Occurence of aflatoxin M1 in two dairy products by ELISA in central part of Iran

Somaye Behnamipour,*Yalda Arast, Majid Mohammadian

1. Msc. of Analytical Chemistry, Research center of Environmental Pollutants, Qom University of Medical Sciences.
2. Instructor of Toxicology, Research center of Environmental Pollutants, Qom University of Medical Sciences.
3. *General Practitioner, Deputy Qom University of Medical Sciences, Qom, Iran*.

E-mail address: arast@gmx.com

Abstract: Aim: Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds that have been implicated as causative agent in human hepatic and extra hepatic carcinogenesis. The aim of this study was conducted to investigate the pasteurized milks and yoghurt contamination with M1 aflatoxin(AFM1) products of main factories that provide some Qom city dairy needs, in terms of contamination with this mycotoxin. **Materials and Methods:** 103 (75 pasteurized liquid milk, 28 yoghurt) sample produced by seven dairy factories were randomly selected during two cold (winter 2009) and warm (summer 2009) seasons and their AFM1 concentration was determined by a competitive Enzyme-Linked Immuno Sorbent Assay (ELISA) method. The main difference analyzed using Excel 2007 in software environment. **Results:** All of the examined samples were contaminated with AFM1 by measurable amounts. Mean of the M1 aflatoxin in whole pasteurized liquid milk samples was 22.44 ng/kg ranging from 8 to 64 ng/kg and in whole yoghurt samples mean of the M1 aflatoxin was 13.55 ng/kg ranging from 5 to 36 ng/kg . AFM1 contamination was higher than Iran National Standard (50ng/kg) only in 8.33% of the summer milk. **Conclusion: High** prevelance of AFM1 contamination in pasteurized milk and yoghurt samples is worrying and notifies the necessity of preventing measures to reduce entrance of B1 aflatoxin to dairy animal's feed and more controlling measures on milk distribution.

[Yalda Arast, Majid Mohammadian, Somaye Behnamipour. **Occurance of aflatoxin M1 in two dairy products by ELISA in central part of Iran**. *Life Sci J* 2012;9(3):1831-1833] (ISSN:1097-8135).
http://www.lifesciencesite.com. 265

Keywords: Aflatoxin M1, pasteurized milk, yoghurt, ELISA

1. Introduction

Aflatoxins are extremely toxic compounds produced by certain species of aspergillus, especially aspergillusflavus, A. parasiticus, and Anomius that contaminate plants and its products. A. flavus produces only B aflatoxins, while the others produce both B and G aflatoxins(1 Pei SC, Zhang YY, Eremin SA, Lee WJ. 2009).Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds that have been implicated as causative agent in human hepatic and extra hepatic carcinogenesis (Oveisi M-R.2007). Aflatoxin M1 (AFM1) is the monohydroxylated of AFB1 metabolized by cytochrome p450 enzyme system in liver and excreted into the milk of lactating livestock which consumed AFB1 contaminated diet (Murphy PA.et.al.2006). It could be appeared in milk whitin 12 h after the first ingestion of AFB1. Following the withdrawal of contaminated source, AFM1 disappeared within 72 h. there is a liner relationship between the AFM1 content in milk and the consumption of AFB1 via foodstuffs(Sassahara M.et.al.2005). It has been estimated that about 0.3-6.2% of AFB1 present in animal feed pass as AFM1 in milk(Creppy EE. 2002).Although the toxicity of AFM1 is less than AFB1, its cytotoxic, genotoxic and carcinogenic effects is well demonstrated. Hence the IARC of WHO initially categorized AFM1 as a group 2 human carcinogen(IARC.1993), but has transferred it to group 1 according to recent investigations(IARC.2002). As milk is the main nutrient for infants and children who are considered to be more susceptible to adverse effect of mycotoxins, the presence of AFM1 in milk is a concern. Infants usually use pasteurized milk more than adults (per kilogram of body weight). Infants in Iran usually consume pasteurized milk after breast weaning, up to three years of age as the main food, so the problem seems to be more important in this age group. On the other hand milk is not consumed as liquid milk, but also utilize for the preparation of infant formula, yoghurt, cheese, and milk based confectionaries including chocolate and pastry. Therefore, it is important to determine AFM1 levels in milk and dairy products in order to protect consumers in various age groups, from its potential hazard(Oveisi M-R.2007)2). The purpose of this study was to determine naturaloccurance and level of AFM1 in pasteurized liquid milk and yoghurt consumed in Qom, Iran.

