Aflatoxin B₁ and ochratoxin A contamination in food and feed products in some provinces in the north of Vietnam

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Summary

One hundred eighty one samples of food and feed products were collected from some provinces in northern Vietnam. Natural occurrence of aflatoxin B₁ and ochratoxin A were surveyed in 26 samples of peanut kernel, 55 samples of animal feed, 20 samples of peanut products including peanut candy and fried peanut, 15 samples of soybean products including soybean powder, compressed soybean cake and soybean meat, 15 samples of maize products including nutrient corn powder and fried maize, 18 samples of coffee products including instant coffee and coffee berry, 16 samples of cashew nut products including cashew nut powder, cashew nut cake and cashew nut pigment powder, and 16 samples of meat products including crustaceous meat. Aflatoxin B₁ and ochratoxin A were analyzed according to an AOAC method and the modified AOAC method of Doronina and Makshimenko. Two of nine samples of peanut products in Hanoi city were contaminated with aflatoxin B₁ at an average concentration of 6 µg/kg. One of five samples of peanut products in Haiphong city and one of six of those in Thanh hoa province were contaminated with aflatoxin B₁ at the level of 4 and 5 µg/kg, respectively. Ochratoxin A was not detected in 20 peanut product samples. Neither aflatoxin B₁ nor ochratoxin A was detected in 80 samples including 15 soybean product samples, 15 maize product samples, 18 coffee product samples, 16 cashew nut product samples and 16 meat product samples. Thirteen of 26 peanut kernel samples and 35 of 55 animal feed samples were contaminated with aflatoxin B₁ at average concentrations of 43 µg/kg and 12 µg/kg, respectively.

Key words : food, feed, aflatoxin B₁, ochratoxin A, Vietnam

Introduction

Vietnam is a tropical country with high temperature and humidity. These conditions are favorable for fungal growth, especially mycotoxin-producing fungi. The distribution of toxigenic fungi and mycotoxin contamination in paddy and rice in Vietnam were reported. The natural co-occurrence of Fusarium toxins and aflatoxin B₁ in corn for
feed in northern Vietnam had been studied, and the occurrence of aflatoxin B₁ and ochratoxin A in maize harvested in Vietnam in 1995-1996 was reported. A study on the infected degree of species of Aspergillus flavus and aflatoxin B₁ in lotus seed in some localities were reported. Although trials for the control of fungal growth and decontamination by the fumigant, methylbromide, have been performed in the laboratory and in large scale, no systematic survey on mycotoxins in food and feed products has been carried out in Vietnam.

In addition, Vietnam is a country with diversity of agricultural products. The determination of mold and mycotoxin contamination in foods is essential for insurance of human health, and food hygiene and safety to meet the demand for domestic consumption and export. Toxicologically, aflatoxin B₁ is well known as a potent initiator of liver cancer, and ochratoxin A is a hepatotoxic and nephrotoxic substance with carcinogenicity. Therefore, we performed the survey of aflatoxin B₁ and ochratoxin A contamination in food and feed products in some provinces in the north of Vietnam.

**Materials and Methods**

*Samples for analysis* One hundred and eighty one samples of food and feed products were collected in Hanoi city, Haiphong city and Thanhhoa province, and stored under 4 °C until analysis. There were 26 samples of peanut kernel, 55 samples of animal feed, 20 samples of peanut products including sweet peanut and fried peanut, 15 samples of soybean products including soybean powder, compressed soybean cake and soybean meat, 15 samples of maize products including nutrient corn powder and fried maize, 18 samples of coffee products including instant coffee and coffee berry, 16 samples of cashew nut products including cashew nut powder, cashew nut cake and cashew nut pigment powder, and 16 samples of meat products including crustaceous meat.

*Standards of mycotoxins* Standard aflatoxin B₁ was purchased from Sigma Co. Lab., and standard ochratoxin A was provided by Dr. K. Tanaka of the Food Safety and Quality Div., National Food Research Institute, Tsukuba, Japan.

