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Prevalence of *Aspergillus* spp. and occurrence of aflatoxins in peanut sauce processing by peanut sauce manufacturers

[1]Farawahida, A. H., [1,2]*Jinap, S., [1]Nor-Khaizura, M. A. R., [1,2]Samsudin, N. I. P., [3]Chin, C. K. and [1,4]Norlia, M.

[1]*Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Laboratory of Food Safety and Food Integrity (FOSFI), Institute of Tropical Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[3]*Food Safety and Quality Division, Ministry of Health Malaysia, Precinct 3, 62675 Putrajaya, Malaysia*
[4]*Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia*

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Abstract

The aims of the present work were to determine the prevalence of *Aspergillus* spp. and occurrence of aflatoxins (AFs) along the peanut sauce processing line from different peanut sauce companies in Malaysia, and to determine to which extent peanut sauce processing steps employed by the peanut sauce industries could efficiently reduce AFs in peanut sauce. Peanut and chili samples were collected at each processing step along the peanut sauce production from three peanut sauce companies which were different in companies' profile. Peanut samples from Companies B (87.5%) and C (100%) were contaminated with AFs. Of these, 12.5% (Company B) and 75% (Company C) samples exceeded the Malaysian regulatory limit. None of the samples from Company A was contaminated. The steps efficient in reducing AFs in peanut sauce identified in the present work were (i) safety monitoring of raw materials, (ii) sorting of raw materials, and (iii) heat treatment of raw materials.

Introduction

Aflatoxins (AFs) are toxic, mutagenic, and carcinogenic compounds (Zain, 2011) that might occur in various types of foods and feedstuffs (Afsah-Hejri *et al.,* 2013). The main producers of AFs are *Aspergillus flavus* and *A. parasiticus* (Zain, 2011). Twenty AFs analogues have been identified thus far, but only AFB_1, AFB_2, AFG_1, and AFG_2 are the primary contaminants of foods and feeds, with AFB_1 being the most toxic (IARC, 1993).

In South East Asia, peanuts are used as the main ingredient in popular foods such as peanut sauce. Peanut sauce is usually consumed with *sate* (traditional skewered grilled meat; Jinap *et al.,* 2013), *nasi impit* (compact rice), and *yong tau foo* (tofu dish). Peanut sauce main ingredients, which are peanuts and dried chili, have been shown to be contaminated with AFs (Kiran *et al.,* 2005; Arzandeh and Jinap, 2011). According to the Malaysian Regulation (Food Act, 1983) and European Commission (EC, 2010), the maximum permitted level of total AFs in peanuts for further processing is 15 ng/g, while only 5 ng/g of AFB_1 and 10 ng/g of total AFs are allowed in chili (EC, 2010).

In Malaysia, the implementation of Hazard Analysis and Critical Control Points (HACCP) principle by the food industries is optional (Standards Malaysia, 2007). The Critical Control Points (CCPs) put in place by established peanut sauce companies associated with microbial hazards (bacteria, not *Aspergillus* spp.) are filling / sealing and retort processing. None of the peanut sauce companies in Malaysia has thus far established the CCPs to prevent *Aspergillus* spp. and AFs contamination.

The aims of the present work were therefore to determine the prevalence of *Aspergillus* spp. and occurrence of AFs along the peanut sauce processing line from different peanut sauce companies in Malaysia; and to determine to which extent peanut sauce processing steps used by peanut sauce industries in Malaysia could efficiently reduce AFs in peanut sauce.

Materials and methods

Sampling

Samples (1 kg each) comprising of peanut,

*Corresponding author.
Email: sjinap@gmail.com ; jinap@upm.edu.my

peanut sauce, dried chili, and chili powder were collected along the processing line from three different peanut sauce manufacturers (Companies A, B, and C) from July 2016 to June 2017 in Selangor, Malaysia. The samples taken followed the ongoing process from different batches randomly, and included at least three different locations of the container. The samples were packed in air-tight polyethylene bags, labelled and stored at -18°C prior to analysis.

Companies' profile

The selected companies were asked to fill up a questionnaire regarding their background and processing activities. To disguise their identities, the companies were coded A, B, and C (Table 1).

Materials

Mixed AFs standards containing AFB_1 and AFG_1 at concentration of 1,000 ng/mL, and AFB_2 and AFG_2 at concentration of 300 ng/mL were purchased from Supelco (Bellefonte, PA, USA). All solvents used in the present work were of HPLC-grade and supplied by Merck (Darmstadt, Germany). AflaTest WB SR (Super Recovery) Immunoaffinity Column (IAC) were purchased from VICAM (Watertown, MA, USA).

