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Determination of Common Spices and Herbs Contamination with Aflatoxin in Al Majmaah Province

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Abstract: Spices and herbs are widely consumed, due to their cooking benefits, unique flavors, colors, aromas and medicinal importance. Unfortunately, they are liable to be heavily contaminated with toxigenic fungi with subsequent production of aflatoxins (AFs). Acute and chronic exposure to aflatoxin is considered a serious health risk problem. This study aimed to determine the level of AFs in some unpacked spices and herbs sold in local markets of Al Majmmah province. 45 unpacked spices and herbs samples including ginger, turmeric, cinnamon, cardamom, cumin, black pepper, chili pepper, cloves, laurel leaves and aniseed were collected randomly from local markets of Al Majmaah province, KSA from March to June 2017. The presence of AFs was determined by HPLC. AFs mainly, AFB1were detected in 13 samples. All results were below the limit of 10 ug/kg for total AFs. AFB1 levels were below 5ug/kg except for three turmeric samples.The levels of total AFs in all contaminated samples are in the permissible European limits. For AFB1, only three turmeric samples were beyond the European limits. Regular monitoring of unpacked spices is required.

Keywords: Mycotoxins, Aflatoxin, Spices, Herbs, fungi.

INTRODUCTION

Mycotoxins are secondary metabolites produced by fungi. Their worldwide occurrence in natural food and animal feeds poses a major risk for human and animal health and, consequently, causes economic losses [1]. There are more than 400 known mycotoxins, of which AF produced mainly by *Aspergillus flavus*, *Aspergillus nomicus* and *Aspergillus parasiticus*, is the most potent toxin found in nature [2].

Aflatoxins were first identified in England following a poisoning outbreak causing 100,000 cases of death [3]. Acute exposure to excessive amounts of AFs is known to cause nonspecific liver damage, malaise and death in few days [4].

Chronic exposure is detrimental to human and animal health as AFs have toxic, mutagenic, carcinogenic, immunosuppressive and teratogenic effects [5].

The most important AFs are AFB1, AFB2, AFG1 and AFG2. However, AFB1 shows the most toxicity. AFB1 is a potent human liver carcinogen, contributed to 25200-15500 cases of hepatocellular carcinoma each year. The majority of these cases occurred in Asia and Sub-Saharan Africa [6]. AFB1 has been classified as group I human carcinogen by the International Agency for Research on Cancer (IARC) [7].Aflatoxins are usually found in various foods and feeds in various concentrations. The most pronounced contamination has been encountered in the tree nuts, peanuts, corn, rice, cottonseeds and spices [8].

Spices and herbs have gained considerable attention as they are widely used for flavoring, coloring and preservation of food or beverage for thousands of years [9]. Most of the spices and herbs traditionally traded through the world are products of tropical environments that is characterized by high temperature, humidity and heavy rainfall [10]. Because of these environmental conditions and the steps involved in their processing, transport and storage, spices have the potential to be heavily contaminated with toxigenic fungi with subsequent production of AFs [11].

Unfortunately, these toxins are not completely eliminated during food processing operations and can contaminate finished processed food products [12-13]. Therefore, several countries have imposed strict guidelines for the limitation of AFs contamination and its ingredients. For instance, the European Commission Regulation (ECR) sets the maximum limits (MLs) for AFB1 and total AFs at 5 and 10 µg/ kg, respectively [14]. On the other hand, USA (FDA and FAO) set the ML for total AFs at 20 µg/ kg [15].

A number of survey and monitoring programs have been carried out in several countries including Turkey, India, Ghana and Poland attempting to obtain general pattern of extent of contamination of various types of food with AFs [16-20].

In Saudi Arabia, the presence of AFB1 (12–40 µg/kg) in *Pimpenella anisum, Piper nigrum, Mentha piperita* was reported in Jeddah region [21]. Another study in Asser region reported heavy contamination of ginger, fennel, fenugreek, red pepper and aniseed with mycotoxin producing fungi [22].

Therefore, this study was conducted to determine the level of AFB1, AFB2, AFG1 and AFG2 in some commonly used spices and herbs (un-packed) sold in local markets of Al Majmmah province and to make people aware of the epidemiological effects of contaminated spices.