2. Material and Methods

103 (75 pasteurized liquid milk, 28 yoghurt) samples produced by seven dairy factories were randomly selected during two cold (winter 2009) and warm (summer 2009) seasons from Qom, Iran.

2-1. Sample preparation:

Add to 10g yoghourt samples 100 ml of warm (20-25ºC) demonized water and shacked for 10 min with shaker in speed of 250min^{-1}. Subsequently, these samples as well as liquid milk samples were centrifuged at 3500 g for 10 min at 4ºC. Aflatoxins are water soluble (desponded 2), so the upper creamy layers were completely discarded and the lower phases were further diluted 20 times (v/v) with demonized water and then were used for the quantitiveteste.

2-2. Analysis of AFM1 in samples by competitive ELISA

The quantity of AFM1 was determined by RIDAscreenaflatoxin M1 test (R-Biopharm GmbH prepared from Rocket International Co. Ltd) which is a competitive enzyme immunoassay based on antigen-antibody reaction. The wells in the micro titer strips were coated with specific antibodies to AFM1. 100 µl of sample solution + 100 µl of standard 10ng/kg (standard addition method was used because of detection limit 5ng/kg) were added to the wells to occupy the binding sites proportionately, then mixed gently and incubated for 60 min at room temperature in the dark. Then the liquid samples were poured out of the wells and the wells were filled with 250 µl distilled water and poured out the liquid. Then other steps were done by the kit instruction and ultimately each well was washed for four times by washing buffer. At most after one hour, light absorption was read at 450 nm by ELIZA reader. The standard curve was used for determination related to the kit and the pasteurized milk and yoghourt samples by competitive ELISA. All of information after study analyzed by using Excel 2007 in software.

3. Results

The sample of pasteurized liquid milk (n= 75) and yoghourt (n=28) showed that the incidence of contamination with AFM1 is 100%, the presence of AFM1 in each group was 100%, ranging between 5-64ng/kg respectively. Iran national standard prescribe the limit of 50 ng/kg in milk and yoghourt.theoccurance and levels of AFM1 in milk and yoghourt samples are depicted in tables 1-4.

Table 1: Occurrence of AFM1 in milk and yoghourt samples in winter

Sample	n	Positive samples	AFM1 contamination	
			Range(ng/kg)	Mean±SD
Milk	39	39(100)	8-43	18/05±8/2
Yoghourt	15	15(100)	5-23	11/8±5/53

Table 2: Levels of AFM1 in winter milk and yoghourt samples.

Sampledistribution of samples n (%)					
	<5ng/kg	5-10ng/kg	10-25ng/kg	25-50ng/kg	>50ng/kg
Milk	0(0)	10(25.64)	4(26.4)	25(64.4)	0(0)
Yoghourt	0(0)	0(0)	9(60)	6(40)	0(0)

Table 3: Levels of AFM1 in summer milk and yoghourt samples

Sample	n	Positive samples	AFM1 contamination	
			Range(ng/kg)	Mean±SD
Milk	36	36(100)	9-64	26.83±14.99
Yoghourt	13	13(100)	5-36	15.3±8.38

Table 4: Occurrence of AFM1 in milk and yoghourt samples in summer

Sampledistribution of samples n (%)					
	<5ng/kg	5-10ng/kg	10-25ng/kg	25-50ng/kg	>50ng/kg
Milk	0(0)	1(2.8)	20(55.6)	12(33.3)	3(8.3)
Yoghourt	0(0)	3(23.0)	9(69.2)	1(7.7)	0(0)

