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Occurrence of Aflatoxin M_1 in Pasteurized and Traditional Cheese Marketed in Southern Khorasan, Iran

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Abstract

Background: Aflatoxin M_1 (AFM_1) is a toxic and carcinogenic mycotoxin which after presence in milk and dairy products such as cheese and butter could make them as contaminated food. The main objective of this study was to evaluate the concentration of AFM_1 in pasteurized and traditional cheese marketed in Southern Khorasan, from December 2011 to January 2012.

Methods: A total of 102 cheese samples (including 43 non-pasteurized traditional and 59 pasteurized cheese) were analyzed for detection of AFM_1. The samples were collected randomly from three major cities of Southern Khorasan province, Iran. A rapid and sensitive indirect competitive Enzyme Linked Immuno Sorbent Assay (ELISA) method using monoclonal antibody was used to measure AFM_1 concentration in the samples.

Results: According to the results of this study, AFM_1 was detected in 14 (32.55%) traditional and 21 (35.59%) pasteurized cheese samples. In total, 25.42% of pasteurized and 27.90% of traditional cheese samples were contaminated at above level of the Iranian standard limits (50 ng/kg).

Conclusion: Considering high contamination level of both pasteurized and traditional cheese products in Southern Khorasan, it is recommended to establish continuous monitoring program of AFM_1 by food safety agencies in order to control the incidence of mycotoxin contamination.

Introduction

Mycotoxins are common toxic metabolites of fungi produced mainly by specific moulds genera such as *Aspergillus*, *Penicillium* and *Fusarium*. Aflatoxins biosynthesized by toxicogenic strains of *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* can contaminate corn and grain crops after harvest during the drying process (Fallah, 2010a; Montaseri et al., 2014). Outbreaks of aflatoxicosis were firstly noted in the 1960s in England, when more than 100000 turkeys on poultry farms died due to consumption of aflatoxin contaminated feed. There are four main types of aflatoxins consist of B_1, B_2, G_1 and G_2. Aflatoxin G_1 (AFG_1) causes cancer in animals (Sidhu et al., 2009), but aflatoxin B_1 (AFB_1) is carcinogen for both animal and human. For achieving this characteristic, AFB_1 must be metabolized (Kursat et al., 2011). Aflatoxin M_1 (AFM_1) which is the hydroxilated metabolite of AFB_1, found in milk and milk-derived products obtained from livestock that have ingested AFB_1 contaminated feed (Sidhu et al., 2009).

Dairy products as valuable sources of calcium and proteins should be placed in a healthy human diet. AFM_1 is more concentrated in cheese compared to its corresponding

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milk; therefore, cheese is considered to be the most potent source of AFM_1 among dairy products (Ardic et al., 2009; Tekinsen and Tekinsen, 2005).

However, AFM_1 is less toxic than AFB_1, but it is known as hepatotoxic and carcinogenic toxin (Lee et al., 2009). Complications of aflatoxicosis consist of anemia, hepatotoxicosis, hemorrhage, teratogenesis, carcinogenesis and mutagenesis (Arast et al., 2012).

Referring to scientific literature, many studies have been carried out in different countries to assess the occurrence of AFM_1 in milk and dairy products. Although numerous researches have been conducted on the incidence of AFM_1 in dairy products in Iran (Alborzi et al., 2006; Fallah, 2010a; Fallah, 2010b; Ghazani, 2009; Hasanzadeh Khayat et al., 1999; Kamkar, 2006; Oveisi et al., 2007; Tajik et al., 2007), there is little information about the occurrence of AFM_1 in cheese. Also, it has been stated that the presence and amount of milk products contamination with AFM_1 show variations according to geographical area, country and season.

Therefore, this study was aimed to evaluate the presence and levels of AFM_1 in pasteurized and traditional cheese marketed in three major cities of Southern Khorasan province of Iran.

Materials and methods

Sampling

This cross-sectional study was carried out on a total of 102 samples of non-pasteurized traditional cheeses (n=43) and marketed pasteurized one (n=59) distributed in Southern Khorasan province of Iran from December 2011 to January 2012.

Non-pasteurized traditional cheeses, from three major cities including Birjand (n=19), Qhaen (n=15) and Ferdows (n=9), and pasteurized one, including three known brands named as brand A (n=19), B (n=20) and C (n=20), were purchased randomly from supermarkets. All samples were stored at 4 °C and analyzed for presence of AFM_1 before their expiry date.

Determination of AFM_1 in the samples by indirect competitive ELISA

The quantity of AFM_1 was determined using the Rocket International test Kit (Rocket International Company, Cat No. EEM005096) with competitive ELISA with ELISA Reader (Anthos device 2020, Italy).

In order to extract the solution, 5 ml of methanol was added to 1 g of each sample and mixed for 5 min at room temperature, centrifuged at 6000 xg for additional 5 min. The sample was left at room temperature for 4-5 min. Then, 1 ml of the upper layer was discarded and the solvent was volatilized. Also, 1 ml of Phosphate Buffered Saline (PBS) buffer was added to the solution and mixed for 1 min. Afterward, the mixture was again centrifuged at 6000 xg for additional 5 min. Finally, the mixtures were diluted (1:2 v/v) by addition of 0.3 ml PBS buffer and then used for quantitative tests. For the next step, 200 µl of enzyme conjugate was added to each well and mixed by shaking the plate manually and then incubated for 15 min at room temperature in the dark. Chromogen stained in red (200 µl) was added to each well and mixed manually for 10 min additional incubation at room temperature in the dark. Stop solution (5 µl) added to each well, led to color change from blue to yellow. The plate was mixed manually again gently by shaking and subjected to absorbance measures at 450 nm. Distilled water (200 µl) was added to the control well and 200 µl of prepared sample solution or AFM_1 standard were added to other wells followed by gently shaking of the plate and 10 min incubation at room temperature in the dark. The liquid was then poured out completely from the wells and the wells were fulfilled with 300 µl of diluted rinsing buffer. The buffer is then discarded out of the wells. This stage of procedure was repeated 2 more times.

