CONTROL OF AFLATOXINS IN THE FOOD INDUSTRY

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ABSTRACT

Certain foods such as nuts and treenuts are preferred media for aflatoxin-producing Penicillia and Aspergilli. About 50 per cent of the mouldy nuts examined contained aflatoxins in amounts of up to several hundred ppb. About 10–20 per cent of the mouldy grain products contained aflatoxins in amounts of up to 100 ppb. Aflatoxin was never detected in jams, pickles or potato chips and crisps, even when mouldy.

Aflatoxin may penetrate several centimetres into foods in the course of only a few days. In general the aflatoxin content of the mycelium is higher than that of the food underneath and decreases with its distance from the mycelium; however, at a certain depth the aflatoxin content increases again.

Even non-mouldy foods or raw materials may contain aflatoxins. Spores can be transferred by insects, especially flies, wasps and bees, or by birds to foods where the spores germinate, produce mycelium, and aflatoxin is excreted. The mycelium can be removed afterwards by technological processes such as excavating, washing, salting, mixing, drying and heating. Such reasons may account for our findings of 5 ppb aflatoxin B_1 in a sample of mould-free salami and 100 ppb in mould-free ham. Seeds can contain aflatoxins by infection of the egg-cells of the flowering plants. Mould-free peanut kernels isolated from their shell contained 5 and 7 ppb aflatoxin B_1, whilst the more frequently occurring mouldy kernels in the shell contained up to 100 ppb. Mould-free peaches can contain mouldy kernels infected by toxin-producing Aspergilli and Penicillia. Their seeds contained up to 10 ppb aflatoxin $B_1 + B_2 + G_1$.

During food processing there are many possibilities for mould infection. Blanched almonds for Marzipan and peach or apricot seeds for Persipan should be processed immediately after blanching. We detected 3 ppb aflatoxin B_1 and traces of B_2 and G_2 in blanched almonds kept for 3 days at 28°C. The ready products 'Marzipan' (almond paste) and 'Persipan' (peach and/or apricot seed paste) can contain aflatoxins in amounts of up to 5 ppb.

55 samples of skim and whole milk powder supplied from 5 different German manufacturers were analysed. The milk powders of one supplier were practically free of aflatoxin M_1, whilst nearly all samples of another supplier contained aflatoxin M_1 in amounts ranging from traces to 4 ppb.

Pellets for hatched trouts contained 500 ppb aflatoxin B_1. We only detected traces of aflatoxin in the trouts' livers, whilst the faeces contained 500 ppb aflatoxin B_1. Canaries' feed contained 300 ppb aflatoxin B_1 and 125 ppb B_2. Mouldy corn used as deer fodder contained 25 ppb aflatoxin B_1 and 25 ppb aflatoxin G_1.

Conditions for controlling aflatoxins in stored products and for preventing mould growth and toxin production are discussed. Storage conditions for certain raw materials are mentioned. The results of aflatoxin assays of hundreds of peanut samples harvested in different countries in the last 5 years are intimated.

INTRODUCTION

The natural occurrence of aflatoxin in food is rare[3]. Results obtained on samples of different foodstuffs examined at our laboratories indicate that those which do contain aflatoxins can be roughly divided into two groups. The first type is more or less contaminated with mould and/or spores, and in the second type neither mould nor spores can be detected.

RESULTS

Spontaneously mouldy foodstuffs

Experimentally, mould growth, with associated toxin production, has been reported in a wide variety of food products[2]. However, under normal (storage) conditions foods are not easily contaminated. Frank and Eyrich[1] identified aflatoxin in only about 10 per cent of spontaneously mouldy foodstuffs. Bösenberg and Eberhardt[5] examined 185 samples of mouldy foods taken in the course of half a year from three different supermarkets; only 3 of the 14 isolated *A. flavus* stems were aflatoxin producers.

With regard to our observations on mouldy foods—meaning some mouldy spots on the food—there seems to be a preference for the aflatoxin-producing Aspergilli and Penicillia to grow and produce aflatoxin on special foods (*Table 1*).

