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Natural Occurrence of Aflatoxins in Chinese Peanut Butter and Sesame Paste

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A study on the natural occurrence of aflatoxins in Chinese peanut butter and sesame paste samples was conducted. Aflatoxin B_1 (AFB_1) was the predominant toxin detected abundantly and frequently at a level up to 68.51 μg/kg in 41 of 50 peanut butter samples and 20.45 μg/kg in 37 of 100 sesame paste samples analyzed by liquid chromatography (LC). Of the AFB_1-positive samples, 15 (37%) and 1 (2%) peanut butter samples with AFB_1 exceed European Union (EU) and Chinese regulations, respectively, whereas 19 and 32% of sesame paste samples contained AFB_1 higher than Chinese and EU regulations, respectively. Fourteen and 1 peanut butter samples and 10 and 7 sesame paste samples, respectively, will be legally claimed as positive, rejected, and even banned with consideration of an uncertainty of 40% for AFB_1, based on EU and Chinese regulations. Seeking to balance health benefits with the potential trade disruptions that regulations can cause is the issue of concern.

KEYWORDS: Aflatoxins; peanut butter; sesame paste; LC; fluorometry

INTRODUCTION

Aflatoxins are a group of potent hepatotoxic, teratogenic, mutagenic, and carcinogenic compounds produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nominus* in a number of foods both pre- and postharvest. They have been most commonly associated with groundnuts and their products, tree nuts, maize, dried fruit, spices, cottonseed, and so forth under specific moisture and temperature conditions. The major aflatoxins of concern and economic importance are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2), with AFB_1 being predominant and the most toxic (*1*). It was documented as a group I carcinogen by the International Agency for Research on Cancer, probably implicated in the etiology of human primary hepatocellular carcinoma (PHC) (*2*). The incidence of PHC varies widely throughout the world. It is one of the most common cancers and a public health issue in China, sub-Saharan Africa, and Southeast Asia (*3*). A 10-year surveillance program from 1980 to 1990 in China revealed that the number of patients diagnosed annually with PHC was 110,200, accounting for approximately 45% of the total PHC cases in the world (*4*). According to the National Center for Health Statistical Information of China, the overall number of deaths and proportionate mortality of PHC ranked first in malignant tumors in rural areas ($25.9/10^5$) and second in urban areas ($22.34/10^5$) in 2006, respectively (*5*). The geographical variation of PHC also suggests that the causes of PHC are multifactorial: genetic susceptibility and environmental exposures including chronic infection of hepatitis B virus (HBV), algal toxins in drinking water, and dietary exposure to AFB_1 are involved in the extraordinarily high rate of PHC in these regions.

China is one of the largest peanut producers in the world. The annual peanut output is 15 million tons, accounting for 42% of the world's output and contributing 25% to the world's gross trade. Peanuts are frequently contaminated with aflatoxins, owing to aflatoxin-producing fungi that have a high affinity to growth in peanut kernels. The earliest survey carried out in China in 1972 showed that 444 of 1689 (26%) raw peanut samples and 560 of 1172 (48%) peanut oil samples, respectively, were contaminated with AFB_1 at concentrations above the limit of detection (LOD) of 5 μg/kg (data not published). According to the results of a nine-year successive surveillance conducted from 1990 to 1999 in Fujian province, 323 (66%) peanut oil samples were positive for AFB_1 and 71 samples with AFB_1 levels exceeded the Chinese legislation of 20 μg/kg (*6*). Hence, it is likely that the contamination of AFB_1 in Chinese peanut and its products is severe.

Peanut butter is popular in some countries such as Canada, the United States, Australia, the United Kingdom, and New Zealand, due to its cholesterol-free, unique flavor, and richness in protein, vitamins, minerals, and unsaturated fatty acids. It is especially consumed with pleasure by children. Omer et al. (*7*, *8*) reported that peanut butter consumption has been identified as a strong risk factor for hepatocellular carcinoma as a result of aflatoxin contamination in Sudan. More reports on natural occurrence of aflatoxins in peanut butter were found in the world, but there are no data from China (*9*−*11*). Additionally, sesame paste is of great importance and popular in Chinese kitchens as sauce for hotpot, vegetable salad, steamed twisted rolls, and so forth.

