing radiation. *Appl. Environ. Microbiol.*, **43**, 1317–1319.

Viquez, O. M., Castell Perez, M. E., Shelby, R. A. & Brown, G. (1994). Aflatoxin contamination in corn samples due to environmental conditions, aflatoxin-producing strains, and nutrients in grain grown in Costa Rica. *J. Agric. Food Chem.*, **42**, 2551–2555.

Waltking, A. E. (1971). Fate of aflatoxin during roasting and storage of contaminated peanut products. *J. Assoc. Off. Anal. Chem.*, **54**, 533–539.

Yousef, A. E. & Marth, E. H. (1985). Degradation of aflatoxin M1 in milk by ultraviolet energy. *J. Food Protect.*, **48**, 697–698.

Yousef, A. E. & Marth, E. H. (1987). Kinetics of interaction of aflatoxin M1 in aqueous solutions irradiated with ultraviolet energy. *J. Agric. Food Chem.*, **35**, 785–789.

Zhen-Zhen, J. I. A. (1989). A review of the study on fungi and mycotoxins in foodstuffs in Beijing during the last 10 years. In *Mycotoxins and Phycotoxins*, eds S. Natori, K. Hashimoto and Y. Ueno. Elsevier Science Publishers, Amsterdam, pp. 135–143.