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Contamination of Aflatoxins in Different Kinds of Foods in China

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Objective To study the contamination of total aflatoxins (AFs) in different kinds of foods including corn, peanut, rice, walnut, and pine nut in six provinces and two municipalities in China. **Methods** A total of 283 samples of corn, peanut, rice, walnut and pine nut were randomly collected from local markets in Fujian, Guangdong, Guangxi, Hubei, Jiangsu, and Zhejiang provinces, as well as in Shanghai and Chongqing municipalities. The samples were ground to which acetonitrile/water solution was added. After filtering, the extract was transferred into a MycoSep™ purifying column and was pressed slowly. Then the purified liquid was derivatized with trifluoroacetic acid (TFA) and assayed using high performance liquid chromatography (HPLC). **Results** AFs were detected in 70.27% of corn samples, with a mean level of 27.44 μg/kg and the highest level of 1098.36 μg/kg. In peanut, the AFs detection rate was 23.08%, with a mean level of 0.82 μg/kg and the highest level of 28.39 μg/kg. Very few rice samples with AFs were detected. The AFs levels were very low in walnut and pine nut. **Conclusion** Corn is the food most seriously contaminated with AFs in China. AFB_1 is the main aflatoxin which is found as a contaminant in foods.

Key words: Aflatoxins; Contamination; Foods

INTRODUCTION

Aflatoxins (AFs) are a group of toxins which are relatively stable and are produced mainly by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*[1-2]. Their carcinogenicity has been evaluated by International Agency for Research on Cancer (IARC) and classified as carcinogenic to humans[3]. Joint FAO/WHO Expert Committee on Food Additives (JECFA) has also evaluated for several times the safety of AFs to humans[4-7]. Peanut, corn, and tree nut are the most common foods contaminated with *A. flavus*, *A. parasiticus*, or *A. nomius*, and thus become the main source of human exposure to AFs[8].

In order to study the contamination of AFs in corn, peanut, rice, walnut, and pine nut, we conducted a survey in 2003 in eight regions in China, including Chongqing, Fujian, Guangdong, Guangxi, Hubei, Jiangsu, Shanghai, and Zhejiang.

MATERIALS AND METHODS

Sampling

All the samples were randomly collected from local food markets in the eight regions. The total sample size for corn, peeled peanut, rice, walnut (peeled and non-peeled), and pine nut (peeled and non-peeled) consisted of 84, 65, 74, 48, and 12 units, respectively; each sample weighed 1000 g. All the samples were packed in a dry and sealed condition, and were transported to the laboratory in Beijing within one week. In Beijing laboratory, each sample was completely milled and mixed evenly. A certain amount of each sample of the milled foods was weighed and subjected to the process for detection of AFs within one day. The rest of the samples that were not processed were packed in a sealed dry plastic bag and stored in a refrigerator under 4℃.

Methodology

Based on *AOAC Official Method 994.08: Aflatoxins in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts-Multifunctional Column (Mycosep) Method*[9], we made some modification in mobile phase in this study.

The HPLC system used in this study was a Waters 600 coupled with a Waters 474 fluorescence detector. The multifunctional purifying columns were MycoSep™ 228 produced by Romer Labs. Other

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vessels included electronic vibrating device, vortex blender, oven, centrifuge, vacuum dryer, *et al.* The chromatographic condition was as follows: HPLC column: Hypersil ODS (125×2.1 mm, 5 μm); column temperature 30℃. Mobile phase: water and acetonitrile; flow rate: 0.5 mL/min; injection volume 25 μL. Fluorescence detector: Ex 360 nm, Em 440 nm.

The standard solutions of AFB_1, AFB_2, AFG_1, and AFG_2 were provided by Romer Labs. The TFA (analytic grade) was provided by Sigma Co. The acetronitrile (analytic grade and HPLC grade) and n-hexane (analytic grade) were obtained from Fisher Co.

