

## Aflatoxin M1 Contamination in Milk and Milk Products in Iran: A Review

R. Kazemi Darsanaki\*, M. Mohammad Doost Chakoosari, M. Azizollahi Aliabadi

Department of Microbiology, Faculty of Science, Lahijan Branch, Islamic Azad University, Lahijan, Iran

(Received: 15 June 2013

Accepted: 15 July 2013)

**Abstract:** Mycotoxins are secondary metabolites of molds and have adverse effects on humans, animals, and crops. Those can cause illnesses and economic losses. Aflatoxin M1 (AFM1) is one of the mycotoxins produced from the hydroxylated metabolite of aflatoxin B1 (AFB1). It can be found in milk or milk products obtained from livestock that have ingested contaminated feed. In this paper, recent studies were reviewed in aflatoxin M1 contamination in milk and milk products in Iran.

**Keywords:** Aflatoxin M1, Milk, Iran, Milk Products.

### INTRODUCTION

Mycotoxins are secondary metabolites of molds and have adverse effects on humans, animals, and crops that result in illnesses and economic losses [1]. AFM1 is a hepatocarcinogen found in milk of animals that have consumed feeds contaminated with AFB1, the main metabolite produced by fungi of the genus *Aspergillus* in particular *A. flavus*, *A. parasiticus* and *A. nomius* [2]. AFM1 remain stable after pasteurization, sterilization, preparation and storage of various dairy products [3]. In the assessment of cancer risk, the infants are more exposed to the risk because the milk is a major constituent of their diet. Therefore the presence of AFM1 in milk and milk products is undesirable and a particular risk for human [4]. The amount of AFM1 excreted as a percentage of AFB1 in feed is usually 1-3%. 12-24h after the first AFB1 ingestion, the toxin can be detected in the milk. When the intake of AFB1 is stopped, the AFM1 concentration in the milk decreases to an undetectable level after 72 hours [5]. The

contamination of milk and milk products with AFM1 display variations according to geography, country and season. The pollution level of AFM1 is differentiated further by hot and cold seasons, due to the fact that grass, pasture, weed, and rough feeds are found more commonly in spring and summer than in winter. At the end of summer, greens are consumed more than concentrated feed, causing a decreased level of AFM<sub>1</sub> in milk and milk products [5, 6]. Aflatoxins are highly toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic compounds. The main target organ for their toxicity and carcinogenicity is the liver. Milk and milk products are major nutrient for humans, especially children. For this reason, AFM<sub>1</sub> in milk and dairy products should be controlled systematically [5, 7]. In this paper, we reviewed recent studies in AFM<sub>1</sub> contamination in milk and milk products in Iran.

#### *Occurrence of AFM1 in Milk*

Milk is a key contributor to improving nutrition and food security particularly in developing

**Corresponding Author:** R. Kazemi Darsanaki, Department of Microbiology, Faculty of Science, Lahijan Branch, Islamic Azad University, Lahijan, Iran. Reza\_kazemi\_d@yahoo.com. Tel: +989366201665

technology offer significant promise in reducing poverty and malnutrition in the world. However, many of the previous studies have indicated the presence of AFM1 at high concentrations in dairy products. AFM1 distribution in milk is not

homogeneous. The European Commission (EC) has approved a maximum admissible level of 50ng/l for AFM1 in Milk [8]. Many researchers from Iran have carried out studies about the incidence of AFM1 in milk (Table 1.).