Moisture content determination

The moisture content (%) of samples was determined by the oven-drying method (AOAC, 1990). Briefly, empty crucibles and lids were labelled and dried in the oven (Memmert 854, Schwabach, Germany) for 30 min and transferred to desiccator to cool before being weighed. Next, 5 g of samples were weighed into the crucibles and spread with spatula. The crucibles with samples and lids were placed in the oven and heated to 105°C. After drying, the crucibles with partially covered lids were transferred to desiccator to cool. The crucibles and their dried samples were weighed daily until constant weight was achieved. Moisture content was determined by using Eq. 1:

$$[(\text{Fresh weight} - \text{Dry weight}) / (\text{Fresh weight})] \times 100 \quad \text{(Eq. 1)}$$

Aflatoxin extraction and immunoaffinity clean-up

AFs were extracted and determined following the AOAC Method 991.31 (AOAC, 2000) with slight modification. Samples in liquid or semi-liquid form were first dried by using freeze dryer (Labconco, Kansas City, Missouri) prior to aflatoxin extraction. Dried samples were then ground using a Waring blender (Waring, Torrington, CT, USA) for 2 min. Next, 25 g of sub-samples and 5 g of sodium chloride (NaCl; Merck) were added to 125 mL of methanol/water (70:30, v/v), homogenized for 2 min, and filtered with fluted filter paper (24 cm Ø; VICAM, Germany). Then, 15 mL of filtrate were diluted with 30 mL of purified water followed by second filtration with a microfiber filter (11 cm Ø; VICAM, USA). Next, 15 mL of filtrate was passed through the AflaTest WB SR (Super Recovery) IAC (VICAM, USA) at a rate of 1 - 2 drop/sec. The column was twice washed with 10 mL of purified water at similar rate. Finally, trapped AFs were eluted with 1 mL of methanol, followed by dilution with 1 mL of purified water.

Determination of aflatoxins by HPLC

The method from Arzandeh *et al.* (2010) was followed in the determination of AFs by High Pressure Liquid Chromatography (Waters 600 Controller; NY, USA) joined with a Multi λ Fluorescence Detector (HPLC-FLD) (Waters 2475; NY, USA) with a post-column Photochemical Reactor for Enhanced Detection (PHRED; Aura Industries, NY, USA) with a slight modification. A reverse-phase symmetry XBridge C_{18} column (25 cm length × 4.6 mm width and 5 µm particle sizes) running on a Waters 2475 HPLC system was used at an excitation wavelength of 365 nm and emission wavelength of 435 nm. Methanol (100%), acetonitrile (100%) (Merck, Germany), and purified water (Elga Purelab Classic UV MK2, UK) were separately filtered through nylon membrane filter (0.45 µm Ø; Merck, Germany), degassed by Microprocess Controlled Bench-top Ultrasonic Cleaner (Powersonic 420, Hwashin Technology, Seoul, Korea) and used as mobile phase for the HPLC-FLD in the ratio of water/methanol/acetonitrile (55:35:10, v/v/v) with a flow rate of 0.6 mL/min. The injection volume was 20 µL. The determination was done in triplicates. Empower 2 Chromatography Data Software (Waters, Milford, MA, USA) was used for data processing.

Linearity, limit of detection (LOD), limit of quantification (LOQ), linear equation, and coefficient of regression (R^2) of the analytical method were also determined. Mixed AFs standards at seven concentrations of 2, 4, 6, 10, 25, 50, 100 ng/mL for AFG_1 and AFB_1, and 0.6, 1.2, 1.8, 3.0, 7.5, 15.0, and 30.0 ng/mL for AFG_2 and AFB_2 were injected to estimate the linearity. The injection was done in triplicates. To determine the recovery, AFs were spiked in peanut, peanut sauce, and chili samples at concentrations of 0.50, 5.00, and 30.00 ng/mL for AFB_1 and AFG_1, and 0.15, 1.50, and 9.00 ng/mL for

Table 1. Profiles of Companies A, B, and C.