Although the number of mouldy foods that we analysed was not very large, one can see from *Table 1* that peanut, hazelnut and walnut kernels, and almonds when mouldy contain aflatoxin in more than 50 per cent of the samples and even show the highest amounts (up to 50000 ppb) of that toxin. This is in good agreement with the observations of Wheeler[4], Frank[10], Frank and Eyrich[1], Hesseltine[13] and Bösenberg and Eberhardt[5] who found that most of the tested groundnuts and groundnut products were contaminated with aflatoxin in extremely high amounts (up to 400 ppb). We found that single peanut and hazelnut kernels can be highly contaminated (30000 and 50000 ppb aflatoxin B_1 respectively). Cucullu[8] reported single peanut kernels which contained from 300 up to 1100000 ppb aflatoxin B_1.

Mouldy grain and corn and their processing products, also seem to be suitable media for aflatoxin production, although the detected aflatoxin contents were not much higher than 100 ppb. In the case of bread the tendency to form aflatoxins seems to depend upon the pH value of the medium[6] and increases with the content of vitamin B_1[7], protein and salts. In mouldy bread samples with low protein, salt and vitamin B_1 content we analysed up to 25 ppb aflatoxin B_1 whilst in mouldy whole-meal bread and shredded wheat the aflatoxin content was more than 100 ppb. Frank and Eyrich[1]

Table 1. Aflatoxin content in spontaneously mouldy foods

Kind of mouldy food	Number of samples	Samples containing aflatoxin	Percentage (roughly)	Aflatoxin B_1 content (ppb)	Kinds of mould isolated, aflatoxin producers are marked with an asterisk*
1. Oilseeds					
Peanut kernels	62	46	70	20–28000	*A. flavus** *M. mucedo*
Hazelnut kernels	29	18	50	5–50000	*A. flavus** *M. mucedo*
Walnut kernels	12	3	25	5–35, 500000	*A. flavus** *A. glaucus**
Cashew nuts	6	1	—	830	*A. flavus**
Almonds, sweet	23	19	80	39–4000	*A. flavus**
Sesame seed	19	4	20	4–10	*A. flavus** *A. glaucus**
2. Spices					
Nutmeg	13	3	20	5–15	*P. glaucum** *M. mucedo*
3. Fruits and vegetables					
Apples	15	1	—	35	*P. expansum** P. species
Oranges	14	4	20	5–50	*P. expansum** *P. digitatum* *P. citromyces strictum var. asymetricum*
Lemons	18	3	15	20–60	*P. digitatum** P. species
Peaches	20	9	40	5–15	*P. glaucum** *A. glaucus** *A. flavus** *A. nidulans** *A. fumigatus*
Cherries	8	1	—	5	*P. expansum** *P. digitatum*
Apricots	11	0	0	0	*M. mucedo* *P. glaucum* A. species
Cucumber	4	2	—	0	P. species
Tomatoes	8	1	—	5	*A. flavus** *M. mucedo* P. species
4. Wheat, rye					
Wheat kernels	7	3	—	10–15	*A. flavus**
Rye kernels	2	1	—	15	*A. flavus**
Maize	3	1	—	25	*A. flavus**
White bread	18	2	10	20–25	*P. glaucum**
German bread 'Landbrot', made from 80% wheat flour 20% rye flour	14	1	10	10	*A. glaucus**
Whole-meal wheat bread	18	4	20	5–60	*P. glaucum**
'Müsli', made from shredded wheat and water ('organic food')	14	4	25	20–120	*A. flavus** P. species

Table 1 (cont.). Aflatoxin content in spontaneously mouldy foods

Kind of mouldy food	Number of samples	Samples containing aflatoxin	Percentage (roughly)	Aflatoxin B_1 content (ppb)	Kinds of mould isolated, aflatoxin producers are marked with an asterisk*
5. Cheese					
Edam type	11	0	0	0	*P. glaucum* *A. flavus*
Cheese, lyophilized, powdered	11	0	0	0	*P. glaucum* *A. flavus*
6. Meat products					
Bacon	10	2	—	1000, 5000	*A. flavus**

found that 10 per cent of the mouldy whole-meal breads contained aflatoxin and also detected 7000 ppb B_1 and 1000 ppb G_1 aflatoxin in a 'Müsli' made from shredded wheat and water, which was supposed to be eaten after standing for 3–5 days at 28–30°C.

