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Incidence of Mycotoxins in Food Stuffs in the Markets

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Abstract: Fungi are widely distributed in nature. They exist whether saprophytic or as normal commensally flora for most animal species and human, attacking the host when the immune status becomes low (opportunistic). Fifty samples including cereals, minced meat, spices, nuts and dairy products collected from Saudi's markets in the current study and presence of mycotoxins – producing molds as well as the produced mycotoxins in the mentioned samples were detected (qualitatively) using Thin Layer Chromatography (TLC) and microbiological examination as screening techniques. Mycotoxins concentrations were compared to the permissible limits approved by WHO. Besides, the quantities of Aflatoxins and Ochratoxins in collected samples were detected using High Performance Liquid Chromatography (HPLC). Results revealed that the highest concentration of Aflatoxins was detected in cereals followed by spices (47.3 ppb and 38 ppb respectively and it showed slight increment in cheese and minced meat (6,9 ppb and 5.7 ppb respectively), while the concentration of Aflatoxins in nuts did not exceed the permissible limit. Occurrence of Ochratoxins was also detected among the samples used in our study. Only spices samples contained the highest concentration that exceeded the permissible limit (12 ppb).

Key words: Mycotoxins · Aflatoxins · Ochratoxins · HPLC · TLC

INTRODUCTION

Molds toxicity has attracted attention, especially in agriculture and food industry. Filamentous fungi often contaminate crops and animal products, considering a source of diseases in man and animals. Each single mold species can produce different toxins, and a given mycotoxin may be produced by more than one species of fungi. Furthermore, toxin-producing molds need certain growth conditions such as temperature, water content and humidity in order to produce toxin [1]. Mycotoxins production is enhanced by intrinsic and extrinsic factors that influence fungal growth and mycotoxins production on certain substrates. The intrinsic factors may include water activity and pH whereas extrinsic factors are relative humidity, availability of oxygen and temperature, toxins – producing fungi can grow on food containing more than 12 –15% moisture. In wet feed staff such as silage, higher moisture levels allow the fungal growth only if oxygen is available. The conditions suitable for molds growth and for mycotoxins formation are not necessarily the same [2].Conditions that is favorable for the production of a given type of mycotoxins may not be suitable to influence the production of another type. For example, aflatoxin produced by *Aspergillus* depends on the concentrations of oxygen, zinc, carbon dioxide, copper, and physical location, while ochratoxin production relates to air exhaustion [3].

Food and feed staff are often contaminated by many species of molds, when the temperature and relative humidity are optimal after contamination; there is a great probability of mycotoxin production [4]. Ochratoxin A is characterized by moderate stability and it is able to resist most food processing unfavorable conditions to certain

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extent and may thus be found in consumer products. The lack of sanitary measures applied as well as the hygienic quality of the added spices and some food additives were considered as the main source of filamentous fungi and mycotoxins that lead to either food spoilage or mycotoxicosis [5].

Acute toxicity cases are rarely reported in humans, while the sub-chronic and chronic mycotoxicosis caused by ochratoxin A are of greatest concern. Ochratoxin A has been shown to be nephrotoxic, hepatotoxic, teratogenicity and immunotoxigenic to animals and as carcinogen in mice and rats causing tumors in liver and kidneys [6]. Ochratoxin A has been reported to be an immune-suppressor in many mammalian species. It causes inhibition of protein biosynthesis and inhibits macrophage migration. Ochratoxin A has been shown to cause DNA damage and chromosomal aberrations in mammalian cells *in vitro* as well as DNA damage *in vivo*. However, the mechanism for genotoxicity is ambiguous and there was no clear justification explaining how it was mediated by direct interaction with DNA [7].

In Saudi Arabia, it was recorded that at least 5000 camels died and thousands more became sick, in a country, which boasted around 862,000 camels in 2005. The affected camels lost control of their movements and a cerebral hemorrhage and total paralysis were reported among them. Owners have attributed the deaths of thousands of camels to the bran used in animal feeding instead of barley and due to ‘‘toxic fodder contaminated with mycotoxins [8].

Fifteen spices samples collected from public markets were examined for their mold growth. A total of 520 fungal isolates, representing 57 species, were isolated and identified from dried and ground spice samples on three different media. The most heavily contaminated spice samples examined were observed in ginger [9].

The aim of this study was to determine the occurrence of mycotoxins in collected cereals, minced meat, as well as spices, nuts and dairy products from Saudi's markets. Microbiological examination was applied on all the samples for the presence of molds, presence of mycotoxins in the mentioned samples was detected (qualitatively) using Thin Layer Chromatography (TLC) as a screening technique. Besides, the quantities of some mycotoxins in collected samples were detected using High Performance Liquid Chromatography (HPLC). Results were analyzed statistically and thus, the health hazards inflicted by these mycotoxins could be assessed.