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Sesame seed is sensitive to aflatoxin-producing fungal invasion as well. Therefore, for the sake of reducing dietary exposure to aflatoxins and protecting human populations from mycotoxin-related sufferings, the objectives of the present study are to study the natural occurrence and distribution of aflatoxins in peanut butter and sesame paste and to evaluate the potential effect of contamination of both foods with aflatoxins on international trade. The data obtained in this study might shed light on ongoing research and the hypothesis about the relationship between human dietary exposure to aflatoxins and high mortality of PHC in China.

MATERIALS AND METHODS

Samples. A total of 100 sesame paste and 50 peanut butter samples manufactured domestically in 2007 were collected from Beijing, Shanghai, Changchun, Chengdu, Shijiazhuang, and Zhengzhou retail markets. For the sesame paste, 59 samples were industrially manufactured and packed in glass bottles with shelf lives between 12 and 24 months, and 15 and 26 bulk samples were freshly prepared and obtained from free markets and restaurants, respectively. For the peanut butter, all samples were packaged either in plastic jars or in laminated paper packs with shelf lives between 9 and 18 months. The varieties included smooth (38 samples) and chunky (12 samples) peanut butter. Two of the smooth peanut butter samples were mixed with cocoa. The chunky peanut butter has peanut pieces blended into the smooth peanut paste. The majority (82%) of peanut butter was produced in Shandong province, the key peanut production base in China. The brands covered >90% of the domestically commercialized peanut butter and sesame paste products. All samples were analyzed for AFB_1, AFB_2, AFG_1, and AFG_2 and stored at 4 °C prior to analysis.

Chemicals and Reagents. Standards for AFB_1, AFB_2, AFG_1, and AFG_2 were purchased from Sigma Chemical Co. (St. Louis, MO). All organic solvents and water used for sample extraction and toxin separation were of HPLC grade. Sodium chloride was of analytical grade. The immunoaffinity column containing monoclonal antibody specific for AFB_1, AFB_2, AFG_1, and AFG_2 was purchased from Vicam Corp. (Watertown, MA).

Extraction of Aflatoxins. The test portion sample (25 g) was blended with 5 g of sodium chloride and 125 mL of a methanol/water (6:4, v/v) mixture for 1 min. Sample extract was filtered, diluted 2-fold with water, and passed through a glass microfiber paper. A 10 mL portion was applied to a conditioned immunoaffinity column. Aflatoxins were separated, purified, concentrated on column, and removed from antibodies with 1 mL of methanol. The eluate was analyzed by both fluorometer and liquid chromatography (LC).

Fluorometer and LC Determination. For the analysis of aflatoxins, all peanut butter and sesame paste samples were qualitatively screened for total aflatoxins with a TorBex model FX-100 fluorometer (Vicam Corp.) after reaction with bromine solution. Samples with a positive fluorometer calibration response (containing ≥1 μg/kg total aflatoxins) were reanalyzed by LC with fluorescence detection and postcolumn iodine derivatization. A Hitachi L-2130 pump equipped with a 200-L2000-000 organizer and a Hitachi L-2485 fluorescence detector with excitation/emission wavelengths of 360/440 nm were used. A 150 mm × 4.6 mm i.d., 5 μm, short Cloversil reverse phase column (Vicam Corp.) thermostated at 30 °C was employed; the mobile phase was methanol/water (50:50, v/v) at a flow rate of 1 mL/min for separation of the four aflatoxins. The injection volume into LC system for both standard and final sample extract was 20 μL. The detection limit for total aflatoxins by fluorometry was 1 μg/kg, and that for AFB_1, AFB_2, AFG_1, and AFG_2 by LC was 0.15 μg/kg, respectively.

Mean recoveries of AFB_1, AFB_2, AFG_1, and AFG_2 from triplicate peanut butter samples were 84, 96, 88, and 68% at 0.25 μg/kg with coefficients of variation (CV) of 4, 4, 5, and 9%, respectively, and 95, 98, 90, and 67% at 1.5 μg/kg with CVs of 4, 4, 4, and 10%, respectively. At 3 μg/kg, the recoveries of AFB_1, AFB_2, AFG_1, and AFG_2 were 98, 96, 93, and 67% with CVs of 5, 5, 5, and 10%, respectively.