Description	Company A	Company B	Company C
Status of company	Medium Enterprise*	Small Enterprise**	Small Enterprise**
Machine / manual processing	Machine	Manual	Manual
Raw materials / processing steps taken	a. Chili paste (L) b. Peanut crush (rough) (D) c. Peanut crush (fine) (D) d. Cooking and stirring (L) e. Filling and sealing (S) f. Sterilization (S) g. Delivery (S)	a. Dried chili (D) b. Cooked chili paste (D) c. Storage 1 (peanut, after receiving) (D) d. Storage 2 (peanut, after sorting) (D) e. Storage 3 (peanut, after oil-less frying) (D) f. Storage 4 (peanut, after grinding) (D) g. During cooking (peanut sauce) (S) h. After cooking (peanut sauce) (S)	a. Chili powder (D) b. Storage 1 (peanut, after receiving) (D) c. Storage 2 (peanut, after sorting) (D) d. Frying (peanut) (D) e. Grinding (peanut and chili powder) (D) f. Mixing (peanut, chili powder , and other ingredie nts) (D) g. Holding (peanut sauce) (D) h. Packaging (peanut sauce) (D)
End products	Ready -to-eat peanut sauce (RTE)	RTE peanut sauce	Pre-mix peanut sauce
Form o f end products	Semi -liquid	Semi -liquid	Dry
Destination of end products	Local + export	Local	Local
Quality certification	GMP, MS ISO 9001, ISO 22000, HACCP (MS 1480), BRC Global Standard	None	1Malaysia Best

*Medium Enterprise generates annual sales of MYR 15 - 50 mil, or 75 - 200 employees. **Small Enterprise generates annual sales of MYR 0.3 to < 15 mil, or 5 - 74 employees. L = liquid; S = semi-liquid; D = dry.

AFB_2 and AFG_2. To estimate the LOD and LOQ, three times standard deviation (SD) and ten times SD were used, respectively. For quantification of AFs in the samples, a calibration curve with seven points was constructed for AFG_2, AFG_1, AFB_2, and AFB_1, respectively.

Enumeration of Aspergillus flavus and A. parasiticus

Enumeration of *A. flavus* and *A. parasiticus* was performed following the method of Pitt *et al.* (1983) with slight modification. Samples were collected from each step of peanut sauce processing and used for *Aspergillus* spp. enumeration. Ten grams of samples were added to 90 mL of 0.1% peptone water and homogenized by BagMixer 400 (Interscience, France) for 2 min. Next, 100 µL of homogenate was inoculated onto *Aspergillus flavus* and *parasiticus* agar (AFPA). Inoculated plates were incubated at 30°C for 48 h. *Aspergillus flavus* and *A. parasiticus* produced orange-yellow reverse colony pigmentation. *A. ochraceus* also exhibits orange-yellow reverse colony but it requires longer incubation period. Light yellow reverse colony indicates the presence of A. niger. However, after prolonged incubation, *A. niger* will produce black conidial heads and is easily differentiated from *A. flavus* (Pitt *et al.,*

1983). The results were presented as log Colony-Forming Unit per gram (log CFU/g).

Statistical analysis

All experiments were performed in triplicates, and readings were reported as mean ± SD. The individual significance probability for each independent variable was shown by *p*-value. $p < 0.05$ was accepted as significant difference. All data were subjected to univariate one way analysis of variance (ANOVA) using Minitab® (version 16.0, Pennsylvania, USA).

Results and discussion

Method validation

The standard curve constructed for all AFs analogues (AFG_2, AFG_1, AFB_2, AFB_1) yielded very good correlation coefficient ($R^2 > 0.999$) as shown in Table 2. This indicated that the quantification procedures were accurate and reliable. Low LOD and LOQ also signified the sensitivity and accuracy of the quantification instruments (Shrivastava and Gupta, 2011).

Table 2. LOD, LOQ, and R^2 for aflatoxin quantification.

Aflatoxins	LOD (ng/mL)	LOQ (ng/mL)	R^2
AFG_2	0.06	0.20	0.9994
AFG_1	0.09	0.30	0.9996
AFB_2	0.01	0.05	0.9994
AFB_1	0.04	0.14	0.9994

Table 3 lists the recovery of AFs on spiked samples using the immunoaffinity columns (IAC). The recovery rates obtained were within the regulation limits for AFs determination (Codex Alimentarius, 1995; EC, 2006) except for AFG_2 in chili. The levels of AFG_2 in the spiked samples were very low with no detection in most of the tested samples. Low detection of AFG_2 might be due to its low affinity with the antibodies in the IAC, depending on the sample matrices and diluents used for samples extraction (Ali *et al.,* 2005; Zhao *et al.,* 2016). Considering that AFG_2 is not as carcinogenic as AFB_1, and that the mean recovery for AFB_1 were within the specified limits, the IAC procedures were accepted for subsequent quantification of AFs.