Fresh fruit such as peaches and cherries (not apples) also seem to be preferred media for the growing of aflatoxin-producing fungi, although the excreted toxin amount is rather low (5–15 ppb). Oranges and lemons showed on their mouldy peels aflatoxin contents of up to 50 ppb.

Vegetables similar to tomatoes for instance (not mentioned in *Table 1*) often show mouldy spots but they are mostly contaminated by *Mucor* which normally does not produce aflatoxin; Aspergillus or Penicillium can only occasionally be isolated. The aflatoxin content, if any, is low.

In some mouldy foods such as jams, raisins, potato chips and crisps, and pickles we never detected any aflatoxin. With regard to jams this confirms the observation of Frank and Eyrich[1]. The high concentration of sucrose in jams and raisins, the sodium chloride content (2 per cent) of potato chips and crisps and the low pH value of 4 in pickles may prevent the germination of the spores and the formation of aflatoxins in these foodstuffs. Aspergillus growing on jams very often shows atypical famine forms.

From the mouldy foods mentioned in *Table 1* we isolated 45 mould stems; 15 of them were *A. flavus*; 7 of them were *P. glaucum*; 5 of them were *M. mucedo*; 4 of them were *A. glaucus* and the rest were other different Penicillia and Aspergilli.

Migration (diffusion) of aflatoxin into foodstuffs

Aflatoxins excreted by the mycelium may deeply diffuse into foodstuffs during a few days.

(*a*) *Coconut*

We inoculated the endosperm of a cross-sectioned coconut with *A. flavus* Linck and kept it at 35°C and 90 per cent relative humidity. The aflatoxin content was estimated two weeks after inoculation. The results are given in *Table 2*.

Table 2. Diffusion of aflatoxin B_1 from inoculated *A. flavus* into coconut endosperm. Three series: 14 days, 35°C

Tested component	Depth (mm)	Content of aflatoxin B_1 (ppb) test 1	test 2	test 3	Mycelium
Spores	0	*	*	5	–
Mycelium	2	20	250	11000	+
Endosperm	10	—	85	12000	+
Endosperm	20	—	835	38000	–

* not detectable

Aflatoxin was identified in the spores in only one of the series. Toxin production in spores seems to depend upon the state of growth and other unknown circumstances. We tested the aflatoxin content of spores of *A. flavus* growing on a single walnut kernel every week and could not detect any aflatoxin in the first two weeks. In the third week, however, we analysed 500 ppm!

The aflatoxin B_1 content of the mycelium was different in the three series: 20, 250 and 11000 ppb respectively. In the endosperm, 10 mm below the mycelium, we found 85 and 12000 ppb respectively; in a depth of 20 mm 835 and 38000 ppb were detected.

(*b*) *Whole-meal wheat bread*

A loaf of 14 cm length was cut in the middle into two pieces. One piece was inoculated with *A. flavus* Linck on the crust and the other one on the crumb, and then they were stored for 9 and 21 days respectively at 35°C and 90 per cent relative humidity. A considerable amount of the toxin migrated into the food to a depth of 7 cm (*Tables 3a* and *3b*).

The results show that aflatoxins are able to penetrate foods several centimetres in the course of only a few days. Migration of aflatoxin in cheese was reported by Frank[10] and the Netherland Yeast and Alcohol Company[12]. Kiermeier and Behringer[9] observed a penetration of 16 cm.