MATERIALS AND METHODS

A total of 50 samples including three types of cereals including wheat, maize and rice (n= 10), three types of spices including turmeric, coriander and thyme (n= 10), 3 types of nuts including hazelnuts, pistachio and almond (n= 10), local cheese as dairy products (n= 10) and minced beef meat (n= 10) were randomly sampled from local markets in three (3) Saudi cities, Qassim, Zulfi and Majmaah from January through March 2014. All samples were ground, mixed and stored at 4 °C prior to analysis. Samples of spices, nuts and cereals were examined grossly (by naked eyes) for the presence of fungal growth.

Microbiological Examination: Samples were prepared and examined for isolation of fungi (molds) according to the technique recommended by APHA [10]; five grams of finely ground meat (or tissues) were added to 45 ml of Sabouraud's Dextrose Broth in stomacher jar (original suspension). One ml from this suspension was transferred to a test tube containing 9 ml of sterile Sabouraud's Dextrose Broth and thoroughly mixed to have a dilution of 1/100. Ten – fold dilutions were prepared and 1 ml of each dilution was poured in sterile Petri dish, then the melted and tempered Sabouraud's dextrose agar was added. The plates were left to solidify at room temperature then incubated at 25°C for 5–10 days. After the end of incubation period, the isolated molds were identified [11].

Chromatographic Analysis: Qualitative assessment was applied on all the samples using TLC, while quantitative assessment analysis was done using HPLC.

Preparation of the Samples for Chromatographic Analysis: Preparation of the samples was done according to the type of the sample as follow:

For Minced Meat and Cheese Sample S [12]: Ten (10) grams were taken from each sample with the help of a sterile scalpel, and 90 ml of diluent (physiological saline) were added to the sample in a sterile homogenizing vessel. The propeller homogenizer with 10.000 revolution was used for homogenization; the homogenization time was 2.5 min. Extraction and cleanup for aflatoxins and ochratoxin analysis were performed also according to Monaci *et al*. [13]which briefly includes a double extraction with acidic ethyl acetate. The organic phase

was removed and extracted with 0.5M NaHCO3, pH 8.4. The aqueous extract was acidified to pH-2.5 with 7M H_3PO_4. Sample was finally back extracted into ethyl acetate (3 mL). The organic phase was evaporated to dryness under N_2 steam, reconstituted in 150 µL mobile phase and a 20 µL aliquot injected. The detection limit for OTA in organs was 0.01 ng/g with a 61% (C.V. =14.5%) mean recovery from artificially contaminated samples at 3 ng/g (n = 3).

For Other Samples (Spices Cereals and Nuts): Ten grams of each sample (fine powder) were added to 90 ml portion of sterile saline solution (0.85%) in 500 ml Erlenmeyer flask and homogenized thoroughly on an electric shaker at constant speed for 30 min. The spice–water suspension was allowed to stand for 10 min with intermittent shaking before being analyzed [14].

Thin Layer Chromatography conditions (TLC): The stationary phase was applied onto the plate uniformly and then allowed to dry and stabilize. With a pencil, a thin mark was made at the bottom of the plate to apply the sample spots. Then, samples solutions were applied on the spots marked on the line in equal distances, mobile phase was poured into the TLC chamber to a leveled few centimeters above the chamber bottom. A moistened filter paper in mobile phase was placed on the inner wall of the chamber to maintain equal humidity, the plate prepared with sample spotting was placed in TLC chamber so that the side of the plate with the sample line was facing the mobile phase. Then the chamber was closed with a lid. The plate was then immersed, allow sufficient time for the development of spots, then the plates were removed and allowed to dry. The sample spots were seen in a suitable UV light chamber.

High Liquid Chromatography Conditions (HPLC): AF and OTA determination was carried out in all the samples according to the instructor: Analysis was performed using an HPLC instrument consisting of Water Binary Pump model 1525, a model Waters 1500 Rheodyne manual injector; Water 2475 multi wave length fluorescence detector and a data workstation with software Breeze. A phenomoex C18 (250 X 4,6 mm I,d), particle size 5 µm from Waters Cooperation USA. Water was purified through a Milli- Q treatment system (Millipore, London, U.K.) and Phosphate Buffered Saline (PBS) was prepared as per Vicam (NaCl 8 g l_1, KCl 0.2 g l_1, Na_2HPO_4 1.15 g l_1, KH_2PO_4 0.2 g l_1; pH 7.4).