Confirmation of Positive Samples. Positive results were confirmed by ultraperformance liquid chromatography tandem mass spectrometry (Waters, Milford, MA). Chromatographic separation of four aflatoxins was performed on a 100 mm × 2.1 mm i.d., 1.7 μm, UPLC BEH C18 column (Waters). A gradient program was used with the mobile phase combining solvent A (10 mmol/L ammonium acetate) used for the ESI^+ mode and solvent B (methanol) as follows: 20% B (initial), 20−85% B (5.5 min), 85−100% B (0.3 min), 100−20% B (1.2 min). A subsequent re-equilibration time (2 min) should be performed before the next injection. The flow rate was 0.3 mL/min, whereas the injection volume was 5 μL. Moreover, the column and sample temperatures were maintained at 35 and 25 °C, respectively. MS/MS was performed on a Micromass Quattro Ultima triple-quadrupole mass spectrometer equipped with an ESI source (Micromass, Manchester, U.K.). The parameters used for the mass spectrometer under the ESI^+ mode were as follows: capillary voltage, 3.5 kV; cone voltage, 45 V; source block temperature, 120 °C; RF lens one, 32; aperture, 0; RF lens two, 0.7; cone gas, 50 L/h; desolvation temperature, 350 °C; desolvation gas, 500 L/h; argon collision gas pressure, 3×10^{-3} mbar; LM lens one, 12.5 V; HM lens one, 12.5 V; ion energy one, 0.6 eV; entrance lens voltage, 10 V; collision gradient, 1.0; exit lens voltage, 12.5 V; LM lens two, 12.5 V; HM lens two, 12.5 V; and ion energy two, 1 eV.

RESULTS AND DISCUSSION

Natural Occurrence of Aflatoxins in Chinese Peanut Butter. The natural occurrence of aflatoxins in Chinese peanut butter samples is shown in **Table 1**. It is observed that aflatoxins are present in most of the samples analyzed with the frequency of 82% and levels ranging from 1 to 74 μg/kg by fluorometry (mean = 9.3 μg/kg, median = 3 μg/kg) and from 0.77 to 70.64 μg/kg by LC (mean = 8.51 μg/kg, median = 3.2 μg/kg). The correlation coefficient of 0.9963 (R^2 = 0.9926) indicates a perfect match in aflatoxin concentration analyzed by both methods. Therefore, to save time and reduce manpower, fluorometry can be employed as an initial screening method backed up by determination of the four individual aflatoxins by LC. This combination is cost-effective in the quantification of these compounds because of the large number of samples analyzed.

According to LC results, AFB_1 and AFB_2 are found in all 41 aflatoxin-positive samples, whereas AFG_1 and AFG_2 are present in 40 and 37 samples, respectively. AFB_1 is the predominant toxin and accounts for on the average of 60% (range = 27−97%) of the total aflatoxins followed by AFG_1 (28%), AFB_2 (12%), and AFG_2 (8%). European Union (EU) regulated dual limits for both AFB_1 (2 μg/kg) and the sum of AFB_1, AFB_2, AFG_1, and AFG_2 (total aflatoxins = 4 μg/kg) in all foods. China established a maximum limit (ML) of 20 μg/kg for AFB_1 alone in peanut and peanut-based products, but without any regulations for total aflatoxins. The EU is the largest importer of Chinese peanut products. Therefore, of the aflatoxin-positive samples, 16 (39%) with total aflatoxins exceed the EU regulation of 4 μg/kg, whereas 15 (37%) and 1 (2%) samples with AFB_1 are higher than EU and Chinese regulations, respectively. It is interesting to note that two samples with high levels of total aflatoxins (50.81 and 20.16 μg/kg) contained higher concentrations of AFG_1 (21.22 and 11.92 μg/kg) than AFB_1 (17.72 and 5.9 μg/kg). Confirmation of positive results was achieved by LC-MS/MS, either total ion chromatogram (TIC) of four aflatoxins in peanut butter or chromatograms of quantified daughter ions fragmentized from four positive ions (**Figures 1** and **2**).

