Aflatoxin Contamination in Foods and Foodstuffs

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田端 節子* : 食品のアフラトキシン汚染

Aflatoxin, a metabolic product of *Aspergillus flavus* and *A. parasiticus*, is one of the strongest carcinogenic compounds, and it is found in several kinds of foods and foodstuffs^{1,2)}. Most countries set limits on aflatoxins, and the regulation level in Japan is 10 ppb for aflatoxin B₁. In our laboratory, aflatoxins in foods and foodstuffs have been tested since 1971, when aflatoxin was first found in peanut cream in Japan. This report summarizes the data on aflatoxin contamination in commercial foods and foodstuffs in Tokyo for 15 years during the period of 1982-1996.

Materials and Methods

Samples A total of 7,500 samples, consisting of nuts, cereals, spices, beans, and dairy products, collected from commercial markets and from the food industry in Tokyo, were examined.

Aflatoxins determination Aflatoxins were determined and confirmed by the method reported earlier³⁾, as follows: 200-1000 g samples were ground; after addition of water, aflatoxins were extracted with chloroform from 20 g of the ground sample, purified by florisil column chromatography, and determined and confirmed by high-performance thin-layer chromatography. Detection limits (ppb) were 0.2 for B₁, 0.1 for B₂, 0.2 for G₁, 0.1 for G₂, and 0.1 for M₁. Recoveries of aflatoxins were 91% for B₁, 89% for B₂, 78% for G₁, 78% for G₂, and 92% for M₁.

Results and Discussion

Aflatoxins in nuts Measurable levels of aflatoxins were found in peanut, pistachio nut, Brazil nut, and sesame seed (Table 1).

Of 459 samples of peanut examined, 35 samples were contaminated with aflatoxins. 3 samples contained over 10 ppb aflatoxin B₁, the regulation level in Japan. The high incidence in peanut was from certain kinds of their products such as peanut butter and peanut powder. The incidence of aflatoxin in whole peanut, the largest part of the peanut sample, was low, but high in crushed, powdered peanut, peanut paste, and peanut oil (Table 2). The reasons were probably as follows. One possible reason was that the whole peanut products were higher grade, and crushed peanuts, whose grade the consumer cannot estimate, were lower grade. The other possible reason was that aflatoxin contamination is very partial, grain by grain. In the case of crushed peanut product, a large amount of peanuts were crushed. Therefore, the probability to include aflatoxin-contaminated grain was high.

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Table 1 Incidence and levels of aflatoxins in nuts and nut products

Sample	No. of sample	No. of positive	Range (ppb)			
			AFB ₁	AFB ₂	AFG ₁	AFG ₂
Peanut	459	35(3)	0.4-21.7	0.1-5.3	0.3-22.1	Tr.-6.8
Pistachio nut	481	9(7)	0.8-1382	0.1-260	306	48.3
Cashew nut	212	0				
Almond	151	0				
Brazil nut	8	1(1)	10.2	0.8	3.2	0.3
Walnut	71	0				
Macadamia nut	20	0				
Pine seed	17	0				
Pumpkin seed	23	0				
Mixed nut	17	0				
Sesame seed	47	5	0.6-2.4	0.2-0.5		
Others	101	0				
Total	1607	50				

() : No. over regulatory limit Tr. : <0.1 ppb

Table 2 Incidence of aflatoxins in peanut and peanut products

Sample	No. of sample	No. of positive	Incidence of AFB ₁ (%)
Whole	391	1(1)	3
Crushed	7	2(1)	29
Powdered	17	11(1)	64
Paste	34	16	47
Oil	11	5	45
Total	459	35	8

() : No. over regulatory limit

Of 481 samples of pistachio nut examined, 9 were contaminated with aflatoxins, and 7 samples contained over 10 ppb aflatoxin B₁ (Table 1). The level of aflatoxin in pistachio nut was very high; the highest level of aflatoxin B₁ was 1382 ppb. The tested samples were from Iran and the United States. A great difference was observed in the incidence and level of aflatoxins depending on the country of origin. Of the positive samples, only 1 sample was from the United States. The other 8 samples were from Iran, although more than half of the samples tested was from the United States. This difference was probably due to different conditions after harvest.