Companies' profiles

The profiles of companies A, B, and C are listed in Table 1. For Malaysia, the definition of Small and Medium Enterprises (SMEs) is based on employment and sales (Ndubisi and Nwankwo, 2013).

Company A

Company A produced 120 kg peanut sauce daily. The process of making peanut sauce started with receiving rough and fine roasted crushed peanut, dried chilies, shrimp paste, tamarind, water, onion, lemon grass, granulated sugar, salt, and palm olein. Next, the raw materials except crushed peanuts were mixed in a tank and cooked at 90 - 95°C for 2 h into gravy. Then, rough and fine crushed peanut were added to the gravy, passed through the piping line, and placed in a holding tank at 65°C for 4 h. The product was filled into an aluminium pouch and

Table 3. Recoveries of aflatoxins in spiked samples.

Samples	Aflatoxins	Concentration of spiked aflatoxins (ng/mL)	Mean recovery ± SD (%)
Peanut	AFG_2	0.15 - 9.00	71.63 ± 2.00
	AFG_1	0.50 - 30.00	94.79 ± 3.7
	AFB_2	0.15 - 9.00	96.36 ± 8.2
	AFB_1	0.50 - 30.00	83.56 ± 3.2
Peanut sauce	AFG_2	0.15 - 9.00	73.70 ± 1. 2
	AFG_1	0.50 - 30.00	82.76 ± 5.5
	AFB_2	0.15 - 9.00	102.21 ± 11.7
	AFB_1	0.50 - 30.00	97.83 ± 8.3
Chili	AFG_2	0.15 - 9.00	44.71 ± 1.0
	AFG_1	0.50 - 30.00	102.12 ± 2.6
	AFB_2	0.15 - 9.00	86.15 ± 2.5
	AFB_1	0.50 - 30.00	85.84 ± 8.4

sterilized at 121°C for 26 min before being cooled (90°C) and dried (70°C, 15 min). The product was then passed through the X-ray machine to detect metal contaminant, and stored in the warehouse at 4°C before being delivered to customers.

Company B

Company B produced 100 kg peanut sauce daily. The process of making peanut sauce started with receiving peanut kernels, dried chilies, palm olein, water, granulated sugar, and salt. Raw peanuts were manually sorted to remove defect peanuts. The sorted peanut kernels were then oil-less fried and ground. Dried chilies were blanched at 90°C for 3 min, sieved and then ground into chili paste. Then, the chili paste was cooked with palm oil at 60°C for 10 min. All the raw ingredients including cooked chili paste were mixed in a big pot and cooked manually for 3 h at 99°C. After cooking, the peanut sauce was left to cool down for 5 min before packing and delivery.

Company C

Company C produced 20 kg peanut sauce daily. The process of making peanut sauce started with receiving palm olein, onion, garlic, shrimp paste, tamarind, peanut kernels, chili powder, salt, monosodium glutamate (MSG), and sugar. Then, the peanut kernels were sorted and stored. Several days after, the sorted peanuts were fried at 120°C for 20 min. After the removal of oil by machine, the fried peanuts were left to cool down for 10 min and mixed with chili powder and ground together. Onion and garlic were sliced, fried, blended, and separately stored in containers. Other ingredients were added to the mixture of ground peanut and chili powder, and mixed by machine. After holding for approximately 4 h, peanut sauce was packed manually by sealer in aluminium pouch (primary packaging) and delivered to regular customers or sold at retail markets.

Moisture contents, aflatoxin levels, and A. flavus and A. parasiticus loads in samples from the companies

Company A

No contamination of AFs, *A. flavus*, and *A. parasiticus* was detected in any of the sample (Table 4). This might be due to the fact that the company has given a specification to the supplier of less than 4 ng/g AFs, and during receiving the concentration was verified again by using RIDA AFs test kit (R-Biopharm AG, Darmstadt, Germany). This company also controlled the temperature during storage and processing (4°C). Since Company A practiced proper storage, it had one time higher in the implementation of hygiene practices as compared to Companies B and C (Mohd Azaman *et al.,* 2016). Other than that, the peanut sauce processing was found to be a continuous process utilizing fully automated machine

Table 4. Moisture content, prevalence of Aspergillus spp. (log CFU/g), and occurrence of aflatoxins (ng/g) in samples from Company A.