MATERIAL AND METHODS

Sample collection: A total of 45 unpacked spices and herbs samples (n=45) were collected randomly from local markets of Al Majmaah province, KSA from March to June 2017. The samples correspond to 45 unpacked spices and herbs including ginger, turmeric, cinnamon, cardamom, cumin, black pepper, chili pepper, cloves, laurel leaves and aniseed. Each sample was kept in plastic bag at -4°C till further analysis.

Chemicals and Reagents: HPLC grade acetonitrile, methanol, ethyl acetate, hexane, chloroform and benzene were purchased from Merck (Germany). De-ionized water was used for the preparation of solutions. Analytical standards of (AFB1, AFB2, AFG1 and AFG2) were purchased from Acros Organics.

Standard preparation: Stock solution of AFs was prepared by dissolving 1 mg of AF standards in 1 ml benzene/acetonitrile. Individual standards of 10 µg/ml of AFB1, AFB2, AFG1 and AFG2 were prepared by diluting appropriate volume of the stock solution in benzene-acetonitrile. Their exact concentration was determined by spectrophotometry according to AOAC [23]. Mixture solution of AFB1, AFB2, AFG1 and AFG2 of 1 µg/ml was prepared by diluting appropriate volume of individual solutions in benzene-acetonitrile and stored in small vials at – 20°C. Working solutions was prepared by evaporation and derivatization of AFs mixture solution using trifluoroacetic acid in a pre-column derivatization step [24].

Analysis of AFs in spices: Test portions of 25 g (ginger, turmeric, cinnamon, cardamom, cumin, black pepper, chili pepper, cloves, laurel leaves and aniseed) were extracted by blending with 200 ml chloroform and 25 ml of sodium chloride solution for 2 minutes in a high-speed blender. Then, the extracts were filtered and an aliquot of 40 ml was evaporated at 40°C. The residue was dissolved in 4 ml hexane-ethyl acetate and 0.5 ml was subjected to gel permeation chromatography (GPC) clean up. 0.5 ml was passed through GPC system at a flow rate of 2.5 ml/min. 32 ml was dumped and 37 ml was collected in a 100 ml pear-shaped flask. The solution was evaporated at 40°C. Then derivatization of AFs was done by adding 100 µl of trifluoroacetic and 200 µl hexane to the residue. The flask was allowed to stand for 5 min at room temperature. 900 µl of acetonitrile-water was added and the flask was shaken occasionally for 0.5 min and let to stand for 5 minutes. Then, 100 µl of the aqueous layer was subjected to HPLC analysis.

HPLC analysis: AFs were separated and quantified by reversed phase-HPLC using C18 analytical column. The mobile phase was deionized water, methanol and acetonitrile. The flow rate was 0.7 ml/min. AFs were detected by fluorescence detector at the excitation and emission wavelengths of 360 and 440 nm respectively. The injection volume was 100 µl.

RESULTS AND DISCUSSION

In this study, the AFs were analyzed in 45 samples of unpacked spice products including ginger, turmeric, cinnamon, cardamom, cumin, black pepper, chili pepper, cloves, laurel leaves and aniseed. The levels of AFs detected in the tested spices are summarized in **Tables 1&2** and **Figures 1&2**. In this study 13 samples were found contaminated with AFs in the range of 0.9- 9.4 ug/kg (**Tab.1**). AFs contamination was detected in ginger, turmeric, cinnamon, cumin and chili pepper samples. The frequency of contamination was 80% in turmeric, 75% in hot chili, 60% in ginger, 40% in cinnamon and 20% in cumin samples (**Fig.1**). Turmeric samples contained the highest mean concentration of total AFs corresponding to 6.20 ug/kg whereas cumin samples had the lowest mean; 1.1ug/kg (**Fig.2**). However, the highest level of total AFs in turmeric samples is lower than the maximum limits (10 ug/kg) as arranged by the ECR (not shown).

Table 2, shows that AFB1 was detected in all contaminated samples. The highest mean concentration of AFB1 was in turmeric samples corresponding to 5.35. For all tested samples only three turmeric samples, representing 6.7% of the total number of samples analyzed exceed the permissible limit of AFB1 (not shown). AFB2 was detected in three turmeric samples with a mean level of 1.65 ug/kg. No contamination with AFG1 or AFG2 was found in all tested samples. These results suggest that AFB1 is the most frequent among AFs.