Stock solution of AFs in acetonitrile (AFB_1, AFG_1: 2.0mg/L; AFB_2, AFG_2: 0.5 mg/L) was diluted to produce serial working standard solutions. The concentration level of AFB_1 was the same as AFG_1, and AFB_2 was the same as AFG_2 in the solutions. The concentrations of AFB_1 (AFG_1) and AFB_2 (AFG_2) included 0.500 μg/L and 0.125 μg/L; 1.000 μg/L and 0.250 μg/L; 3.000 μg/L and 0.750 μg/L; 5.000 μg/L and 1.250 μg/L; 10.000 μg/L and 2.500 μg/L; 25.000 μg/L and 6.250 μg/L; 50.000 μg/L and 12.500 μg/L; 100.000 μg/L and 25.000 μg/L.

The ratio of acetonitrile (analysis grade) to water in the extracting solution was 84:16. The solution to resolve the residue before HPLC detection was water/acetonitril (HPLC grade) (85/15).

The experiment was conducted on the basis of the following procedure: put 20 g ground sample into 250 mL flask; add 80 mL acetonitrile/water solution (84/16) to the flask; shake it for 30 min on the electronic vibrating device; filter the solution into a glass tube; transfer 8 mL extracted solution into the glass tube of the multifunctional purifying column; press the MycoSep™ 228 column slowly and collect the cleaned extract solution; transfer 2 mL of the clean extract solution into a vial, and blow it mildly with nitrogen in 60℃ water; add 200 μL n-hexane and 100 μL TFA solution to the residue left, and cap the vial immediately; shake the vial for 30 s, and derivatize the solution at 40℃ for 15 min in an oven; completely evaporate the solution at room temperature, and then dissolve the residue in 200 μL water/acetonitrile (85/15); vibrate the vial on the vortex blender for 30 seconds; centrifuge the solution in a centrifuge tube vial for 15 min at 1000 rpm; pipet the supernatant into a HPLC vial for injection.

In this method, AFs were eluted in the following order: AFG_1, AFB_1, AFG_2, and AFB_2. The detection limits (LOD) for AFB_1, AFB_2, AFG_1, and AFG_2 were 0.012 μg/kg, 0.008 μg/kg, 0.036 μg/kg, and 0.024 μg/kg, respectively. The recoveries of AFB_1, AFB_2, AFG_1, and AFG_2 were more than 80%, and the RSD were less than 3.0%. Figure 1 shows the chromatograph of the aflatoxin standard solution, in which the concentrations of AFB_1, AFB_2, AFG_1, and AFG_2 were 25 μg/L, 6.25 μg/L, 25 μg/L, and 6.25 μg/L, respectively. Figure 2 shows the result of a corn sample, in which only AFB_1 (40.69 μg/kg) and AFB_2 (3.09 μg/kg) were detected.

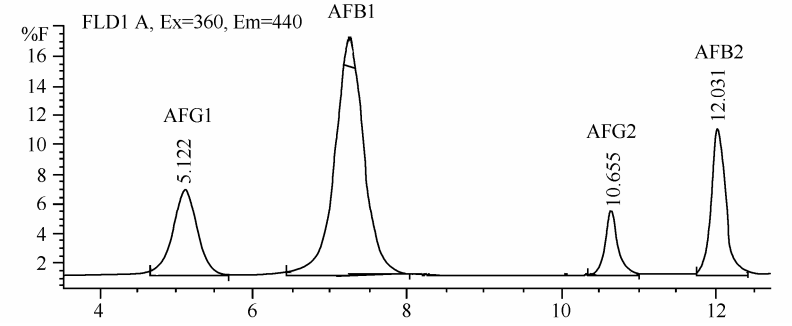

FIG. 1. Chromatograph of AFs standard solution.