Company A	Raw materials / processing steps	Moisture content	Log CFU/g	AFG_2 (ng/g)	AFG_1 (ng/g)	AFB_2 (ng/g)	AFB_1 (ng/g)	Total AFs (ng/g)
Raw m aterials*	Chili paste (L)	82.38 ± 0.40^a	< 2.00 ± 0.00^a	ND^a	ND^a	ND^a	ND^a	ND^a
	Peanut crush (rough) (D)	4.74 ± 0.64^b	< 2.00 ± 0.00^a	ND^a	ND^a	ND^a	ND^a	ND^a
	Peanut crush (fine) (D)	4.19 ± 0.71^b	< 2.00 ± 0.00^a	ND^a	ND^a	ND^a	ND^a	ND^a
Processing steps*	Cooking and stirring (L)	83.19 ± 0.93^a	< 2.00 ± 0.00^a	ND^a	ND^a	ND^a	ND^a	ND^a
	Filling and sealing (S)	61.86 ± 0.87^b	< 2.00 ± 0.00^a	ND^a	ND^a	ND^a	ND^a	ND^a
	Sterilization (S)	62.59 ± 0.75^b	< 2.00 ± 0.00^a	ND^a	ND^a	ND^a	ND^a	ND^a
	Delivery (S)	62.59 ± 0.75^b	< 2.00 ± 0.00^a	ND^a	ND^a	ND^a	ND^a	ND^a

ND: not detected. Different letters within the same column indicate significant difference ($p < 0.05$). *Separate statistical analyses were conducted on raw material and processing steps. L = liquid; S = semi-liquid; D = dry.

which further reduced the risk of cross-contamination usually brought about by manual labour. The owner and production manager of the company possess the knowledge about AFs and food safety. Since Company A had quality certifications such as Good Manufacturing Practices (GMP) and HACCP, it had four times likelihood towards minimizing AFs contamination as compared to Companies B and C (Mohd Azaman *et al.,* 2016).

Company B

12.5% samples were contaminated with *A. flavus* and *A. parasiticus*, and exceeded the regulatory limit (Table 5). During Storage 1, peanuts received had exceeded log CFU/g of *Aspergillus* spp. and total AFs. However, during Storage 2, sorting of peanuts reduced *Aspergillus* spp. and AFs below the regulatory limits. 87.5% of the samples were contaminated with total AFs (0.58 - 32.91 ng/g), with 12.5% of them exceeding the regulatory limit. The total AFs in dried chili far exceeded the regulatory limit of 5 ng/g (Food Act, 1983) due to the favourable condition for mould growth when the moisture content was above 11% (Toontom *et al.,* 2012). However, after cooked into chili paste, the total AFs was reduced by 88% to an acceptable level. This might be due to the heat treatment applied during cooking and lower moisture content of cooked chili paste. According to Farawahida *et al.* (2017), oil-less frying of chili powder could reduce 33 - 41% of AFs, with higher reduction could be achieved with moist samples (Rustom, 1997). Reduction of *Aspergillus* spp. and AFs (95%) were also observed in peanuts after manual sorting as compared to the received raw materials. Since very low concentrations of AFs (0.58 ± 0.20 ng/g) were detected after sorting, oil-less frying of peanuts further reduced AFs to a non-detectable level. Arzandeh and Jinap (2011) reported that roasting peanut kernels at 130 - 150°C for 120 min reduced 57 - 70% of AFB_2 while 78 - 80% reduction of AFB_1 was achieved when peanuts were roasted at 150°C for 120 min. Studies from Yazdanpanah *et al.* (2005) showed that roasting peanut kernels at similar duration and temperature reduced > 95% of AFB_1. However, storage of roasted peanut after grinding saw a significant increase in AFs. This might be due to improper storage practices which subsequently led to cross-contamination and increase in AFs production (Udomkun *et al.,* 2018).

The five-fold increase in AFs noted during cooking of peanut sauce mixture (as compared to during storage 4) might be explained by the addition of AFs-contaminated chili paste. Besides, unsanitised big pot used (from previous cooking) can be another factor contributing to increasing AFs contamination. After cooking process significantly reducing 41% of AFs, the peanut sauce was fully cooked at temperature 99°C for 3 h.