In the next step, 200 µl of conjugate enzyme was added to each well but not to the control well. Afterward, they were mixed by shaking the plate manually and incubated for 15 min at room temperature in the dark. Rinsing the wells with diluted buffer was performed for 3 times. Then, 200 µl chromogen was added to each well and incubated for 10 min at room temperature in the dark. Following, 50 µl of the stop reagent was added to each well, leading to color change from blue to yellow. The light absorption was read at 450 nm.

Statistical analysis

The results were analyzed with SPSS software version 16 using independent t-test. *P* value of <0.05 was considered as significant.

Results

Findings of this survey revealed presence of AFM_1 in 35 (34.3%) of 102 cheese samples (Table 1). AFM_1 was found above detectable level (5 ng/kg) in 32.55% and 35.59% of traditional and pasteurized cheese samples, respectively. Totally, 27.90% of traditional and 25.42% of pasteurized cheese samples exceeded Iran legal regulation (50 ng/kg).

AFM_1 levels were above Iranian national standard in 31.6%, 20% and 33.3% of traditional cheese obtained from Birjand, Qhaen and Ferdous, respectively. This rate in pasteurized cheese samples were 31.6%, 20% and 25% in brand A, B and C, respectively. There was no significant difference in AFM_1 level between traditional and pasteurized cheese ($p>0.05$).

Table 1: AFM_1 level in cheese samples distributed in Southern Khorasan province of Iran

Sample type	Sample size	Distribution percentage of the levels of AFM1 (ng/kg)						Max (ng/kg)	Mean (ng/kg)	Min (ng/kg)
		<5	5-50	51-150	151-250	251-500	>500			
Birjand TC*	19	63.2	5.3	31.6	0	0	0	146.42	31.4	4.77
Qhaen TC	15	73.3	6.7	20	0	0	0	120.66	24.81	4.54
Ferdows TC	9	66.7	0	33.3	0	0	0	130.32	35.21	5.6
PC** (Brand A)	19	57.9	10.5	15.8	10.5	5.3	0	313.85	57.32	9.34
PC (Brand B)	20	70	10	5	10	5	0	294.53	43.6	9.04
PC (Brand C)	20	65	10	15	5	5	0	339.61	43.84	8.8

* TC: Traditional Cheese

** PC: Pasteurized Cheese

Table 2: AFM_1 contents of cheese samples reported in the other previous studies carried out in different countries

Country of origin	Sample size	Positive sample (%)	Range of contamination (ng/kg)	References
Turkey	100	81	51->800	Sarimehmetoglu et al., 2004
Finland	10	0	Not determined	Kokkonen et al., 2005
Brazil	75	74.6	20-6920	Prado et al., 2000
Turkey	127	28.3	70.61-770.97	Kav et al., 2011
North Africa	20	75	110-520	Elgerbi et al., 2004
Iran (Esfahan)	88	53.4	82-1254	Rahimi et al., 2009
Iran (Central part of Iran)	72	81.9	30-1200	Fallah et al., 2010b
Iran (Lighvan village)	75	65.3	30-313	Fallah et al., 2011
Iran (Tehran)	50	60	40.9-374.0	Tavakoli et al., 2012

Discussion

Milk and dairy products play major role in a healthy human diet and should be highly consumed by infants and children because of their rich mineral contents (Baskaya et al., 2006). According to high toxicity and carcinogenicity of AFM_1, its incidence in cheeses, as a concentrated dairy product is of great concern. ELISA, High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) are the most common methods for the detection of mycotoxins in food and feeds (Fallah, 2010a; Fallah, 2010b). Institute of Standards and Industrial Research of Iran (ISIRI, 2005) set the maximum permissible level of 50 ng/l for AFM_1 in liquid milk. According to this survey, AFM_1 was found in 35 (34.3%) of 102 cheese samples indicating high importance from public health point of view.

According to Table 2, several researchers have reported the presence of AFM_1 in various cheese types; the AFM_1 levels were varied from one study to another (Hassanzadeh Khayat et al,. 1999). The differences of the AFM_1 concentration in dairy products can be attributed to multiple variables such as type of cheese, cheese-making procedures, analysis method, season and geographical area (Fallah et al., 2009; Fallah et al., 2011; Tavakoli et al., 2012).

In this survey, no statistical significant difference was observed in AFM_1 level between traditional and pasteurized cheese. This finding may be attributed to this fact that in some regions of Iran, the origin of raw milk used to prepare both traditional and pasteurized cheese, is the same.

According to results obtained from this study, AFM_1 level of 26.47% of samples was above the maximum permissible level set by ISIRI (2005). As occurrence of AFM_1 contamination, even below maximum permissible levels, can lead to health complications, AFM_1 contamination of dairy products, especially cheese, in Iran seems to be a serious public health problem especially in children.

Conclusion

The results of present study showed high frequency of AFM_1 contamination in cheese marketed in Southern Khorasan province of Iran. Therefore, it seems critical to keep animal feeds free from contamination by AFB_1. The best way to deal with this problem is to reduce AFB_1 contamination in animal food stuffs by improved processing and storage practices. In addition, continuous monitoring program by food safety agencies is highly recommended to control the incidence of aflatoxin contamination in dairy products of this country.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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