Table 1. Natural Occurrence of Aflatoxins in Chinese Peanut Butter and Sesame Paste Analyzed by LC

					concn of aflatoxins (μg/kg)				
food	no. of samples	no. of positives	positive %	statistical parameter	AFB_1	AFB_2	AFG_1	AFG_2	total
peanut butter	50	41	82	range	0.39−68.51	0−5.52	0−21.22	0−6.36	0.77−70.64
				average	6.12	0.67	2	0.46	8.51
				median	1.58	0.29	0.41	0.08	3.2
sesame paste	100	37	37	range	0.39−20.45	0−4.92	0−26.28	0−5.75	0.54−56.89
				average	4.31	0.63	1.44	0.37	6.75
				median	1.46	0.18	0.14	0.04	2.15

Because heavy contamination of peanut-based products with aflatoxins is regarded as a risk factor for hepatacellular carcinoma (*7*), the natural occurrence of aflatoxins in peanut butter has been evaluated in some countries of the world. Yentür et al. (*11*) conducted aflatoxin analysis of 20 peanut butter samples from local markets in Ankara, Turkey, where mean levels of AFB_1, AFB_2, and AFG_1 were found to be 15.76, 1.23, and 9.69 μg/kg, respectively. A survey of aflatoxins in peanut butter in Tokyo from 1991 to 1996 showed that 16 of 197 samples were positive for AFB_1 and 2 samples contained AFB_1 above the Japanese action limit of 10 μg/kg (*12*). Afterward, Yoshiko (*10*) analyzed 21 peanut butter samples, and 10 had maxima of 2.59, 0.52, 0.81, and 0.46 μg/kg for AFB_1, AFB_2, AFG_1, and AFG_2, respectively. Offiah et al. (*13*) examined 32 peanut butter samples from Trinidad, and all were negative for aflatoxins. In a survey in the Republic of Cyprus, 21 of 74 (28%) peanut butter samples were contaminated with AFB_1 at a maximum of 73 μg/kg (*14*). Siame et al. (*15*) reported that the average aflatoxin concentration in Botswana peanut butter was 23 μg/kg. Therefore, peanut butter contamination with aflatoxins in the present study, in terms of levels and frequencies, is higher than that found in any other surveys reported previously, with the exception of one sample from the Republic of Cyprus. Different geographical and environmental conditions might be responsible for the differences in aflatoxin contamination and distribution mentioned above. As a result of aflatoxin limits varying from country to country, international trade is made more difficult. Hence, seeking to balance health benefits with the potential trade disruptions and economic losses that regulations can cause is an issue of concern worldwide.

Natural Occurrence of Aflatoxins in Chinese Sesame Paste. Aflatoxins were found in 37 samples (37%) at concentrations between 0.54 and 56.89 μg/kg (mean = 6.75 μg/kg, median = 2.15 μg/kg) by LC (**Table 1**). As shown in **Table 1**, AFB_1 is predominant at concentrations from 0.39 to 24.45 μg/kg (mean = 4.31 μg/kg, median = 1.46 μg/kg). It accounts for 36−100% of the total aflatoxins (mean = 77%, median = 82%) followed by AFG_1 (range = 0−46%, average = 11%, median = 8%), AFB_2 (range = 0−26%, mean = 10%, median = 10%), and AFG_2 (range = 0−12%, average = 3%, median = 2%). It should be noted, moreover, that 19% of the samples contained AFB_1 at levels higher than the Chinese regulation of 5 μg/kg for AFB_1. Of 37 aflatoxin-positive samples, 9 with total aflatoxin levels exceeded the European regulation of 4 μg/kg (4.32−56.89 μg/kg, 1.08−14.22 times higher). One third (32%) of the samples were found to be contaminated with AFB_1 at an average of 5.4 times (range = 1.03−10.23-fold) as high as the European regulation of 2 μg/kg. Besides AFB_1, AFB_2 at levels ranging from 0.02 to 4.92 μg/kg (mean = 0.65 μg/kg) was simultaneously detected in 36 samples. Twenty-nine samples were also positive for AFG_1 at levels between 0.05 and 26.28 μg/kg (average = 2.57 μg/kg). AFG_2 was found in 22 samples, although at low levels.

