JOURNAL OF FOOD QUALITY & HAZARDS CONTROL
SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCES & HEALTH SERVICES

Journal of Food Quality and Hazards Control 2 (2015) 11-14

Aflatoxin M_1 Contamination in Industrial and Traditional Yogurts Produced in Iran

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Article type
Original article

Keywords
Aflatoxin M_1
Yogurt
Iran

Received: 29 Aug 2014
Revised: 5 Oct 2014
Accepted: 13 Nov 2014

Abstract

Background: Aflatoxin M_1 (AFM_1) is a major carcinogenic compound that may be existed in milk and dairy products. This study was aimed to determine the presence of AFM_1 in industrial and traditional yogurt samples produced in Iran.

Methods: A total of 80 yogurt samples (40 industrial, 40 traditional) was collected from supermarkets in Yazd city, Iran. AFM_1 content of the selected samples was determined by enzyme linked immunesorbent assay (ELISA).

Results: Analysis of the data indicated that 96.25% of the yogurt samples were contaminated with AFM_1 in concentration levels ranging from <5 to 91 ng/kg. The AFM_1 content of 5% of the samples was higher than the limit accepted by European Union (50 ng/kg). The mean concentration of AFM_1 in traditional yogurt (33.6 ng/kg) was significantly (p<0.05) higher than industrial ones (24.55 ng/kg).

Conclusion: High level of AFM_1 contamination of yogurt produced in Iran could be a serious risk for the public health.

Introduction

Aflatoxins are the main class of mycotoxins produced by *Aspergillus* species (*A. flavus*, *A. parasiticus* and rarely *A. nomius*) that occur in some food and feeds including tree nuts, spices, dried fruits and cereals during agriculture activities (Pitt, 2000). Aflatoxin B_1 (AFB_1) is the most toxic among different types of aflatoxins (Creppy, 2002; Montaseri et al., 2014; Prandini et al., 2009; Tajkarimi et al., 2008).

Aflatoxin M_1 (AFM_1), as the major metabolite of AFB_1, may be existed in milk and dairy products (Khodadadi et al., 2014; Prandini et al., 2009). It has been stated that about 0.5-5% of AFB_1 content in ingested feed of lactating animals converse to AFM_1 excreting in milk (Murphy et al., 2006; Neal et al., 1998). The International Agency for Research on Cancer (IARC, 2002) includes AFM_1 in group 1 carcinogen toxins.

According to the European Commission (EC, 2006), AFM_1 concentration up to 0.05 µg/kg has been determined in milk and other types of dairy products. The Food and Drug Organization (FDA, 1996) of United States has established an acceptable limit of AFM_1 in 0.5 µg/l from whole fat, low fat and skimmed milk. According to Institute of Standard and Industrial Research of Iran (ISIRI, 2010), the accepted limit of AFM_1 in milk and yogurt has been set up to 0.5 µg/kg. When dairy products are manufactured from AFM_1 contaminated milk, the toxin could be detected in

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these products (Bakirci, 2001), because AFM_1 is a stable compound that persists against most of food processing stages (Fallah, 2010a; Iha et al., 2013).

Previous investigations revealed relatively high occurrence of AFM_1 in milk samples of Iran (Fallah et al., 2011; Montasri et al., 2014). However, studies on AFM_1 contamination of yogurt, as a popular dairy product, are very limited. Thus, the objective of this work was to assess the level of AFM_1 in yogurt samples produced in Iran.

Materials and methods

Sampling

A total of 80 yogurt samples (40 industrial, 40 traditional) collected from supermarkets in Yazd, central part of Iran, during spring and summer 2013. Appropriate amount of each sample was prepared and stored at -20 °C until the time of analysis. All samples analyzed before their expiry date.

Determination of AFM_1

AFM_1 detection was performed using the enzyme linked immunesorbent assay (ELISA) kit named RIDAScreen aflatoxin M_1 (Biopharm, #R1101). Most of the used reagents were supplied by the kit manufacturer. The other chemicals such as methanol, chloroform, dichloromethane and heptane in analytical grade obtained from Merck (Darmstadt, Germany). For conducting recovery study, AFM_1 as a standard was purchased from Sigma Chemical Company (USA). The stock solution of AFM_1 (10 mg/ml) was prepared in methanol. Appropriate portions of the stock solution were evaporated and diluted with methanol/chloroform (1:1 v/v) to give a concentration of 50 µg/ml.