Table 1: Incidence and range of total aflatoxins (AFs) levels in spices and herbs

Sample	Number of samples (n)	Number of AF-contaminated samples (n)	Range of total AFs *(µg/kg)*
Ginger ((*Zingiber officinale)*	5	3	0.9-1.6
Turmeric (*Curcuma longa)*	5	4	1.8-9.4
Cinnamon	5	2	1.3-2.7
Cardamom	5	0	ND
Cumin (*Cuminum cyminum)*	5	1	1.1
Black pepper (*Piper nigrum)*	4	0	ND
Chili pepper	4	3	2.7-4.6
Cloves (*Syzygium aromaticum)*	4	0	ND
Laurel *(Laurus nobilis)*	4	0	ND
Aniseed (*Pimpinella anisum)*	4	0	ND
Total	45	13	

ND: not detected

Table 2: Mean of individual Aflatoxins (AFs) in contaminated samples of spices and herbs

Sample	AFB1 *(µg/kg)*	AFB2 *(µg/kg)*	AFG1 *(µg/kg)*	AFG2 *(µg/kg)*
Ginger (*Zingiber officinale)*	1.55±0.21	ND	ND	ND
Turmeric (*Curcuma longa)*	5.35±2.49	1.65±0.21	ND	ND
Cinnamon	1.86±0.73	ND	ND	ND
Cumin (*Cuminum)*	1.1	ND	ND	ND
Chili pepper	3.70±0.95	ND	ND	ND

ND: not detected

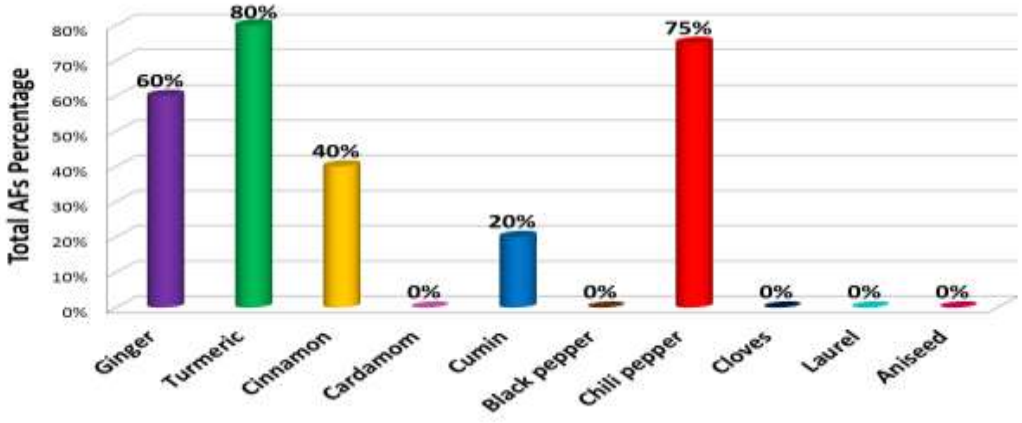

Figure 1: Total AFs percentage in tested spices and herbs samples

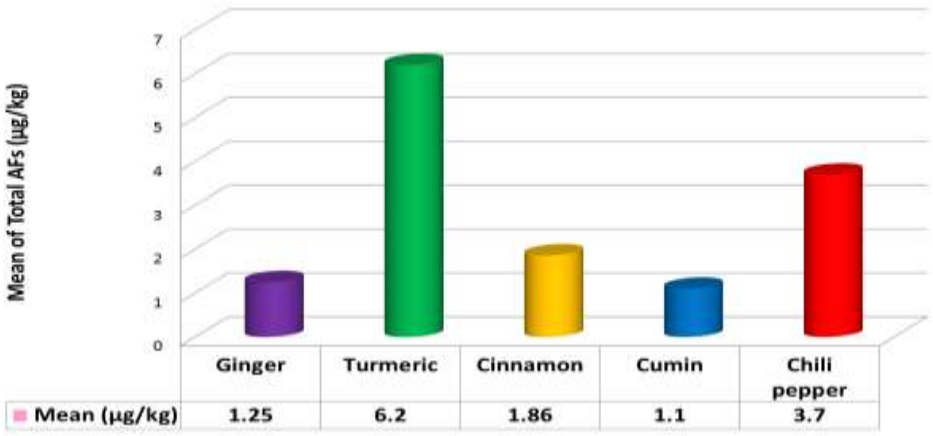

Figure 2: Mean of total Aflatoxins (AFs) in contaminated samples of spices and herbs