Company C

37.5% of the samples were contaminated with *A. flavus* and *A. parasiticus* which exceeded the regulatory limit (log 3.44 - 5.05 CFU/g; Table 6). Similar pattern of *Aspergillus* spp. prevalence has been observed. *Aspergillus* spp. was detected in chili powder, thus their total AFs increased. Besides, *Aspergillus* spp. was found in Storage 1 (after peanut receiving) and Storage 2 (after peanut sorting). Meanwhile, all the samples were contaminated with total AFs (1.71 - 537.09 ng/g), with 75% of them exceeding the regulatory limit. This company did not have a Standard Operating Procedure (SOP) for incoming raw materials, no record-keeping, and did not hire Production Supervisor or Quality Control Manager to oversee the processing line. The owner and operators of the company also did not possess any knowledge about AFs. Therefore, they were three times less likely to implement high level of hygienic practices (Mohd Azaman *et al.,* 2016).

Similar to Company B, the chili powder from Company C too had high level of AFs. It could be deduced that either the dried chili used to prepare the chili powder was contaminated with the fungi, or the fungi colonized the chili powder during storage prior to arrival at the company. The levels of AFs during storage of peanut kernels (Storage 1) were also very high (537.09 ± 2.39 ng/g), which was 36 times higher than the permitted level. This could be due to several factors. The peanut sauce was manually processed in batches and in the traditional way. The temperature in the storage and production areas (32.8°C) provided optimal growth condition for *Aspergillus* spp. (Hocking, 1997). For warehouses dealing with food commodities, they are recommended to have a refrigeration temperature (0 - 10°C) to inhibit any microbial contamination (Codex Alimentarius, 2004). During manual sorting, it was found that approximately 1.5% of peanuts were rejected (based on the physical appearance), with reduction of AFs by 21%. This low rate of rejection could have been translated to the high carryover of AFs after sorting (426.92 ± 7.93 ng/g). Based on preliminary study, about 7.4% of peanuts could be removed by manual sorting. Other studies stated that 29 - 38% of total AFs could be reduced by electronic colour sorting (Whitaker *et al.,* 2005), while manual sorting could reduce 40 - 80% of total AFs (Park, 2002).

Table 5. Moisture content, prevalence of Aspergillus spp. (log CFU/g), and occurrence of aflatoxins (ng/g) in samples from Company B.

Company B	Raw materials / processing steps	Moisture content	Log CFU/g	AFG_2 (ng/g)	AFG_1 (ng/g)	AFB_2 (ng/g)	AFB_1 (ng/g)	Total AFs (ng/g)
Raw materials*	Dried chili (D)	17.67 ± 0.58^{a}	$< 2.00 \pm 0.00^{a}$	ND^{a}	ND^{a}	2.49 ± 0.34^{a}	30.42 ± 0.50^{a}	32.91 ± 0.53^{a}
	Cooked chili paste (D)	7.27 ± 0.42^{b}	$< 2.00 \pm 0.00^{a}$	ND^{a}	ND^{a}	0.25 ± 0.06^{b}	3.67 ± 0.11^{b}	3.92 ± 0.16^{b}
Processing steps*	Storage 1 (peanut, after receiving) (D)	10.10 ± 0.87^{a}	3.04 ± 0.06^{a}	ND^{a}	ND^{a}	2.78 ± 0.04^{a}	10.00 ± 0.11^{a}	12.78 ± 0.15^{a}
	Storage 2 (peanut, after sorting) (D)	11.65 ± 0.49^{b}	$< 2.00 \pm 0.00^{b}$	ND^{a}	ND^{a}	0.09 ± 0.04^{b}	0.49 ± 0.16^{b}	0.58 ± 0.20^{b}
	Storage 3 (peanut, after oil-less frying) (D)	8.70 ± 0.13^{c}	$< 2.00 \pm 0.00^{b}$	ND^{a}	ND^{a}	ND^{b}	ND^{c}	ND^{c}
	Storage 4 (peanut, after grinding) (D)	9.11 ± 0.57^{c}	$< 2.00 \pm 0.00^{b}$	ND^{a}	ND^{a}	0.30 ± 0.15^{c}	1.43 ± 0.69^{d}	1.72 ± 0.84^{d}
	During cooking (peanut sauce) (S)	67.85 ± 0.07^{d}	$< 2.00 \pm 0.00^{b}$	ND^{a}	ND^{a}	1.45 ± 0.16^{d}	7.10 ± 0.53^{e}	8.55 ± 0.69^{e}
	After cooking (peanut sauce) (S)	68.60 ± 0.35^{d}	$< 2.00 \pm 0.00^{b}$	ND^{a}	ND^{a}	0.64 ± 0.05^{e}	4.44 ± 0.07^{f}	5.08 ± 0.10^{f}

ND: not detected. Different letters within the same column indicate significant difference ($p < 0.05$).
*Separate statistical analyses were conducted on raw material and processing steps. S = semi-liquid; D = dry.