The distribution of aflatoxins in sesame paste from different sources is given in **Table 2**. It should be pointed out that both total aflatoxins and AFB_1 in 26 samples from restaurants were all below the EU regulations. Seven of 15 samples purchased from free markets were positive for both AFB_1(range = 0.86−14.56 μg/kg, average = 5.32 μg/kg) and total aflatoxins (range = 1.1−40.49 μg/kg, mean = 12.24 μg/kg). Among them, 2 samples were contaminated with AFB_1 at levels 2.31 and 2.91 times the Chinese tolerance limit of 5 μg/kg and 5.78 and 7.28 times the EU regulation of 2 μg/kg, respectively. It is worth noting that heavy contamination of aflatoxins was found in samples from supermarkets, varying from 0.39 to 20.45 μg/kg (average = 5.73 μg/kg) for AFB_1 and from 0.54 to 56.89 μg/kg (average = 9.44 μg/kg) for total aflatoxins. Of the positive samples, 5 with AFB_1 content of 2.51−4.09 times the Chinese regulation and 10 with AFB_1 concentration of 1.03−10.23-fold EU regulation, respectively, were observed. Additionally, 7 samples with total aflatoxins were in excess of the EU regulation of 4 μg/kg. It needs to be emphasized that two sesame samples with high AFB_1 concentration (20.3 and 14.56 μg/kg) were cocontaminated with a higher level of AFG_1 (26.28 and 16.52 μg/kg).

Distribution of Aflatoxins in Chinese Peanut Butter and Sesame Paste. The distribution of AFB_1 in peanut butter and sesame paste, as well as comparison of the impact of two different hypothetical standards (Chinese and EU regulatory limits) on the percentage of samples rejected, are evaluated. EU Commission Directive 2003/121/EC (*16*) states that for groundnuts, nuts, dried fruit, and cereals intended for direct human consumption the measurement uncertainty and correction for recovery should be taken into account, if one or more of the subsamples exceeds the maximum limit beyond reasonable doubt. On the basis of the relative expanded uncertainty of 39.9% for AFB_1 in nuts using LC with fluorescence detection provided by Maroto (*17*), the absolute expanded uncertainties are 0.8 and 2/8 μg/kg, respectively, for AFB_1 at 2 μg/kg (EU regulatory limit) and 5/20 μg/kg (sesame/peanut products, Chinese regulatory limit). These mean that no sample can be claimed as positive if the concentration of AFB_1 is below 2.80 or 7/28 μg/kg, respectively. According to the rules mentioned above, 14 samples will be rejected, even banned, if peanut butter that contains AFB_1 > 2.8 μg/kg is to be eliminated. However, the application of a 28 μg/kg AFB_1 level will result in only one peanut butter sample rejection. Compared to peanut butter,