Statistical Analysis: Descriptive statistics of the data set were performed with a standard programmed and included arithmetic mean, standard deviation, coefficient of variation, minimum, maximum. Statistical differences in the mean levels of OTA contamination across the three groups of positive samples were determined by one-way ANOVA. Significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Fungal Contamination of Foodstuffs: The growth of different fungal colonies was determined *in vitro* as a parameter for contamination of food samples. Different fungi were isolated with percentages of 35% that differed according to the food stuff and the fungus. Presence of mycotoxins is not linked with the presence of molds growth, as it is well known that the favorable conditions for growth of molds are not necessary be favorable for the production of mycotoxins [15]

Aflatoxins are poisonous and cancer-causing chemicals that are produced by certain moulds (*Aspergillus flavus* and *Aspergillus parasiticus*) which grow in soil, decaying vegetation, hay, and grains. They are regularly found in improperly stored staple commodities. In our results as depicted in Table (1), Aflatoxins concentrations were measured in all the positive cereal samples, the variation among the types of cereals was not significant. Aflatoxins concentration was 47.3 ppb in average and this is considered as the highest average increment above the permissible limit (20 ppb).This is reflecting the bad unacceptable hygienic measures applied during transporting, processing and storage, whereas, grains and peanut were reported as a good substance for aflatoxins producing fungi, this may be due to the intrinsic factors offered by this food staff . Thus, it was imperative to investigate these intrinsic as well as the extrinsic factors favoring the growth of producing molds and the resulted toxins and give more attention to the arising health hazards on human being. The aflatoxin standards for cereals, dried fruits, and nuts intended for direct human consumption are even more stringent, and the range of aflatoxins concentrations in food samples analysed in our study were beyond the safe limits for human consumption as recommended by WHO and FDA (Food and Drug Administration, of United States) [16].In contrary to our results, a market research of cereals from Qatar revealed no detected levels of aflatoxin contamination in rice and wheat [17], while a study on Turkish wheat samples revealed 60% contamination level, in a very low range [18].

Table 1: Occurrence of total Aflatoxins in the examined samples

Item	n	Av. Content of Aflatoxins, ppb	Max. content, ppb	Average /Max, %	(Max-Ave)/ Max
Cereals	10	47.3 a	20	236.5 a	1.365 a
Spices	10	38 b	20	190.0 b	0.900 b
Cheese	10	6.9 c	5	138.0 c	0.380 c
Minced meat	10	5.7 d	5	114.0 d	0.140 d
LSD at 5% level	1.50		6.124	0.06	
Nuts	10	7.6	20		
Total	50				

Nuts samples content of Aflatoxin was detected in low levels and below the permissible limit (7.6 ppb), but indeed if we put in consideration the cumulative effect of mycotoxins, we have to give more attention to control mycotoxicosis that causes many health – related problems even with low concentrations. The European commission has set the limits on groundnuts subject to further processing at 15 ppb for total aflatoxins and 8 ppb for aflatoxin B1, and for nuts and dried fruits subject to further processing at 20 ppb for total aflatoxins and 5 ppb for aflatoxin B1. In the previous studies fifty six samples of stored rice were tested and 12 were positive for aflatoxin as concluded by Prasad *et al.* [19]. Levels of aflatoxins ranged from 184 to 2830 g/kg, while aflatoxin contamination rate of 65 µg/kg in groundnut samples from Bangladesh was reported by Dawlatana *et al.* [20], an aflatoxin level of 162 µg kg-1 was reported in Gambian ground nut samples [21,22] and the highest incidence (1706 mg/kg) of total aflatoxin in foods was found in Brazilian ready to eat peanuts [23].On the other hand, the results obtained by Reddy *et al.* [24] indicated that only 2% of tested rice samples were contaminated with Aflatoxins; similar results were recorded by Hesseltine *et al.*[25] and Dorner *et al.*[26] and Cotty [27] whereas these studied agreed with our results where the increment of Aflatoxins concentrations was not high (concentrations in cheese and minced meat were 6.9 and 5.7 ppb respectively). Toxin production by the genus *Aspergillus* has become now of major importance in human and animal diseases because of the direct toxicity and long term carcinogenic effect of its certain metabolites such as aflatoxins [28], Aflatoxins are a pervasive environmental risk, control and precise surveillance should be applied adequately and will require a multifaceted approaches. In contrary to the incidence of aflatoxicosis in cereals, the incidence of aflatoxicosis in spices screened in the current study was 50% with a degree of similarity to the results of Martins *et al*. [29] who detected Aflatoxin B (AFB) in 34 samples (out of 79 samples) of pre-packaged spices (43.0%) from supermarkets and ethnic shops in Lisbon (Portugal). This incidence percentage may be attributed to the strong fungicidal effect of some of herbs and spices [30].

