

Occurrence of Aflatoxin M₁ in Pasteurized and Traditional Cheese Marketed in Southern Khorasan, Iran

M. Khodadadi¹, R. Khosravi¹, A. Allahresani², Y. Khaksar³, L. Rafati⁴, B. Barikbin^{1*}

1. Social Determinants of Health Research Center, Birjand University of Medical Science, Birjand, Iran

2. Chemistry Faculty, Birjand University, Birjand, Iran

3. Faculty of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

4. Department of Environmental Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Article type

Original article

Keywords

Aflatoxin M₁

Cheese

Iran

Received: 28 Mar 2014

Revised: 15 May 2014

Accepted: 19 June 2014

Abstract

Background: Aflatoxin M₁ (AFM₁) 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 AFM₁ 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 AFM₁. 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 AFM₁ concentration in the samples.

Results: According to the results of this study, AFM₁ 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 AFM₁ 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 B₁, B₂, G₁ and G₂. Aflatoxin G₁ (AFG₁) causes cancer in animals (Sidhu et al., 2009), but aflatoxin B₁ (AFB₁) is carcinogen for both animal and human. For achieving this characteristic, AFB₁ must be metabolized (Kursat et al., 2011). Aflatoxin M₁ (AFM₁) which is the hydroxilated metabolite of AFB₁, found in milk and milk-derived products obtained from livestock that have ingested AFB₁ contaminated feed (Sidhu et al., 2009).

Dairy products as valuable sources of calcium and proteins should be placed in a healthy human diet. AFM₁ is more concentrated in cheese compared to its corresponding

*Corresponding author
E-mail: b_barikbin@yahoo.com

milk; therefore, cheese is considered to be the most potent source of AFM₁ among dairy products (Ardic et al., 2009; Tekinsen and Tekinsen, 2005).

However, AFM₁ is less toxic than AFB₁, 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 AFM₁ in milk and dairy products. Although numerous researches have been conducted on the incidence of AFM₁ 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 AFM₁ in cheese. Also, it has been stated that the presence and amount of milk products contamination with AFM₁ show variations according to geographical area, country and season.

Therefore, this study was aimed to evaluate the presence and levels of AFM₁ 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 AFM₁ before their expiry date.

Determination of AFM₁ in the samples by indirect competitive ELISA

The quantity of AFM₁ 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 AFM₁ 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 AFM₁ in 35 (34.3%) of 102 cheese samples (Table 1). AFM₁ 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).

AFM₁ levels were above Iranian national standard in 31.6%, 20% and 33.3% of traditional cheese obtained from Birjand, Qhaen and Ferdows, 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 AFM₁ level between traditional and pasteurized cheese (*p*>0.05).

Table 1: AFM₁ level in cheese samples distributed in Southern Khorasan province of Iran

Sample type	Sample size	Distribution percentage of the levels of AFM ₁ (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: AFM₁ 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 AFM₁, 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 AFM₁ in liquid milk. According to this survey, AFM₁ 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 AFM₁ in various cheese types; the AFM₁ levels were varied from one study to another (Hassanzadeh Khayat et al., 1999). The differences of the AFM₁ 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 AFM₁ 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, AFM₁ level of 26.47% of samples was above the maximum permissible level set by ISIRI (2005). As occurrence of AFM₁ contamination, even below maximum permissible levels, can lead to health complications, AFM₁ 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 AFM₁ contamination in cheese marketed in Southern Khorasan province of Iran. Therefore, it seems critical to keep animal feeds free from contamination by AFB₁. The best way to deal with this problem is to reduce AFB₁ 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.

Acknowledgement

The authors would like to thank Birjand University of Medical Sciences, Iran for financial and technical supports during this study.