Table 3a. Diffusion of aflatoxin B_1. *A. flavus* inoculated on the crust of whole-meal bread. Two series; 9 and 21 days respectively, 35°C

Tested part	Depth (mm)	Content of aflatoxin B_1 (ppb) series 1	series 2	Mycelium
Crust	5	130	284000	+
Crumb	25	55	137000	+
	45	10	900	+
	65	35	4100	+
	70	420	45000	–

Table 3b. Diffusion of aflatoxin B_1. *A. flavus* inoculated on the crumb of wholemeal bread: 21 days, 35°C

Tested part	Depth (mm)	Content of aflatoxin B_1 (ppb)	Mycelium
Crumb	5	208 000	+
	25	72 000	+
	45	200	−
	65	1400	−
Crust	70	600	−

The mycelium of a mouldy food generally contains more aflatoxin than the food underneath. The aflatoxin content first decreases with its distance from the mycelium. However, at a certain depth the aflatoxin content increases again. This correlates with the observations of Frank[10] in apple juice and of Kiermeier[11] and Kiermeier and Behringer[9] in cheeses (*Table 4*).

Table 4. Diffusion of aflatoxin B_1 in Tilsiter cheese[9]

Depth (mm)	Aflatoxin B_1 (ppb; mean $\bar{x}$)
0	19
20	4
40	5
60	2
80	3
100	5
120	17

We do not have a satisfactory explanation for this phenomenon. The rather high aflatoxin level at the end of the migration point may be a concentration effect. In the regions below the mycelium other and more slowly migrating[10] substances which partly destroy the aflatoxins may be produced. Or, the aflatoxin itself may undergo unknown reactions during migration.

Aflatoxin-containing food with no visible mould or spore infection

Even non-mouldy food may contain aflatoxins: the reasons can be different.

(*a*) *Infection by insects*

A. flavus as well as *A. parasiticus* can be transferred to foods by insects[14], especially flies[15] and birds[16]. The spores germinate, hyphae grow out and aflatoxin is excreted into foods such as ham, meat, bread and cheese. Afterwards the mycelium could be removed by technological processes such as cutting out, washing[24], salting, mixing, drying and heating. This may account for our findings of 5 ppb aflatoxin B_1 in a sample of mould-free salami and 100 ppb in a mould-free ham.

(b) Infection of flowering plants

Mould spores can germinate on the stigmae of the pistil of flowering plants. The pollen tube grows down the style infecting the egg-cells in the embryo sac of the ovary (*Figure 1*) with mould and/or aflatoxin. Mould-free peanut kernels isolated from their shell contained 5 and 7 ppb aflatoxin B_1, whilst more frequently occurring mouldy kernels in the shell may contain more than 100 ppb aflatoxin B_1.

A. flavus and/or P. species infected kernels can be found in mould-free peaches. Their seeds can contain up to 10 ppb aflatoxin $B_1 + B_2 + G_1$.

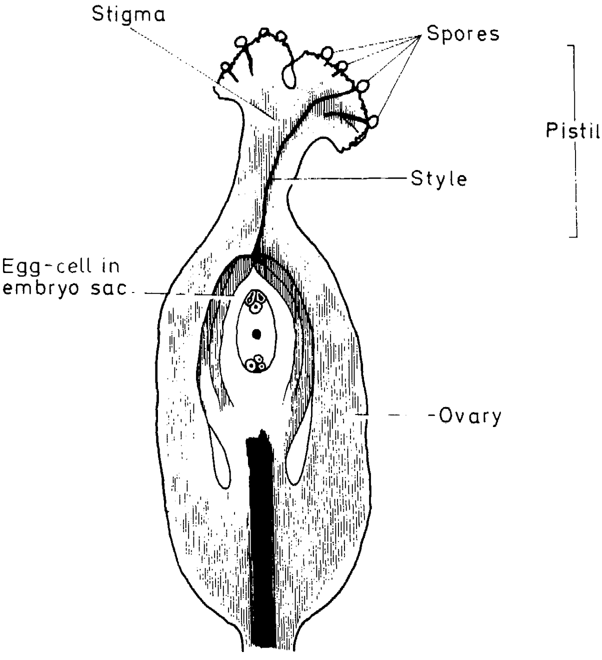

Figure 1. Infection of the ovary by mould spores

(c) Formation of aflatoxin during food processing

There are two typical German sweet goods called Marzipan and Persipan. They are produced from almonds and apricot or peach seeds respectively, by mixing these with equal parts of sugar and drying the obtained mass at a temperature of 90–95°C to a water content of 17 per cent (Marzipan) or 20 per cent (Persipan).