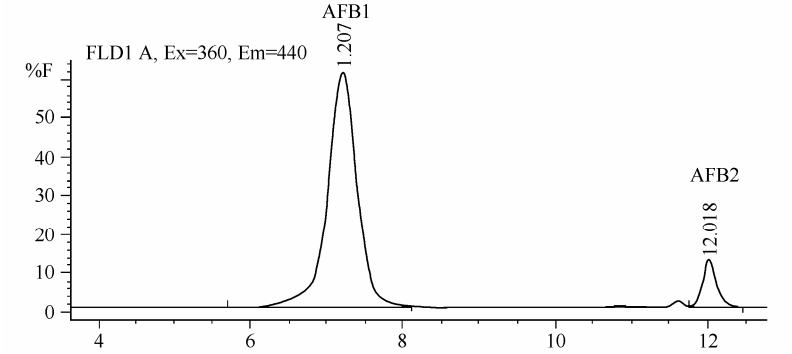

FIG. 2. Chromatograph of aflatoxins in a corn sample.

RESULTS

Contamination of AFs in Corn Samples

The contamination levels of AFs in different foods are listed in Table 1 and those in different areas are listed in Table 2. A total of 74 samples were tested, of which 52 were contaminated with AFs, with a contamination rate of 70.27%. The average content was 27.44 μg/kg, and the highest level was 1098.36 μg/kg.

TABLE 1

Contamination Levels (μg/kg) of AFs in Different Foods

Food	Sample (*n*)	AFB1		AFB2		AFG1		AFG2		AFs	
		Positive Samples (*n*)	Content (μg/kg)	Positive Samples (*n*)	Content (μg/kg)	Positive Samples (*n*)	Content (μg/kg)	Positive Samples (*n*)	Content (μg/kg)	Positive Samples (*n*)	Content (μg/kg)
Corn	74	46	0.14-970.32	41	0.02-128.04	9	0.36-4.76	0	-	52	0.02-1098.36
Peanut	65	9	0.15-22.39	5	0.03-6.00	4	0.42-11.73	3	0.12-2.36	15	0.03-28.39
Rice	84	16	0.15-3.22	3	0.06-0.24	7	0.36-1.59	0	-	23	0.15-3.88
Walnut	48	21	0.14-0.32	12	0.02-0.70	8	0.36-0.83	10	0.07-0.12	31	0.02-1.20
Pine Nut	12	2	0.19-0.23	1	0.02	0	-	0	-	2	0.19-0.25
Total	283	94	0.14-970.32	62	0.02-128.04	28	0.36-11.73	13	0.07-2.36	123	0.02-1098.36

Note. -: No aflatoxins was detected.

TABLE 2

Contamination Levels of AFs in Corn in Different Areas

Region	Sample (*n*)	AFB1			AFB2			AFG1		
		Positive Samples (*n*)	Content (μg/kg)	Average Content (μg/kg)	Positive Samples (*n*)	Content (μg/kg)	Average Content (μg/kg)	Positive Samples (*n*)	Content (μg/kg)	Average Content (μg/kg)
Chongqing	10	9	1.58-774.06	116.06	9	0.13-45.03	6.86	1	4.76	4.76
Fujian	8	4	0.19-2.54	0.85	3	0.08-0.18	0.11	2	0.42	0.42
Guangdong	10	5	0.15-970.32	161.95	5	0.03-128.04	21.38	2	0.39-0.43	0.41
Guangxi	10	10	0.25-38.49	11.52	10	0.03-2.33	0.68	0	-	-
Hubei	10	6	0.14-89.82	16.13	6	0.02-7.91	1.45	1	0.44	0.44
Jiangsu	6	6	0.37-38.94	12.25	6	0.02-2.81	0.67	0	-	-
Shanghai	10	2	0.15-0.17	0.16	1	0.04	0.04	3	0.36-0.72	0.49
Zhejiang	10	4	0.15-0.38	0.25	1	0.08	0.08	0	-	-
Total	74	46	0.14-970.32	49.07	41	0.02-128.04	5.00	9	0.36-4.76	0.93