All yogurt samples were prepared according to the method outlined in the ELISA kit for cheese. Two grams of a representative samples were added to 40 ml of dichloromethane. The mixture was extracted by shaking for 15 min. Suspension was filtered and 10 ml of that was evaporated at 60 °C under weak N_2 gas stream. The oily residue was dissolved in 0.5 ml methanol, 0.5 ml Phosphate Buffer Saline (PBS, pH 7.2 containing of 0.05% Na_2HPO_4, 0.28% Na_2HPO_4 and 0.9% NaCl), and 1 ml heptane. The mixture was centrifuged for 15 min in 2700 xg at 15 °C. The upper layer of heptane was removed and 100 µl of the aliquot were diluted with 400 µl of kit buffer.

For all standards and samples, microtiter plates were inserted into the microwell holder as indicated by manufacturer's instructions. A 100 µl of each standard solution (0, 5, 10, 20, 40, 80 and 250 ng/l) and prepared samples were added separately and mixed gently by manually shaking the plate and then incubated for 30 min at ambient room temperature in the dark. At the end of incubation step, the liquid in the wells was poured out; then, to remove the remainder of the liquid the microwell holder was tapped upside down on an absorbent paper. Using 250 µl washing buffer, each well was washed twice. Then, 100 µl of the enzyme conjugate (peroxidase conjugated AFM_1) was added to each well and mixed quietly and incubated for 15 min at ambient room temperature in the dark. The wells were then washed three times with 250 µl washing buffer and 100 µl substrate/chromogen were added to each well and incubated for 15 min at ambient room temperature in the dark. The bond enzyme conjugate converted the chromogen to a blue product and 100 µl of the stop solution was added to each well that led to a yellow discoloration of the chromogen. The AFM_1 measurement was performed photometrically at 450 nm (Elx800 series, microplate reader, BioTek, Vermont, USA). The obtained calibration curve was found to be virtually linear in the 10–250 ng/l range.

In order to determine the AFM_1 concentration of yogurt samples in ng/kg, the concentration read from the calibration curve was multiplied by a dilution factor of 5. The detection limit of the method was 5 ng/kg in the yogurt samples.

Validation of the ELISA method was carried out by determination of recoveries and the mean variation coefficient for yogurt spiked with different concentrations of AFM_1 (20, 50 and 200 ng/kg). Spiking was carried out in 3 samples for each of the mentioned levels. Analytical values were not corrected for recovery.

Statistical analysis

Statistical analysis of results was performed with SPSS (version 16.0) software. The mean AFM_1 concentration in traditional and industrial yogurt was compared by t-test. *P* value of <0.05 was considered as significant.

Results

AFM_1 was detected in 96.25% of the tested yogurt samples by average concentration of 29.07 ng/kg (Table 1 and 2). Four samples (5%) including 1 traditional and 3 industrial samples had higher AFM_1 level than the admissible level (50 ng/kg) established by the Commission of the European Communities, while none of the samples exceeded ISIRI and FDA regulations.

The range of contamination level varied from <5 to 71 ng/kg in industrial yogurt samples and from 6 to 91 ng/kg in traditional yogurt samples. The results demonstrate that mean contamination levels in traditional yogurts was higher than industrial ones ($p<0.05$).

Table 1: Method validation data for AFM_1 determination in yogurt by ELISA

Spiking level (µg/kg)	AFM_1 found (ng/l)	SD	Recovery (%)	RSD (%)
0.02	0.021	0.002	110	9.1
0.05	0.053	0.005	93	9.2
0.20	0.21	0.015	87	7.4

Table 2: Occurrence of AFM_1 in yogurt samples produced in Iran

Yogurt type	Sample Size	No. of contaminated samples (%)	Mean±SD (ng /kg)	No. of contaminated samples (%)				No. of Exceeded regulation (%)	
				< 5 ng/kg	5–50 ng/kg	50-100 ng/kg	> 100 ng/kg	EU	ISIRI, FDA
Traditional	40	40 (100)	33.6±15.95	0 (0)	37 (92.5)	3 (7.5)	0 (0)	1 (2.5)	0 (0)
Industrial	40	37 (92.5)	24.55±13.65	3 (7.5)	36 (90)	1 (2.5)	0 (0)	3 (7.5)	0 (0)
Total	80	77 (96.25)	29.07±15.43	3 (3.75)	73 (91.25)	4 (5)	0 (0)	4 (5)	0 (0)