In the production of Marzipan (almond paste) the almonds are liberated from their skin (seed-coat) by washing with hot water. In the production of Persipan (peach and/or apricot seed paste) the seeds are first liberated from their skin and their hydrocyanic acid content by watering for 24–48 hours at 12°C. If these blanched almonds and peach and apricot seeds are not processed very soon after blanching an infection by mould spores and subsequent poisoning by aflatoxin can occur. (Blanched almonds kept for

3 days at 28°C contained 3 ppb aflatoxin B_1 and traces of B_2 and G_2). This is one of the reasons why Marzipan and Persipan often contain aflatoxins. Moreover, with Persipan the rate of infection (10 out of 20 samples) and the actual aflatoxin amount (up to 5 ppb) was much higher than with Marzipan where the rate of infection was 4 out of 16 samples and the actual aflatoxin amount did not exceed 2 ppb (*Table 5*).

Table 5. Analysis of almond paste (A) and apricot seed paste (B)*

	Number of samples	Amount of aflatoxin (ppb) B_1	B_2	G_1
A	12	†	†	†
A	3	traces	†	†
A	1	2	traces	†
B	10	†	†	†
B	1	traces	†	†
B	3	traces	traces	†
B	2	1	†	†
B	2	2, 5	†	traces
B	1	3	†	†
B	1	5	†	†

* Aflatoxin G_2 was not detected in any of the samples
† not detectable

(*d*) *Aflatoxin in non-mouldy animal food products*

Animal fodder—If animals receive mouldy fodder there is always the probability of aflatoxin intake and passage and/or transformation into human food.

Non-mouldy pellets, the feed for hatched trouts, contained 500 ppb aflatoxin B_1. In the livers of the trouts fed with these pellets we detected only traces of aflatoxin, whilst the faeces contained 500 ppb aflatoxin B_1. However, there is always the possibility that the flesh may contain aflatoxins and tumours[19–21].

In non-mouldy feed for birds, consisting of hemp, sun-flower seeds, millet, barley, maize and 'coloured' peanut kernels, we detected 300 ppb aflatoxin B_1 and 125 ppb B_2. Aflatoxin-containing feed of birds may be one of the reasons for the kidney and liver cancers very often observed in canaries.

Mouldy corn which was to have been used as stag or deer fodder contained 25 ppb aflatoxin B_1 and 25 ppb aflatoxin G_1.

Milk powder—If cows are fed with aflatoxin-containing fodder, less than 5 per cent of the aflatoxin B content is converted to aflatoxin M[23], which is excreted with the milk.

During the period February to August 1972 we tested 27 samples of spray dried skim milk powder and 28 samples of spray dried whole milk powder. These samples were supplied by five different German manufacturers. The results are shown in *Table 6*.

Supplier D was the only one whose samples—with the exception of one—did not contain any aflatoxin M_1. In contrast practically all samples from supplier A contained aflatoxin M_1 and even showed amounts of up to 4 ppb.

Table 6. Analysis of aflatoxin M_1 content of commercial milk powders from five different German manufacturers

Manufacturer	Number of samples		Aflatoxin M_1 not detected		Aflatoxin M_1 traces		Aflatoxin M_1 1 ppb		Aflatoxin M_1 2–4 ppb	
	S*	W†	S	W	S	W	S	W	S	W
A	8	7	1	1	4	3	1	2	2	1
B	—	14	—	5	—	9	—	—	—	—
C	6	—	3	—	1	—	2	—	—	—
D	5	—	4	—	1	—	—	—	—	—
E	8	7	2	3	5	4	1	—	—	—

* S = skim milk powder
† W = whole milk powder

These observations confirm the findings of Neumann–Kleinpaul and Terplan[17]. They analysed 80 samples of spray and drum dried skim and whole milk powders, and 56 samples of dried milk for dietary purposes, babies and children, and found aflatoxin M_1 contents of between 0.67 and 2 ppb in 6 per cent of the samples. Purchase and Vorster[18] investigated fluid milk samples for aflatoxin M_1 content and found that 2 out of 21 contained about 0.16 ppb and 3 contained traces.