Both AFB_1 and AFB_2 were identified in every sampled province or municipality. In total, 46 samples were contaminated with AFB_1. The contamination rate was 62.16% with an average contaminant content of 29.05 μg/kg. The average content of AFB_1 in corn from Chongqing and Guangdong was higher than that in the other six regions. However, the contamination rate was highest in Guangxi (100%, 10/10). AFB_2 was identified in 41 samples. The contamination rate was 55.41% with an average contaminant content of 2.00 μg/kg. Five out of ten samples from Guangdong were contaminated with AFB_2, with an average contaminant content of 21.38 μg/kg. AFB_2 was detected in all of the corn samples from Guangxi at a level ranging from 0.03 to 2.33 μg/kg.

No AFG_1 was detected in the corn samples in Guangxi, Jiangsu or Zhejiang. In the other regions, the highest AFG_1 contamination rate within a region was 30.00%. In sum, only 9 out of 74 samples were contaminated with AFG_1, with an average contamination rate of 12.16%. The average contaminant content was 0.97 μg/kg, and the highest level was 4.76 μg/kg. In all of the corn samples, no AFG_2 was detected.

In AFB_1 positive corn samples, there were 11

samples (23.91%) with AFB_1 level higher than 20.00 μg/kg, which is the maximum limit for AFB_1 in corn and corn-based products in China[10] (see Table 3).

TABLE 3

AFB_1 Contamination Levels in Corn

Level (μg/kg)	Positive Samples (*n*)	Positive Rate (%)
< 20.00	35	76.09
⩾ 20.00	11	23.91

Contamination of AFs in Peanut Samples

Table 1 shows that out of 65 peanut samples, 15 were contaminated with AFs with a contamination rate of 23.08% and an average contaminant content of 0.82 μg/kg.

Nine samples were contaminated with AFB_1, with a contamination rate of 13.85%. The average contaminant content was 0.40 μg/kg. AFB_2 was identified in five samples, with a contamination rate of 7.69% and an average contaminant content of 0.10 μg/kg. AFG_1 was detected in four samples, with a contamination rate of 6.15% and an average contaminant content of 0.28 μg/kg. Only three samples were contaminated with AFG_2, with a contamination rate of 4.62% and an average contaminant content of 0.04 μg/kg.

Contamination of AFs in Rice Samples

AFB_1, AFB_2, and AFG_1 were detected in rice, but no AFG_2 was identified. The contamination rate of AFs was 27.38%, with an average contaminant content of 0.79 μg/kg. The contamination rates of AFB_1, AFG_1, and AFB_2 were 19.05%, 8.33%, and 3.57%, respectively. The content of AFB_1 (3.22 μg/kg) was the highest among the four aflatoxins in rice (Table 1).

Contamination of AFs in Walnut Samples

Of the 48 walnuts samples, 31 were contaminated with AFs, a contamination rate of 64.58%. The average and highest contents were 0.23 μg/kg, and 1.20 μg/kg respectively. Although all of the four aflatoxins were found in walnut, the levels of each kind of aflatoxin were all less than 1.00 μg/kg (Table 1).

Contamination of AFs in Pine Nut Samples

AFs were found only in 2 out of 12 pine nut samples, with a contamination rate of 16.67%. The average and highest contaminant contents were 0.22 μg/kg and 0.25 μg/kg, respectively. The main contaminant was AFB_1, with a contamination rate of 16.67%, but the level was very low (<0.30 μg/kg). Neither AFG_1 nor AFG_2 was detected in pine nuts samples (Table 1).

DISCUSSIONS

Among the foods examined, corn is the food most seriously contaminated with AFs, which not only has high contamination rate (70.27%) and high contaminant content (highest: 1098.36 μg/kg), but also is contaminated in wide geographic areas. Contaminated samples were identified in all the sampled regions, and the contamination rates ranged from 40.00% to 100.00%. The contamination of AFs in peanut should not be neglected, which has a contamination rate of AFs 23.08%, and the highest content is 28.39 μg/kg. The contamination of AFs in rice, walnut, and pine nut are not severe.