4. Discussions

Iran national standard prescribe the limit of 50 ng/kg in milk and milk products(INA. 2001). There are differences in maximum permissible limit of AFM1 in various countries. For example European Communities and Codex Alimentations prescribe a limit of 50ng/kg(Commission E. 2001) and US regulation fixed the limit to a maximum of 500ng/kg for milk(Food U. Drug Administration, 1996). The occurrence and distribution of AFM1 concentration

obtained are presented in tables 1-4. Almost all of the samples were below the Iranian limit but the incidence of contamination even below standard limit is a serious problem for public health. Aflatoxicosis cause anemia, reduction of immune function, hepatotoxicosis, hemorrhage, teratogenesis, carcinogenesis and mutagenesis. The most prevalent symptoms of aflatoxicosis in animals are reduced growth rate and poor intellectual and behavioral performance. The liver is considered a target organ for the toxic and carcinogenic effects of aflatoxin(Kav K.et.al.2011). Milk and dairy products provide major nutrition's for human because many people especially children, frequently include them in their diets (Baskaya R. 2006).by consider to high toxicity and carcinogenic properties of AFM1, it's presence in milk is a concern. AFM1 is resistant to thermal inactivation, pasteurization. Autoclaving and other varieties of food processing procedures(Park DL. 2002). So to produce high quality milk, it is essential to keep feeds free from contamination by AFB1 (AFM1 mother molecol). The concentration of AFB1 in animal feed can be reduced by goob manufacturing practice and good storage practices. If preventive measure fails, however, AFB1 can be reduced in feed that has lower concentrations or by chemical, physical or biological treatment (Signorini M. 2011-Fallah AA.et.al.2009). The new approach to solve this Problem is the use of non-nutritionally inert adsorbents that can seqester the aflatoxins and reduce the absorption of these toxins from the gastro-intestinal tract(Oveisi M-R.2007). These finding show a potensial risk for consumers, especially in the absence of strict hygiene control. So finding new safe thecniqes for decontamination AFM1 from milk can be good alternatives for this problem .on the other hand, increasing the intake of antioxidants, and vitamins with the diet in order to prevent carcinogenesis should be involved in the prevention strategies.

Acknowledgements:
Authors are grateful to Qom University of Medical Sciences for financial support
to carry out this work.

Corresponding Author:
Yalda Arast
Instructor of Toxicology, Research center of Environmental Pollutants, Qom University of Medical Sciences.
Email: arast@muq.ac.ir

7/22/2012

References

1. Baskaya R. 2006. Aydın A, Yıldız A, Bostan K. Aflatoxin M1 levels of some cheese varieties in Turkey. Medycyna Weterynaryjna. 2006;62(7):778-80
2. Commission E. 2001.Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities L.;77:1-13.
3. Creppy EE. 2002.Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicology letters.;127(1-3):19-28.
4. Fallah AA, Jafari T, Fallah A, Rahnama M. 2009. Determination of aflatoxin M1 levels in Iranian white and cream cheese. Food and chemical toxicology;47(8):1872-5.
5. Food U. Drug Administration, 1996, Sec. 527.400 whole milk, low fat milk, skim milk-aflatoxin M1 (CPG 7106.210). FDA Compliance Policy Guides. 1996:219.
6. INA. 2001.Maximum validity Mycotoxins in human food. Tehran Institute of Standard and Industrial Research of Iran.
7. IARC.1993. Some naturally occurring substances: Food items and constituents hetrocyclic aromatic amines and mycotoxins. IARC monographs on the evaluation of carcinogenic risk to humans International Agency for Research on Cancer. p. 451-89.
8. IARC.2002. Some mycotoxins, naphthalene and styrene. IARC monographs on the evaluation of carcinogenic risk to humans: International Agency for Research on Cancer;. p. 171-300.
9. 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.
10. Murphy PA, Hendrich S, Landgren C, Bryant CM. 2006. Food mycotoxins: an update. Journal of food science.;71(5):R51-R65.
11. 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. [doi: 10.1016/j.foodcont.2006.07.021].;18(10):1216-8.
12. Park DL. 2002.Effect of processing on aflatoxin. Mycotoxins and Food Safety:173-9.
13. Pei SC, Zhang YY, Eremin SA, Lee WJ. 2009.Detection of aflatoxin M1 in milk products from China by ELISA using monoclonal antibodies. Food Control.;20(12):1080-5.
14. Sassahara M, Pontes Netto D, Yanaka E.2005. Aflatoxin occurrence in foodstuff supplied to dairy cattle and aflatoxin M1 in raw milk in the North of Parana state. Food and chemical toxicology. 2005;43(6):981-4.
15. Signorini M. 2011.Gaggiotti M, Molineri A, Chiericatti C, Zapata de Basílico M, Basílico J, et al. Exposure Assessment of Mycotoxins in Cow´ s Milk in Argentina. Food and chemical toxicology.