Table 6. Moisture content, prevalence of Aspergillus spp. (log CFU/g), and occurrence of aflatoxins (ng/g) in samples from Company C.

Company C	Raw materials / processing steps	Moisture content	Log CFU/g	AFG_2 (ng/g)	AFG_1 (ng/g)	AFB_2 (ng/g)	AFB_1 (ng/g)	Total AFs (ng/g)
Raw materials*	Chili powder (D)	11.57 ± 0.15	3.54 ± 0.00	ND	ND	1.18 ± 0.14	21.77 ± 0.51	22.95 ± 0.39
Processing steps*	Storage 1 (peanut, after receiving) (D)	11.55 ± 0.43^{a}	3.44 ± 0.08^{a}	1.49 ± 0.05^{a}	ND^{a}	87.24 ± 0.53^{a}	447.84 ± 2.54^{a}	537.09 ± 2.39^{a}
	Storage 2 (peanut, after sorting) (D)	11.69 ± 0.81^{a}	5.05 ± 0.00^{b}	1.34 ± 0.03^{a}	2.66 ± 0.05^{b}	69.86 ± 0.97^{b}	353.05 ± 6.88^{b}	426.92 ± 7.93^{b}
	Frying (peanut) (D)	5.87 ± 0.88^{b}	< 2.00 ± 0.00^{c}	ND^{b}	ND^{a}	0.41 ± 0.03^{c}	1.30 ± 0.41^{c}	1.71 ± 0.38^{c}
	Grinding (peanut and chili powder) (D)	4.72 ± 0.72^{c}	< 2.00 ± 0.00^{c}	ND^{b}	ND^{a}	2.40 ± 0.87^{d}	9.70 ± 4.03^{cd}	12.10 ± 4.90^{d}
	Mixing (peanut, chili powder, other ingredients) (D)	7.84 ± 0.89^{d}	< 2.00 ± 0.00^{c}	ND^{b}	ND^{a}	4.04 ± 2.30^{e}	16.07 ± 9.48^{cd}	20.11 ± 11.77^{e}
	Holding (peanut sauce) (D)	6.82 ± 0.24^{e}	< 2.00 ± 0.00^{c}	ND^{b}	ND^{a}	8.27 ± 0.13^{f}	25.57 ± 0.33^{de}	33.84 ± 0.24^{f}
	Packaging (peanut sauce) (D)	6.67 ± 0.64^{be}	< 2.00 ± 0.00^{c}	0.21 ± 0.23^{c}	ND^{a}	14.42 ± 0.33^{g}	44.91 ± 4.88^{e}	59.53 ± 4.99^{g}

ND: not detected. Different letters within the same column indicate significant difference ($p < 0.05$).
*Separate statistical analyses were conducted on raw material and processing steps. D = dry.

However, these high levels of AFs were greatly reduced more than 99% to permissible limits by frying the peanuts. Peanut frying and roasting have been shown to be very effective in reducing AFs (Razzazi-Fazeli *et al.,* 2004; Mutegi *et al.,* 2013). Grinding peanuts with chili powder increased the level of AFs due to the addition of the AF-contaminated chili powder, and this increasing trend was continuously observed in the subsequent steps (*i.e*., mixing of ingredients, holding, and packaging). Several factors might have contributed to this such as the use of contaminated container during grinding; the use of stand fan during holding; and the increase of humidity during packaging (Abou-Arab *et al.,* 1999).

Conclusion

In the present work, the prevalence of *Aspergillus* spp. has been detected during raw materials (peanuts and chili) receiving and sorting. Manual sorting and heat treatment of peanut kernels (oil frying or oil-less frying) and cooking of chili paste or peanut sauce have been shown to significantly reduce the AFs levels. Moreover, it is also important to ensure that the peanuts and chili powder used in peanut sauce manufacturing meet the AFs guidelines before entering the manufacturing process.

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