Several studies have reported AFs contamination in spices and herbs. Vrabcheva [25] reported that AFs are more frequently found in chili, paprika and ginger. O´Riodran and Wilkinson [26] detected AFs in 96% of commercial spice samples collected from Irish markets. Twaruzek etal. [20] detected AFs in turmeric, ginger, chili powder and nutmeg. For chili and ginger, the frequency of contamination was 60 and 80%. All samples of turmeric were contaminated. For all samples, total AFs content was within the permissible limits except for one chili sample (12.1ug/kg). In another study done by Cho etal. [27], AFs was found in in pepper, curry and ginger samples at a level between 0.8-4.66 ug/kg in 13.6 % of the analyzed samples.

It was observed that the levels of AFs vary among different spices from area to another. In this study, the level of total AFs in chili samples were in the permissible limits of ECR. In contrast, several studies determined higher levels of AFs in chili samples. For instance, In Turkey, Ozkan etal. [28] analyzed 180 samples of red chili for AFs and AFB1. They found that total AFs and AFB1 in 20.5% and 27.5 % of samples was higher than the legal limits of Turkish food codex. In Pakistan, Khan et al [8] analyzed 331 red chili samples and reported that 96.7% of samples were positive for AFs, with an average of 17.2 µg/ kg. In 50% of samples, the levels of AFs were beyond the ML as assigned by ECR. Another study from Pakistan reported that 65.9 of chili samples was higher than the ML of ECR for total AFs and AFB1 collected from urban area[29]. In Irish, O'Riordan and Wilkinson[26] found that among tested samples the highest level of AFs level was in chili powder corresponding to 27.5 µg/ kg .

On the other hand, Naz etal.[30]detected AFB1 in unpacked spice samples including cinnamon, turmeric and cumin seeds with higher contamination in turmeric and cinnamon. The mean levels of AFB1 exceed the maximum levels of ECR (7.3ug/kg in turmeric and 6.8ug/kg in cinnamon). Moreover, Tosum and Arslan [31] found heavy contamination of organically produced spices with AFB1. It was detected in cumin, ginger, cinnamon, black& red pepper and laurel leaves in a range of 0.5-53ug/kg. The highest level of contamination was in cinnamon. In contrast, Zinedine etal. [32] reported contamination of cumin and ginger samples with AFB1 within the permissible limits (1-5 ug/kg and 0.63 ug/kg. respectively).

The current study revealed that cardamom, black pepper, cloves, laurel leaves and aniseed were not contaminated with AFs. The absence of AFs in these samples may be due to intrinsic characteristic of spice or herbs such as essential oils, which inhibit the toxin production [33]. Moreover, the antioxidant property of

some of these spices and herbs may play a role. Narasaiah et al.[34]demonstrated that the presence of active oxygen is favorable for AFB1 production. Hence, the antioxidant property of these spices and herbs is detrimental to secondary metabolism of fungi and thus has the natural potential for inhibiting mycotoxin. From this point of view, several plants have been extensively studied as anti-fungal and anti-mycotoxin agents [35-36.]

Several studies have reported the antifungal effect of cinnamon, ginger, cumin and chili pepper against different types of fungi [37]. However, this effect may be not sufficient to prevent mycotic multiplication in highly contaminated samples with subsequent production of AFs.

The broad-spectrum antimicrobial activity for turmeric including antibacterial, antiviral and antifungal activities has been also reported. This activity is due to the presence of essential oil, curcumins, curcuminoids, turmeric oil, turmerol and veleric acid [38.] Although, curcumin showed significant effect against many types of fungi, it did not affect the growth of *Aspergillus* species [39]. This may explain high levels of AFs in turmeric samples in the present study.

The levels of AFs detected in this study were relatively low as compared to the levels reported in previous studies. All results were below the limit of 10 ug/kg for total AFs. AFB1 levels were also below 5ug/kg except for three turmeric samples. This may be attributed to the relative low humidity in AlMajmaah province, which is unfavorable for growth of toxigenic fungi and AF production.

CONCLUSION

The levels of total AFs in contaminated samples of turmeric, chili pepper, ginger, cinnamon and cumin are lower than the European limits. This may have no risk on public health. AFB1 level exceeds European permissible limits in three turmeric samples. As the most frequently identified toxin is AFB1, regular monitoring of the unpacked spices and herbs in AL Majmaah Markets is required.

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