One of the 8 brazil-nut samples contained over 10 ppb aflatoxin B₁. The sample was from Brazil.

Of 47 samples of sesame seed examined, 5 contained aflatoxins. Most of the positive samples were from China.

No aflatoxins were detected from cashew nut, almond, or walnut, although they were imported from India, the United States, and China, areas suitable for producing aflatoxins.

Aflatoxins in cereals and sugars Measurable levels of aflatoxins were found in coix seed, corn, buckwheat, and sugar (Table 3).

The positive sample of rice was powdered rice, called "shiratama-ko" in Japan.

Table 3 Incidence and levels of aflatoxins in cereals, sugar, and their products

Sample	No. of sample	No. of positive	Range (ppb)			
			AFB ₁	AFB ₂	AFG ₁	AFG ₂
Rice	170	1	0.5			
Wheat	352	0				
Barley	276	0				
Oat	39	0				
Rye	21	0				
Coix seed	212	48(1)	0.1-14.9	Tr. -1.8	0.3-0.7	
Corn	474	4	0.1- 0.4			
Buckwheat	252	23	0.1- 8.8	0.1-0.9	0.2-0.8	Tr. -0.1
Mixed flour	81	0				
Sugar	31	12	1.0- 1.5	0.1-0.2		
Others	78	1	0.4			
Total	1991	92				

() : No. over regulatory limit Tr. : <0.1 ppb

Coix seed is a traditional herbal medicine. During 1980s, it was in great demand as a healthy food in Japan, and a large amount of coix seed was imported from other Asian countries. Over 20% of samples examined were contaminated with aflatoxins. 1 sample contained over 10 ppb aflatoxin B₁. The positive samples were imported from Thailand.

Aflatoxin B₁ was found at levels of 0.1-0.4 ppb in 4 of 474 corn samples examined. Of the positive samples, 2 were corn grits and 2 were corn snacks. We reasoned that the source of aflatoxin might be corn grits.

Of 252 buckwheat samples examined, 23 were contaminated with aflatoxins. The highest level of aflatoxin B₁ was 8.8 ppb, near the regulatory limit in Japan. All the positive samples were buckwheat powder, packed 200-300 g in small paper bags, and sold at supermarkets for cooking at home. Buckwheat is a staple food in Japan, used in foods such as buckwheat noodles, and intake is large. Therefore, if commercial buckwheat noodles were contaminated with aflatoxins, it would be a serious problem. So we started to examine commercial buckwheat noodles. No aflatoxin was detected. We believe that the buckwheat powder contaminated with aflatoxin was on a different commercial route.

Of 31 sugars examined, 12 were contaminated with aflatoxins. All of the positive samples were mucovado (crude sugar) from the extreme south of Japan. No aflatoxin was detected in refined sugar.

Aflatoxins in Spices Measurable levels of aflatoxins were found in white pepper, red pepper, paprika, nutmeg, and mixed spice (Table 4).

Of 220 white pepper samples examined, 21 were contaminated with aflatoxins. The level of aflatoxin B₁ was 0.1-2.3 ppb, which was not very high.

A high incidence (38%) and high levels of aflatoxins were found in red pepper. 3 samples imported from China contained over 10 ppb aflatoxin B₁.

Over 50% of paprika samples examined were contaminated with aflatoxins. Some of them were imported from Spain.