*Qualitative and quantitative analysis of aflatoxin B₁ in peanut, maize, soybean and meat products* Aflatoxin B₁ in peanut, maize and soybean products was analyzed by the modified AOAC Official Method 968.22 except for the clean up with Sep-pak Plus Silica Cartridges instead of column chromatography. The residue of the eluate was dissolved in 100 μl chloroform with ultrasonic waves, applied on a thin layer chromatograph (TLC) plate (Silicagel Whatman, Germany), which was then developed with chloroform-acetone (9:1, v/v). The confirmatory test for aflatoxin B₁ was carried out using trifluoroacetic acid anhydride. Detection limit was 4 μg/kg.

*Qualitative and quantitative analysis of aflatoxin B₁ in coffee products* Aflatoxin B₁ in coffee products was analyzed by the modified AOAC Official Method 970.46 (See
Qualitative and quantitative analysis of aflatoxin B, in cashew nut products  Aflatoxin B, was analyzed according to the method of Doronina and Makshimenko. Briefly, 200 g of cashew nut products was milled using a laboratory miller and mixed thoroughly. Twenty-five grams of the sample was added with a mixture of 100 ml acetone and 25 ml 10% NaCl, and homogenized in a homogenizer (Heidolph Diax 600) for 5 min. The suspension was filtered using a filter paper (Whatman No 1). Fifty ml of the filtrate was mixed with 10% Pb(CH₃COO)₂, a complexing agent, and filtered. Eighty ml of the filtrate was transferred to a separatory funnel, and the fatty portion was removed by extraction with 40 ml hexane (2 times). Forty ml chloroform was added to the aqueous layer to extract aflatoxin B, (3 times). The chloroform layer was evaporated using a vacuum evaporator. TLC was performed with the same procedure as above. Detection limit was 4 µg/kg.

Qualitative and quantitative analysis of ochratoxin A in coffee and maize products  Aflatoxin B, in peanut, maize and soybean products was analyzed by the same method as the modified AOAC Official Method 975.38 except that clean up was done using Sep-pak Plus Silica Cartridges instead of column chromatography. The residue of the eluate was dissolved in 100 µl acetic acid-benzene with ultrasonic waves, applied on a thin layer chromatography (TLC) plate (Silicagel Whatman, Germany) and developed with toluene-ethyl acetate-formic acid (5:4:1, v/v/v). The confirmatory test for ochratoxin A was carried out by spraying alcoholic NaHCO₃ solution. Detection limit was 4 µg/kg.

Ochratoxin A in peanut products, soybean products, cashew nut products and meat products  Ochratoxin A was analyzed according to the method of Shimomura and Ishikuro. Briefly, 200 g samples were ground and mixed thoroughly. Twenty five grams of the ground samples were homogenized with a mixture of 12.5 ml 5% acetic acid and 125 ml chloroform in a homogenizer for 5 min. The suspension was filtered using a filter paper (Whatman No 1) through anhydrous Na₂SO₄. Fifty ml of the filtrate was evaporated to dryness in a vacuum evaporator. Sep-pak Plus Silica Cartridge was used for clean-up. The residue dissolved in 2 ml toluene was loaded into the cartridge, which was then rinsed with 10 ml toluene and 6 ml chloroform-methanol (97:3, v/v). Ochratoxin A was eluted with 10 ml toluene-acetic acid (9:1, v/v) (two to four times). The eluate was evaporated to dryness under stream of N₂. TLC was performed as described above. Detection limit was 4 µg/kg.

Results and Discussion

As shown in Table 1, 20 samples of peanut products for food were collected from the markets in Hanoi city, Haiphong city and Thanh-hoa province. Two of nine peanut
product samples from Hanoi city were contaminated with aflatoxin B$_1$ at the level of 4 $\mu$g/kg to 8 $\mu$g/kg. One of five samples of peanut products from Haiphong city and one of six samples of peanut products from Thanh-hoa province were contaminated with aflatoxin B$_1$ at the level of 4 $\mu$g/kg and 5 $\mu$g/kg, respectively. The results in Table 1 also show that ochratoxin A was not detected in any peanut product sample.

