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Aflatoxin, a metabolic product of *Aspergillus flavus* and *A. parasiticus*, is one of the strongest known carcinogens, and it is found in several kinds of foods and foodstuffs\(^1\),\(^2\). Most countries set limits on aflatoxins, and the maximum permitted level in Japan is 10 ppb for aflatoxin B\(_1\). 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 6 years during the period of 1991–1996, and the results are compared with the data of the previous period.

### Materials and Methods

**Samples**
A total of 2,047 samples, consisting of nuts, cereals, spices, beans, and dairy products, collected from commercial markets and from the food industry in Tokyo during the period of 1991–1996, were examined.

**Aflatoxins determination**
Aflatoxins were determined and confirmed by the method reported earlier\(^3\), as follows. Samples of 200–1,000 g were ground; after addition of water, aflatoxins were extracted with chloroform from 20 g of the ground sample, purified by Florisil column chromatography, and deter-

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of sample</th>
<th>No. of positive</th>
<th>Range (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AFB(_1)</td>
</tr>
<tr>
<td>Peanut</td>
<td>197</td>
<td>16 (2)</td>
<td>0.8–10.9</td>
</tr>
<tr>
<td>Pistachio nut</td>
<td>221</td>
<td>4 (2)</td>
<td>0.8–128</td>
</tr>
<tr>
<td>Cashew nut</td>
<td>75</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Almond</td>
<td>62</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Brazil nut</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Walnut</td>
<td>34</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Macadamia nut</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pine seed</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pumpkin seed</td>
<td>11</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mixed nut</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sesame seed</td>
<td>24</td>
<td>3</td>
<td>0.3–1.9</td>
</tr>
<tr>
<td>Others</td>
<td>31</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>673</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

\(\): No. over regulatory limit
mined 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, and sesame seed (Table 1). Of 197 samples of peanut examined, 16 samples were contaminated with aflatoxins. 2 samples contained over 10 ppb aflatoxin B1, the maximum permitted level in Japan. No aflatoxin was detected from commercial whole peanut product. The aflatoxin-positive samples were peanut butter and crushed or powdered peanut. This was probably due to the reason we reported before; commercial whole peanuts were of higher grade, and crushed peanuts, whose grade the consumer cannot estimate, were of lower grade2).

Of 221 samples of pistachio nuts examined, 4 were contaminated with aflatoxins. Two samples, imported from Iran, contained over 10 ppb aflatoxin B1, and the highest level was 128 ppb. The tested samples were from Iran and the United States. Of the positive samples, only 1 sample was from the United States. In the previous period, all the aflatoxin-positive samples were from Iran8). This difference between the 2 countries was probably due to the conditions after harvest.

Of 24 samples of sesame seed examined, 3 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, including areas where conditions are suitable for producing aflatoxins.

Aflatoxins in cereals and sugars

Measurable levels of aflatoxins were found in coix seed, and sugar (Table 2).

Coix seed is a traditional herbal medicine. About 50% of the samples examined were contaminated with aflatoxins. One sample contained 7.7 ppb aflatoxin B1. The positive samples were imported from Thailand.

Of 27 sugars examined, 9 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 3).

No aflatoxin was detected from black pepper.
Of 112 white pepper samples examined, 3 were contaminated with aflatoxins. The level of aflatoxin B₁ was 1.2–1.6 ppb, which is not very high. The reason for the difference in aflatoxin contamination between white and black pepper...
seemed to be 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.

A high incidence and high levels of aflatoxins were found in red pepper. One sample imported from China contained over 10 ppb aflatoxin B<sub>1</sub>. The highest incidence of aflatoxins was found in paprika. Over 75% of paprika samples examined were contaminated with aflatoxins. Some of them were imported from Spain.

Aflatoxins were found from a half of the nutmeg samples examined. Most of them were imported from Indonesia.

A great difference was found in aflatoxin contamination between nutmeg and mace, although they are parts of the same plant. The reason might be the difference in the aptitude as substrates for aflatoxin-producing fungi.

No aflatoxins were detected in thyme, laurel, clove, sage, or other spices.

**Aflatoxin contamination in beans**

No aflatoxins were detected in beans for bean jam (from which high levels of aflatoxin were detected in the previous period<sup>1,2</sup>), soybeans, coffee beans, cacao beans, or other beans.

**Aflatoxin contamination in dairy foods**

No aflatoxins were detected in natural cheese processed cheese, or other dairy foods.

**Aflatoxin contamination in other foods**

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

**Annual change in aflatoxin contamination**

Comparing these results with the results during the period of 1982–1990<sup>1,2</sup>, the aflatoxin contamination in foods was not constant (Fig. 1).

In 1982, 34% of natural cheese was contaminated with aflatoxin, then the incidence decreased year by year, and no aflatoxin has been detected since 1985. Aflatoxin M<sub>1</sub> in dairy food is a metabolite of aflatoxin B<sub>1</sub> 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 B<sub>1</sub> in feedstuffs for dairy cattle was reduced from 20 to 10 ppb.

Aflatoxin contamination in buckwheat was found in 1982–1985. The highest incidence was 46%, found in 1985. After 1985, no aflatoxins as detected. The reason for the change in buckwheat might be that buckwheat from Brazil was not imported after 1985.

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 the high incidence in peanut butter.

**Conclusion**

Aflatoxins are found in several kinds of foods and foodstuffs every year, and some of them contain over 10 ppb aflatoxin B<sub>1</sub>, the regulatory limit in Japan. Also, most aflatoxins in foods remain after cooking, and are ingested by humans<sup>6</sup>. Therefore, it is important to develop a method for preventing aflatoxin contamination, and to check carefully for aflatoxins in foods and foodstuffs.

**References**