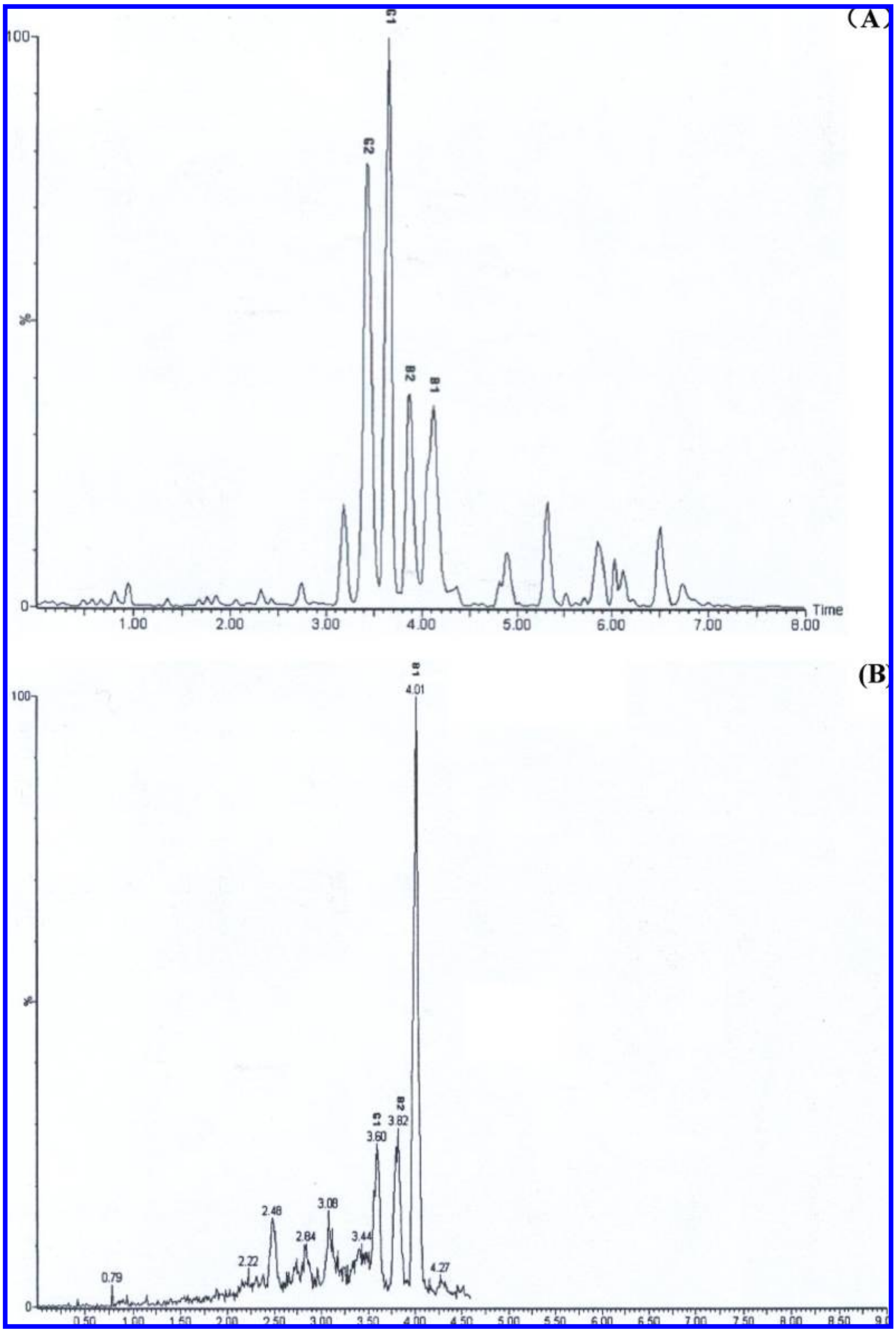

Figure 1. Total ion chromatogram of four aflatoxin standards (**A**) and aflatoxins in peanut butter sample (**B**).

10 and 7 sesame paste samples will face the possibility of rejection if sesame paste that contains $AFB_1 > 2.8$ or 7 μg/kg, respectively, is to be excluded.

This is the first report on the natural occurrence of aflatoxins in Chinese peanut butter and sesame paste. Heavy contamination of Chinese sesame paste with aflatoxins is an issue of concern because sesame paste is one of the most popular sauces in China and the annual consumption accounts for one-fourth of the world's total consumption. Generally, sesame grains are rarely invaded by aflatoxin-genic fungi because they are very small and resistant to mechanical damage. It should be pointed out that samples collected from supermarkets packed in the bottles were contaminated with higher levels of aflatoxins than those from restaurants and free markets. This may due to the fact that the factories stockpiled large quantities of sesame seeds for production and the seeds were easily invaded by aflatoxin-producing fungi under the poor storage practices. On the contrary, the restaurants and free markets make the sesame paste at the time of consumption. This may not only keep the