Table 4 Incidence and levels of aflatoxins in spices

Sample	No. of sample	No. of positive	Range (ppb)			
			AFB ₁	AFB ₂	AFG ₁	AFG ₂
Black pepper	120	0				
White pepper	220	21	0.1- 2.3	0.1-0.3		
Red pepper	81	31(3)	0.2-27.7	0.1-1.2	0.1- 2.1	0.1-0.2
Paprika	44	26	0.2- 6.5	0.1-0.3		
Nutmeg	257	155(17)	0.2-60.3	0.1-6.5	0.1-15.8	0.1-0.4
Mace	56	0				
Caraway	21	0				
Thyme	25	0				
Cinnamon	18	0				
Coriander	13	0				
Laurel	23	0				
Clove	18	0				
Hop	52	0				
Ginger	22	0				
Sage	27	0				
Curry powder	18	0				
Mixed spice	161	28	0.2-1.9			
Others	128	0				
Total	1304	261				

() : No. over regulatory limit

Table 5 Incidence and levels of aflatoxins in beans and bean products

Sample	No. of sample	No. of positive	Range (ppb)			
			AFB ₁	AFB ₂	AFG ₁	AFG ₂
Soy bean	113	0				
Beans for beanjam	916	14(4)	0.1-254	0.4-8.5		
Coffee bean	77	0				
Others	100	0				
Total	1206	14				

() : No. over regulatory limit

The highest incidence and levels of aflatoxins were found in nutmeg. 17 samples contained over 10 ppb aflatoxin B₁. Most of them were imported from Indonesia.

No aflatoxins were detected in black pepper, mace, or laurel.

A remarkable difference was found in the incidence and level of aflatoxins between white and black pepper, and between nutmeg and mace, although they are a part of the same plant.

There are two possible reasons. One is the difference in the treatment after harvest. To make white pepper, the pepper is soaked in filthy ponds to remove the skin at the producing district, in a tropical area. The other reason seemed to be the difference between the spices as substrates for aflatoxin producing fungi.

Aflatoxins in beans 0.1-254 ppb of aflatoxin B₁ was found in beans for bean jam, which are called butter bean and which come from Myanmar (Table 5). Aflatoxin was also detected in

bean jam, the product of the butter bean.

No aflatoxins were detected in soybean, coffee bean, cacao bean, or other beans.

Aflatoxins in dairy foods Of 354 samples of natural cheese examined, 44 contained 0.1-1.2 ppb of aflatoxin M₁. No aflatoxins were detected in processed cheese, or other dairy foods.

Aflatoxins in other foods No aflatoxins were detected in dried fruit, tea, herbs, meat, eggs or other foods.

Annual change in aflatoxin contamination The contamination of aflatoxin in foods was not constant during the 15-year period (Fig. 1).

The incidence of aflatoxin in natural cheese and buckwheat was high in 1982-1985. After 1985, no aflatoxins were detected in these foods. Aflatoxin M₁ in dairy food is a metabolite of aflatoxin B₁ in dairy cattle. Therefore, these results indicated that aflatoxin contamination in feed for dairy cattle decreased after 1985. The reason for this seems to be that the number of countries with legislation controlling aflatoxin in feedstuffs increased from 22 in 1981 to 35 in 1986. Also, the European Community directive was tightened in 1984 when the tolerance for aflatoxin B₁ in feedstuffs for dairy cattle was reduced from 20 to 10 ppb. The reason for the change in buckwheat was probably that buckwheat from Brazil was not imported after 1986.

Until 1992, the incidence of aflatoxins in white pepper was over 30%, but no aflatoxins have been detected in recent years.

A high incidence was found in nutmeg throughout the period, reaching over 80% during 1985-1990.

The constant incidence in peanut was due to peanut butter.

Fig. 1 Change in incidence of aflatoxin
---△--- Natural cheese, ---◇--- Buckwheat, ---○--- White pepper, ---●--- Nutmeg,
---▲--- Peanut, ---□--- Pistachio nut, ---■--- Coix seed

Conclusion

Aflatoxins are found in several kinds of foods and foodstuffs every year, and some of them contain over 10 ppb aflatoxin B₁, the regulatory limit in Japan. Also most aflatoxins in foods remained after cooking, and ingested by humans⁴⁾. Therefore, it is important to develop a method for preventing aflatoxin contamination, and to check carefully for aflatoxins in foods and foodstuffs. We must try to eliminate aflatoxin-contaminated foods, to reduce the risk of aflatoxins.

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