Concentration of Aflatoxins in spices came in the second degree after these of cereals with an average of 38 ppb as depicted in Table (1), with a significance degree less when compared with that in cereals (0.900 and 1.365 respectively). Minor concentrations of aflatoxins contaminated various kind of spices were previously reported by Majerus *et al.* [31], 5.2~24 µg/kg; Misra [32], 3~37 µg/kg; Misra and Batra (1987) [33], 8~19 and El-Kadey *et al.* [34], 8~35 µg/kg.

Ochratoxins are a group of mycotoxins produced by some *Aspergillus* species (mainly *A. ochraceus*, but also by 33% of A. *niger*) and some *Penicillium* species, especially *P. verrucosum* and *P. carbonarius*. Among the different subtypes, Ochratoxin A is the most prevalent and relevant fungal toxin of this group, while ochratoxin B and C are of lesser importance. Ochratoxin A is known to occur in commodities such as cereals, coffee, dried fruit, and red wine [5].

Contamination with Ochratoxin was detected in the current study but with low level. Our results revealed that ten samples (40%), were contaminated with Ochratoxin and the variation among the cereal types was not significant.

Although 3.33% contamination with Ochratoxin were detected in Spices and nuts as shown in Table 2, the highest average concentration of Ochratoxin was recorded in spices (12 ppb). Contamination with Ochratoxin was nil in cheese and minced meat samples (0 ppb in both), the risk of ochratoxicosis is limited to monogastric species, because ruminants can hydrolyze the amidic bond of OTA into phenylalanine and ochratoxin a, which is generally considered to be non-toxic [35].

Although the concentrations of Ochratoxins in cereals and nuts (2.4 ppb and 1,7 ppb respectively) did not exceeded the permissible levels that were approved by WHO, a great awareness should be increased about

Table 2: Occurrence of total Ochratoxin in the examined samples

Item	n	Positive (%)	Av. Content of mycotoxin	Max. content
Cereals	10	10 (40%)	2.4 ppb	3 ppb
Spices	10	3 (33.3%)	12 ppb	10 ppb
Hazelnuts	10	1 (33.3%)	1.7 ppb	5 ppb
Cheese	10	0 (0%)	---------	-----
Minced meat	10	0 (0%)	---------	-----
Total	50	-----		

ochratoxicosis as their effect on general health is very severe, whereas, Ochratoxin is a moderately stable molecule and is able to survive most food processing to some extent and may thus reach the consumer's products and it is of special interest as it can be accumulated in the meat of animals. Besides, it is one of the human carcinogens. Exposure to ochratoxin through diet can cause acute toxicity in mammalian kidneys. It has been approved to be nephrotoxic, hepatotoxic, teratogenic and immunotoxic to several species of animals and carcinogenic in mice and rats causing tumors of the kidney and liver [36]. Ochratoxin A has been reported to be an immunosuppressor and affects the immune system in a number of mammalian species, as it has great ability to cause inhibition of protein biosynthesis and inhibition of macrophage migration[7].

Different strategies have been applied for elimination or inactivation of aflatoxins, problems still remain with the efficacy, safety and cost requirements for these methods [37]. Although preventing mycotoxin production at farm level is the best way to control mycotoxins contamination [38], chemical, biological and physical methods have been tried to inactivate aflatoxins or reduce their content in foodstuffs [39-41]. Advances in molecular techniques and other decontamination methods such as gamma irradiation and microwave heating could help in this respect [42]. Further studies are needed to help demonstrate and develop more effective decontamination methods.

With increasing awareness of mycotoxins as one of the sources of health hazard to both human and animals, application of advanced methods such as DNA biosensors and infrared spectroscopy for rapid and accurate detection of mycotoxins and related fungi increased. Furthermore, efforts have been made to eliminate the toxin or reduce its content in foods and feedstuffs to significantly lower levels.

CONCLUSION

Results revealed that the highest concentration of Aflatoxins was detected in cereals followed by spices (47.3 ppb and 38 ppb respectively and it showed slight increment in cheese and minced meat (6,9 ppb and 5.7 ppb respectively), while the concentration of Aflatoxins in nuts did not exceed the permissible limit. Occurrence of Ochratoxins was also detected among the samples used in our study. Only spices samples contained the highest concentration that exceeded the permissible limit (12 ppb). According to these results, control of mycotoxin is a matter of importance not only for health implications, but also for improvement of economy in the affected countries. Since most of mycotoxins are stable and generally resistant to heat and processing, control of toxins contamination lies in the control of the growth of the toxin-producing fungi. Effective prevention of mycotoxins contamination therefore depends on good farming and agricultural practices. Good Agricultural Practices including methods to reduce fungal infection and growth during harvest, storage, transport and processing provide the primary line of defense against contamination with mycotoxins in addition to periodical qualitative and quantitative evaluation and measuring of mycotoxins.

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