The above results have a practical bearing on the feeding of animals and men. There is a danger of aflatoxin intake from animal food for human consumption if the animals receive mouldy fodder. The absence of aflatoxins cannot be guaranteed when the industrially produced fodder is not free of aflatoxins and the farmers, who often give mouldy feed to the animals, do not know about the dangerous effects of mycotoxins.

DISCUSSION

Although the natural occurrence of aflatoxins is rare there are many possibilities by which humans and animals can be harmed by unconscious intake of mycotoxins with their food. Aflatoxins occur in the spores, in the mycelium, or as separate exotoxins in the nutritive medium of the fungi, i.e. the food.

Certain special foods such as peanuts, almonds, treenuts, wheat, rye and maize kernels, or whole-meal products seem to be preferred media for the growing of aflatoxin-producing fungi. On the other hand, fruit and vegetables if contaminated contain aflatoxins in only small amounts, if at all. Aflatoxins were never detected in some naturally mouldy foods such as jams, potato chips and crisps, and pickles.

We found that the following conditions for storing raw materials and foods effectively controlled aflatoxins and prevented mould from growing (and toxin production).

Relative humidity <65 per cent; mould fungi require a relative humidity of 65 per cent.

Temperature <10°C. Mould fungi are able to grow at -3°C, however, aflatoxin production induced by Aspergillus and Penicillium ceases at 13 and 4°C, respectively.

Moisture content <12 per cent; aflatoxin synthesis can commence with 12 per cent substrate humidity.

This is the reason for our proposal of very closely kept storage conditions for some raw materials which are easily susceptible to mould growth and aflatoxin formation (*Table 7*).

Table 7. Storage conditions for some raw materials which are susceptible to mould growth and aflatoxin formation; storage time 6 months

Raw material	Substrate humidity (% H_2O)	Temperature (°C)	Relative humidity (% φ)
Wheat kernels, Rye kernels, Maize kernels	<15	6	60
Peanut kernels	6	4	<65
Cashew kernels	6	8	<65
Hazelnut kernels	6	8	<65
Walnut kernels	6	4	<65
Almonds	6	6–10	<65

Tables 8a and *8b* show how far harvesting and storage conditions ('aflatoxin control') may influence the quality of exported peanuts. We never found aflatoxins in Chinese samples. The peanuts are grown in the northern part of China and are harvested during a 3 month period in which there is usually no rain fall. The quality of peanuts from the Sudan has much improved during the course of the last few years. Peanuts from the USA always had particular samples with aflatoxin amounts above 100 ppb. Peanuts from

Table 8a. Aflatoxin content in peanut kernels of different provenances; 1970

Provenance	Number of samples	Aflatoxin B_1 (ppb)
South Africa	543	*
	1	20
	2	10
	6	5
	1	5
USA	54	*
	1	10
	1	125
Sudan	19	*
	1	40
Angola	1	625 B_1 180 B_2 315 G_1 40 G_2
Brazil	1	*
China	1	*
Malawi	2	*
Senegal	2	*

* not detectable

South Africa were practically free of aflatoxins because of the intensive hand picking and the hard control work done by the Oil Seeds Control Board. Their toxin content never exceeded 20 ppb.

Table 8b. Aflatoxin content in peanut kernels of different provenances; 1971

Provenance	Number of samples	Aflatoxin B_1 (ppb)
China	8	*
Sudan	12	*
	1	5
	1	20
South Africa	258	*
	1	20
USA	35	*
	1	5
	1	10
	1	625

* not detectable

The food industry and farmers should observe all conditions attached to the so-called (food) processing hygiene[22]. This includes sanitation of raw materials and their manufacturing. Mouldy foodstuff should neither be used for the production of human food nor for feeding animals. Otherwise there can be no guarantee given that the food products are free from aflatoxins.

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