The AFs contamination levels in different foods rank in the following order: AFB_1>AFB_2>AFG_1 in corn; AFB_1>AFB_2>AFG_1>AFG_2 in peanut; AFB_1>AFG_1>AFB_2 in rice; AFB_1>AFB_2>AFG_2>AFG_1 in walnut, and AFB_1>AFB_2 in pine nut. Therefore, AFB_1 is the main aflatoxin contaminating foods, followed by AFB_2, AFG_1, and AFG_2.

In a previous study conducted by Feng-Qin LI *et al.*[11] in China in 1998, the contamination rate of AFB_1 in corn was 85% (17/20), with a contaminant range between 9 and 2496 μg/kg (mean, 460 μg/kg). In positive samples, 76% (13/17) contained AFB_1 at a level of >20 μg/kg (71-2496 μg/kg, mean, 598 μg/kg). AFB_2 in corn at a level ranging from 11 to 320 μg/kg (mean, 82 μg/kg) was simultaneously detected in 65% samples. AFG_1 in nine samples was detected, with a contaminant range of 12-21 μg/kg and a mean level of 15 μg/kg. There was no AFG_2 detected. In that study, HPLC analysis was used with a detection limit of 1 μg/kg for AFB_1 and AFB_2, 5 μg/kg for AFG_1 and AFG_2. In comparison with the previous study, it can be seen that the AFs levels (Table 2) and their positive rates at a level of >20 μg/kg (Table 3) in foods in China are much lower than before. However, much will have to be done for the control of AFs contamination to protect people's health. The Good Agricultural Practices (GAP) should be implemented widely to include the selection and breeding of new seeds for anti-contamination of AFs and measures to avoid contamination by some fungi producing AFs in field, harvest, transportation and storage. It is also very important to establish maximum limits for AFs in foods, not only for AFB_1, in China. The findings of this study also indicate that special attention should be paid to corn that is highly contaminated

with AFs.

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REFERENCES

1. Maurice O, Moss (2002). Risk assessment for aflatoxins in foodstuffs. *International Biodeterioration & Biodegradation* **50**, 137-142.
2. Goto T, Wicklow D J, Ito Y (1996). Aflatoxin and cyclopiazonic acid production by a sclerotium-producing *Aspergillus tamari* strain Applied and Environmental. *Microbiology* **62**, 4036-4038.
3. IARC (1993). Summaries & Evaluations, volume 56.
4. JECFA (1987). Evaluation of Certain Food Additives and Contaminants, Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization: Geneva, 33-37.
5. JECFA (1997). Evaluation of Certain Food Additives and Contaminants, Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization: Geneva, 45-46.
6. JECFA (1999). Evaluation of Certain Food Additives and Contaminants, Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization: Geneva, 69-77.
7. JECFA (2002). Evaluation of Certain Mycotoxins in Food, Fifty-sixth report of the Joint FAO/ WHO Expert Committee on Food Additives. World Health Organization: Geneva, 1-51.
8. Williams J H, Phillips T D, Jolly P E, *et al.* (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *Am J Clin Nutr* **80**(5), 1106-1122.
9. AOAC International (2001). AOAC Official Method 994.08. Aflatoxins in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts. In: William Horwitz, Official Methods of Analysis of AOAC International, 17th Edition Vol. Ⅱ, Chapter 49, AOAC INTERNATIONAL Published, pp. 26-27.
10. National Center for Health Inspection and Supervision (2005). GB2761-2005, Maximum levels of mycotoxins in foods. Standards Press of China: Beijing. (In Chinese)
11. Feng-Qin LI, Takumi Yoshizawa, Osamu Kawamura, *et al*. (2001). Aflatoxins and Fumonisins in Corn from the High-Incidence Area for Human Hepatocellular Carcinoma in Guangxi, China. *J Agric Food Chem* **49**, 4122-4126.

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