Table 3: Occurrence of AFM_1 in yogurt samples reported form some region in the world

Country	Samples	Incidence (%)	Range	Exceeded regulation	References
Italy	120	61	0.001-0.32	0	Galvano et al. (2001)
Portugal	48	4.1	43-45	0	Martins and Martins (2004)
Lebanon	64	32.81	ND*	6.25	El Khoury et al. (2011)
Brazil	30	0	0	0	Sylos et al. (1996)
South Korea	60	50	17-124	ND	Kim et al. (2000)

* ND: Not Determined

Discussion

In this study a total of 80 yogurt samples analyzed for AFM_1 by ELISA. ELISA is a rapid and simple method for determination of AFM_1 in dairy products. Rosi et al. (2007) showed that ELISA method (RIDAScreen AFM_1) have good results in comparison to high performance liquid chromatography (HPLC) for determination of AFM_1 in milk. The results of validation study that expressed in Table 1 are comparable to data from the RIDAScreen AFM_1 manual, which gives recovery rate of 102% and a mean variation coefficient of 11% for yogurt. According to Table 3, the incidence of AFM_1 in tested yogurt samples in the present survey was relatively higher than the results obtained from the other regions of the world (El Khoury et al., 2011; Galvano et al., 2001; Kim et al., 2000; Martins and Martins, 2004; Sylos et al., 1996), but not very different from the results obtained from other studies in Iran (Fallah et al., 2011; Tabari et al., 2012). In the previous similar published research conducted in northern Iran, Tabari et al. (2012) reported that all tested yogurt samples in Guilan province were contaminated with AFM_1 in range of 4.2-78.9 ng/kg. Around 13.33 % of the tested samples had contamination levels above the European regulation (50 ng/kg).

Besides the industrial yogurt, production and consumption of traditional yogurt are common in Iran. Industrial yogurts are manufactured in dairy industry but traditional ones are made in ranches or small dairy shops (Fallah et al., 2011) and even in homes. The milk that used for production of traditional yogurt is mainly supplied by traditional dairy farms; in this type of dairy farms crop residues, weeds, wheat and barley stubble are the sources of animal feed (Tajkarime et al., 2008).

Higher level of AFM_1 in traditional yogurt might be due to high level of AFB_1 in feedstuff that had been used in the feeding of dairy cattle in traditional husbandry practices. Another possible reason for higher level of AFM_1 in traditional yogurt could be found in the yogurt manufacturing method. It should be indicated that in traditional yogurt production in Iran, the increasing of solid not fat of base milk are gained only by excessive evaporating of milk; so, this concentration process may led to increasing the level of AFM_1 in final product. Lower level of AFM_1 in industri-

al yogurt may also be associated with a dilution effect of contaminated milk with non-contaminated milk in dairy industry.

The mean concentration of AFM_1 in yogurt samples of this study was relatively lower than the levels of AFM_1 in milk samples that observed in the same area. Fallah (2010b) showed that the mean concentration of AFM_1 in pasteurized and UHT milk in central part of Iran were 52.8 and 46.4 ng/l, respectively. In the mentioned study AFM_1 content of 26.7% and 17.4% of pasteurized and UHT milk showed to exceed EU regulations. Lower level of AFM_1 in yogurt might be attributed to factors such as low pH, formation of organic acids and other fermentation by-products (Govaris et al., 2002). It has been shown that lactic acid bacteria that ferment milk to yogurt are capable to remove AFM_1 from milk. El Khoury et al. (2011) reported that the yogurt bacteria, *L. bulgaricus*, *Streptococcus thermophilus* and a combination of these two bacteria reduced AFM_1 content of milk as 58.5%, 37.7% and 46.7%, respectively, after incubation in 37 °C for 6 h. So, it appears that fermentation process of milk could be a practical approach to reduce the risk of this toxin.

Conclusion

AFM_1 is a serious health threatening component that can be found in milk and dairy products. Our study offers a limited data collected in 2013 from Iran. However, the results showed that AFM_1 contamination of yogurt is relatively high and could be a serious health problem. So, it is essential to have continues monitoring program over milk and dairy products by governmental food inspection agencies. Reducing the levels of AFB_1 in animal feedstuffs by improved processing and storage practices can be initial approach to deal with this problem.

Conflicts of interest

None declared.

Acknowledgment

We thank the authorities of Shahid Sadoughi University of Medical Sciences, Yazd, Iran for financial assistance.

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