References

- Alborzi S., Pourabbas B., Rashidi M., Astaneh B. (2006). Aflatoxin M₁ contamination in pasteurized milk in Shiraz (south of Iran). *Food Control*. 17: 582–584.
- Arast Y., Mohammadian M., Behnamipour S. (2012). Occurrence of aflatoxin M₁ in two dairy products by ELISA in central part of Iran. *Life Science*. 9: 1831-1833.
- Ardic M., Karakaya Y., Atasever M., Adiguzel G. (2009). Aflatoxin M₁ levels of Turkish white brined cheese. *Food Control*. 20: 196-199.
- Baskaya R., Aydın A., Yildiz A., Bostan K. (2006). Aflatoxin M₁ levels of some cheese varieties in Turkey. *Medycyna Weterynaryjna*. 62: 778-780.
- Elgerbi A.M., Aidoo K.E., Candlish A.A.G., Tester R.F. (2004). Occurrence of aflatoxin M₁ in randomly selected North African milk and cheese samples. *Food Additives and Contaminants*. 21: 592-597.
- Fallah A.A. (2010a). Assessment of aflatoxin M₁ contamination in pasteurized and UHT milk marketed in central part of Iran. *Food and Chemical Toxicology*. 48: 988-991.
- Fallah A.A. (2010b). Aflatoxin M₁ contamination in dairy products marketed in Iran during winter and summer. *Food Control*. 21: 1478-1481.
- Fallah A.A., Jafari T., Fallah A., Rahnama M. (2009). Determination of aflatoxin M₁ levels in Iranian white and cream cheese. *Food and Chemical Toxicology*. 47: 1872-1875.
- Fallah A.A., Rahnama M., Jafari T., Saei-Dehkordi S.S. (2011). Seasonal variation of aflatoxin M₁ contamination in industrial and traditional Iranian dairy products. *Food Control*. 22: 1653-1656.
- Ghazani M.H.M. (2009). Aflatoxin M₁ contamination in pasteurized milk in Tabriz (northwest of Iran). *Food and Chemical Toxicology*. 47: 1624–1625.
- Hassanzadeh Khayat M., Mortazavi A., Kohestani G.H., Bakhtiarizadeh K., Akhavan H. (1999). Determination of aflatoxin M₁ concentration in pasteurized milk in Mashhad, northeast of Iran. *Iranian Journal of Basic Medical Sciences*. 2: 24–29.
- Institute of Standards and Industrial Research of Iran (ISIRI). (2005). Milk and milk products-raw milk-specifications and test methods. National Standard No. 164. URL: <http://www.isiri.org/portal/files/std/164.doc>. Accessed 23 January 2014.
- Kamkar A. (2006). A study on the occurrence of aflatoxin M₁ in Iranian feta cheese. *Food Control*. 17: 768-775.
- Kav K., Col R., Kaan Tekinsen K. (2011). Detection of aflatoxin M₁ levels by ELISA in white-brined Urfa cheese consumed in Turkey. *Food Control*. 22: 1883-1886.
- Kokkonen M., Jestoi M., Rizzo A. (2005). Determination of selected mycotoxins in mould cheeses with liquid chromatography coupled to tandem with mass spectrometry. *Food Additives and Contaminants*. 22: 449-456.
- Kursat K., Ramazan C., Kaan T. (2011). Detection of aflatoxin M₁ Levels by ELISA in white-brined Urfa cheese consumed in Turkey. *Food control*. 22: 1883-1886.
- Lee J.E., Kwak B.M., Ahn J.H., Jeon T.H. (2009). Occurrence of aflatoxin M₁ in raw milk in South Korea using an immunoaffinity column and liquid chromatography. *Food Control*. 20: 136–138.
- Montaseri H., Arjmandtalab S., Dehghanzadeh G., Karami S., Razmjoo M.M., Sayadi M., Oryan A. (2014). Effect of production and storage of probiotic yogurt on aflatoxin M₁ residue. *Journal of Food Quality and Hazards Control*. 1: 7-14.
- Oveisi M.R., Jannat B., Sadeghi N., Hajimahmoodi M., Nikzad A. (2007). Presence of aflatoxin M₁ in milk and infant milk products in Tehran, Iran. *Food Control*. 18: 1216-1218.
- Prado G., Oliveira M.S., Pereira M.L., Abrantes F.M., Santos L.G., Veloso T. (2000). Aflatoxin M₁ in samples of "Minas" cheese commercialized in the city of Belo Horizonte-Minas Gerais/Brazil. *Ciencia e Tecnologia de Alimentos*. 20: 398-400.
- Rahimi E., Karim G., Shakerian A. (2009). Occurrence of aflatoxin M₁ in traditional cheese consumed in Esfahan, Iran. *World Mycotoxin Journal*. 2: 91-94.
- Sarmehmetoglu B., Kuplulu O., Celik T.H. (2004). Detection of aflatoxin M₁ in cheese sample by ELISA. *Food Control*. 15: 45–49.
- Sidhu O.P., Chandra H., Behl H.M. (2009). Occurrence of aflatoxin in mahua (*Madhuca indica Gmel*) seed: synergistic effect of plant extracts on inhibition of *Aspergillus flavus* growth and aflatoxin production. *Food and Chemical Toxicology*. 47: 774-777.
- Tajik H., Rohani S.M.R., Moradi M. (2007). Detection of aflatoxin M₁ in raw and commercial pasteurized milk in Urmia, Iran. *Pakistan Journal of Biological Sciences*. 10: 4103–4107.
- Tavakoli H.R., Riazipour M., Kamkar A., Rafati Shaldehi H., MozaffariNejad A.R. (2012). Occurrence of aflatoxin M₁ in white cheese samples from Tehran, Iran. *Food Control*. 23: 293-295.
- Tekinsen K.K., Tekinsen O.C. (2005). Aflatoxin M₁ in white pickle and *Van otlu* (herb) cheeses consumed in southeastern Turkey. *Food Control*. 16: 565–568.