Eighty samples of maize, soybean, coffee, cashew nut and meat products were collected from the markets in Hanoi city, Haiphong city and Thanh hoa province. The results in Table 2 show that neither aflatoxin B$_1$ nor ochratoxin A was detected in all the samples. Chau et al. [8] have studied the occurrence of aflatoxin B$_1$ in peanut products in Vietnam in 1995-1996, and found aflatoxin B$_1$ contamination in 7 of 10 samples from Hanoi city at the level of 5 - 450 $\mu$g/kg with the average level of 73 $\mu$g/kg, and in 10 of 15 samples from Thanh hoa province at the level of 5 - 950 $\mu$g/kg with the average level of 130 $\mu$g/kg. Only one of 20 samples of maize kernel collected from some provinces in Vietnam was contaminated with ochratoxin A at the level of 90 $\mu$g/kg. Chau et al. also reported that the concentration of ochratoxin A in maize kernel from the north of Vietnam in 1994 ranged from 0 $\mu$g/kg to 36 $\mu$g/kg, the average concentration being 10 $\mu$g/kg.

The contamination levels of ochratoxin A in the samples investigated were low compared with the levels reported in other countries. Contamination at the level of 15 - 200 $\mu$g/kg [10] and 10 - 50 $\mu$g/kg [11] have been observed in maize in France and in mixed feed in Poland, respectively. In the USA, contaminations at the level of 20-360 $\mu$g/kg [12] and 83-166 $\mu$g/kg [13] have been observed in coffee beans and maize, respectively. In Hungary in the year 2001, the average and the ranges of ochratoxin A levels found in feeding wheat, feeding maize and feeding barley samples were 12.2 and 0.3-62.8 $\mu$g/kg, 4.9 and 1.9-8.3 $\mu$g/kg, and 72 and 0.14-212 $\mu$g/kg, respectively. Sixty-six percent of the coffee samples were contaminated with ochratoxin A (average level: 0.57 $\mu$g/kg, ranges: 0.17-1.3 $\mu$g/kg) [14]. Contamination levels varying from traces to 400 $\mu$g/kg were found in
Table 2. Aflatoxin B1 and ochratoxin A contaminations in food commodities in some provinces in the north of Vietnam.

<table>
<thead>
<tr>
<th>Commodities</th>
<th>Location of sampling</th>
<th>Numbers of analyzed samples</th>
<th>Aflatoxin B1 concentration</th>
<th>Ochratoxin A concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize products</td>
<td>Hanoi</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Haiphong</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Thanh hoa</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Soybean products</td>
<td>Hanoi</td>
<td>6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Haiphong</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Thanh hoa</td>
<td>4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Coffee products</td>
<td>Hanoi</td>
<td>9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Haiphong</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Thanh hoa</td>
<td>4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cashew nut products</td>
<td>Hanoi</td>
<td>6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Haiphong</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Thanh hoa</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Meat products</td>
<td>Hanoi</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Haiphong</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Thanh hoa</td>
<td>6</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: not detected

Various feeds in all seasons, except in summer of the first research year, in Yugoslavia.153 Non-detectable levels of aflatoxin B1 and ochratoxin A in food product samples from some provinces in the north of Vietnam can be attributed to the following people in that area are aware of the danger of mycotoxins, and that mycotoxin contamination in food products should be controlled before food processing to insure safety of food and human health. When raw material is contaminated with high concentrations of aflatoxin B1 or ochratoxin A, we mix it with other raw materials which are not contaminated with the toxins or contaminated at a very low level with the toxins, and use the mixed material for animal feed. In addition, extraction and roasting procedures usually used for processing of maize, soybean, coffee and cashew nut may reduce aflatoxin concentration in maize. Chau et al. reported that aflatoxin B1 concentrations in peanut decreased remarkably after roasting154. In order to find the actual extent of aflatoxin B1 and ochratoxin A contaminations in food in Vietnam at present, it may be necessary to analyze larger numbers of raw material samples for the toxin contamination.

As shown in Table 3, 81 samples of peanut kernel and animal feed collected from the markets in some provinces in the north of Vietnam were contaminated frequently with aflatoxin B1. Thirteen of 26 peanut kernel samples were contaminated at the level of 5 to 237 µg/kg, the average level being 43 µg/kg. Thirty five of 55 animal feed samples were contaminated at the level of 5 - 950 µg/kg, the average level being 12 µg/kg.
Frequent contaminations in animal feed and in peanut kernel indicate that a monitoring program for aflatoxin contamination in raw materials is needed in Vietnam.

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References