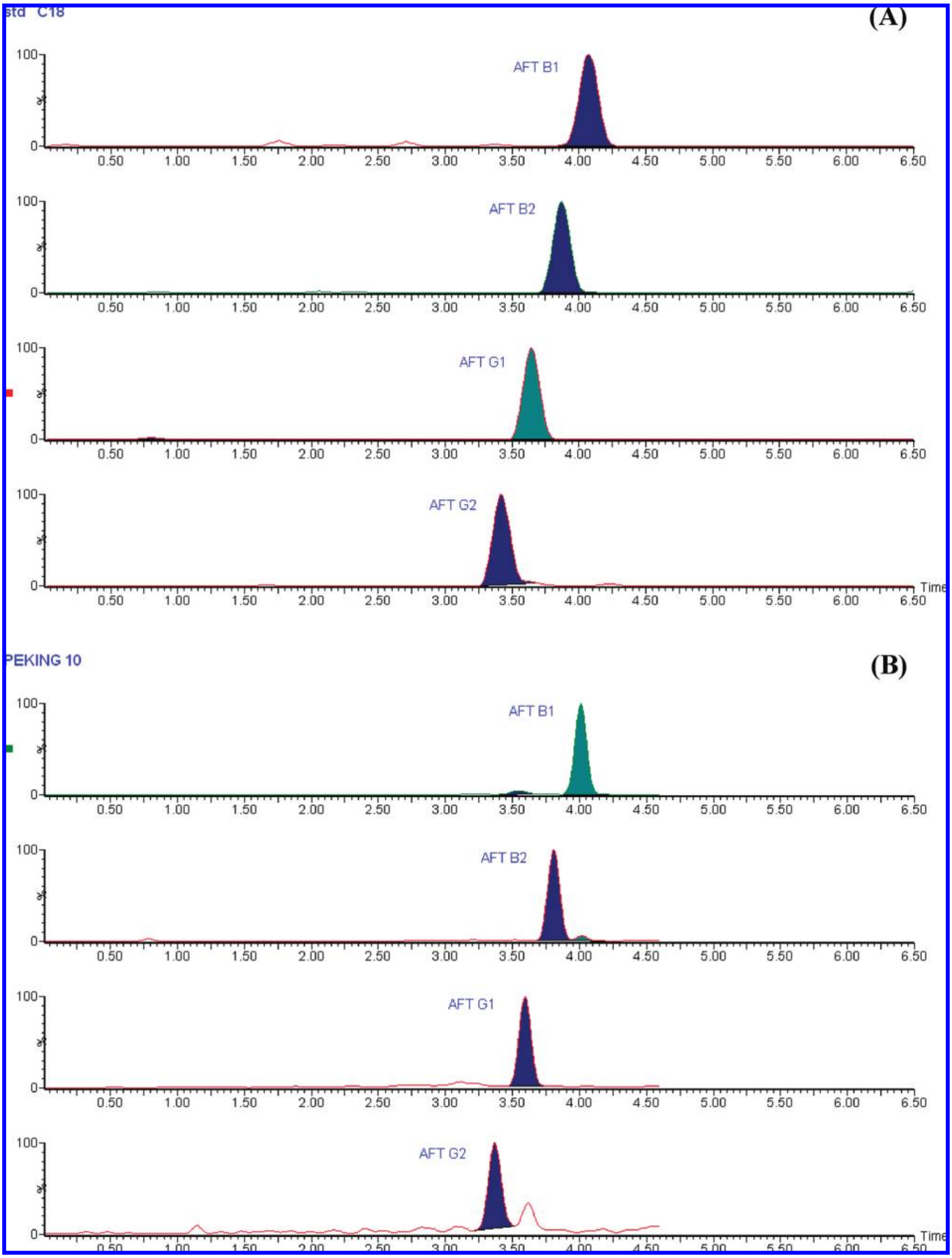

Figure 2. Ion chromatograms of $[M + H]^+$ plus IS^+ of four aflatoxin standards (**A**) and peanut butter sample (**B**).

paste fresh but also prompts the more rapid consumption of sesame seeds. Hence, contamination of aflatoxins in bulk sesame paste from both sources is much lower, in comparison with those from supermarkets. Improper agricultural practices and adverse storage and processing conditions are involved in the invasion of peanut and sesame kernels by aflatoxin-producing fungi. Therefore, peanut and sesame kernels must be subjected to analysis of aflatoxins at each

Table 2. Distribution of Aflatoxins in Sesame Paste from Different Sources

source	no. of samples	no. of positives	positive %	range (μg/kg) AFB$_1$	range (μg/kg) AFs	average (μg/kg) AFB$_1$	average (μg/kg) AFs	median (μg/kg) AFB$_1$	median (μg/kg) AFs	no. of samples with AFB$_1$ ≥ 5 μg/kg[a]	no. of samples with AFB$_1$ ≥ 2 μg/kg[b]	no. of samples with AFs ≥ 4 μg/kg[b]
restaurant (in bulk)	26	8	30.77	0.9−1.78	1−2.18	1.29	1.51	1.31	1.46	0	0	0
free market (in bulk)	15	7	46.67	0.86−14.56	1.1−40.49	5.32	12.24	1.46	2.3	2 (2.31−2.91)	2 (5.78−7.28)	2 (4.81−10.12)
supermarket (bottling)	59	22	37.29	0.39−20.45	0.54−56.89	5.73	9.44	1.93	2.56	5 (2.51−4.09)	10 (1.03−10.23)	7 (1.08−14.22)

[a] Chinese regulation. [b] European regulations. Range of multiples exceeding the tolerance limit are shown in parentheses.

step of production to keep the final products from contamination by aflatoxins.

SAFETY

Aflatoxins are known as potent carcinogenic compounds. Consequently, solvent extracts and associated preparation of standards should be handled with care. All experimental performance should be kept from light because aflatoxins are sensitive to light.

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