Table 1. AFM1 in milk samples

Year	Region	Technique	Sample	Positive samples	Reference
1999-2000	Hamedan	ELISA	186 Raw Milk	63.97%	[9]
2003	Shiraz	ELISA	624 Pasteurized Milk	100%	[10]
2004	Zanjan, Tehran, Mashhad, Lorestan, Khorasan, Khoozest, Kerman, Esfahan, Hamedan, Golpaygan, Golestan, Gilan, Fars, Az.Sharghi, Az. Gharbi	HPLC	319 Raw Milk	54%	[2]
2004-2005	Gonabad	ELISA	90 Pasteurized Milk	100%	[11]
2005	Babol	ELISA	111 Pasteurized and UHT Milk	100%	[12]
2005	Khoram Abad	HPLC	82 Raw and Pasteurized Milk	69.51%	[13]
2005	Chahar-Mahal-Bakhtiari	ELISA	86 Raw Milk	47.67%	[54]
2005-2006	Urmia	ELISA	72 Pasteurized Milk	100%	[14]
2006	Babol	ELISA	72 Pasteurized Milk	100%	[15]
2006	Mashhad	ELISA	110 Pasteurized Milk	100%	[16]
2006	Babol	ELISA	120 Raw milk	56.7%	[17]
2006	Sanandaj	ELISA	84 Pasteurized Milk	65.91%	[53]
2006-2007	Sanandaj	ELISA	257 Raw and Pasteurized Milk	94.48%	[18]
2007	Tehran	ELISA	42 Powdered Milk	100%	[19]
2007	Tehran	ELISA	328 Pasteurized and Infant Milk	96.3%	[20]
2008	Tabriz	ELISA	49 UHT Milk	100%	[21]
2008	Khorasan	ELISA	196 Milk	100%	[22]
2008-2009	Tehran	ELISA	50 Pasteurized Milk	84%	[23]
2009	Tehran, Esfahan, Shiraz, Yazd	TLC	91 Pasteurized Milk	72.5%	[24]
2009	Tabriz	ELISA	10 Ewe's Milk	30%	[25]
2009	Ahvaz	HPLC	100 Pasteurized Milk	100%	[26]
2009	Qom	ELISA	75 Pasteurized Milk	100%	[27]
2009	Central Part of Iran	ELISA	225 Pasteurized and UHT Milk	67.1%	[28]
2009	Esfahan	HPLC	74 Raw and Pasteurized Milk	85.14%	[29]
2009-2010	Qazvin	ELISA	2160 Raw Milk	100%	[49]
2009-2010	Sarab	TLC	100 Raw Milk	84%	[50]
2010	Ardebil	ELISA	122 Raw Milk	100%	[30]
2010	Tehran	ELISA	52 UHT milk	100%	[31]
2010	Kermanshah	ELISA	320 Raw Milk	100%	[32]
2010-2011	Ahvaz	ELISA	60 Pasteurized Milk	40%	[33]
2011	Urmia	ELISA	100 Raw Milk	100%	[34]
2011	Yazd	ELISA	149 Pasteurized and UHT Milk	95.3%	[35]
2011	Mashhad	ELISA	42 Milk Samples	97.6%	[36]
2011	Mazandaran	ELISA	75 Pasteurized Milk	94.66%	[37]
2011	Guilan	ELISA	90 Raw Milk	65.55%	[38]
2011	Mashhad	ELISA	60 Milk	100%	[39]
2011	Sari	HPLC	136 Breast Milk	0.7%	[55]
2011	Sari	ELISA	136 Breast Milk	3.7%	[55]
2011-2012	Ilam	ELISA	102 Raw and 42 Pasteurized Milk	43.75%	[51]
2012	Shush	ELISA	120 Raw Milk from cow and buffalo	69%	[56]

### Occurrence of AFM1 in Cheese

Occurrence of AFM1 in cheese can be due to three possible causes: (1) AFM1 present in raw milk because of carryovers of AFB1 from contaminated cow feed to milk, (2) Synthesis of AF (B1, B2, G1 and G2) by *A. flavus* and *A. parasiticus* growing on cheese and (3) Occurrence of these toxins in

dried milk used to enrich the milk which is being used in the production of cheese. The incidence of positive cheese samples for AFM1 (Table 2) seem to be widely variable. The European Commission (EC) has approved a maximum admissible level of 250ng/l for AFM1 in Cheese.

Table 2. AFM1 in Cheese samples

Year	Region	Technique	Sample	Positive samples	Reference
2008	Esfahan and Yazd	ELISA	210	76.6	[40]
2008	Hamedan	ELISA	118	70.7%	[52]
2009	Shahrekord and Esfahan	ELISA	70	64.3%	[41]
2009	Tehran, Esfahan, Shiraz, Yazd	TLC	72	81.9%	[24]
2010-2011	Ahvaz	ELISA	61	55.73%	[42]
2011-2012	Iran market	ELISA	80	86.3%	[43]
2011	Guilan	ELISA	90	86.66%	[5]
2012	Shahrekord	ELISA	40	40%	[3]
2012	Rafsanjan	ELISA	82	47.6%	[44]

### Occurrence of AFM1 in Yoghurt

Yoghurt has strong medicinal properties. Yoghurt is rich in potassium, calcium, protein and B vitamins, including B-12. Research shows yoghurt strengthens and stabilizes the immune system.

Many researchers from Iran have carried out studies about the incidence of AFM1 in Yoghurt (Table 3.). The European Commission (EC) has approved a maximum admissible level of 50ng/l for AFM1 in yoghurt.

Table 3. AFM1 in Yoghurt samples

Year	Region	Technique	Sample	Positive samples	Reference
2009	Mazandaran	ELISA	50	100%	[45]
2009	Qom	ELISA	28	100%	[27]
2009	Tehran, Esfahan, Shiraz, Yazd	TLC	68	66.1%	[24]
2011	Guilan	ELISA	60	98.33%	[46]
2011	Tehran	ELISA	100	70%	[57]
2011-2012	Iran market	ELISA	80	80%	[43]
2012	Guilan	HPLC	120	100%	[47]
2012	Shahrekord	ELISA	40	35%	[3]

### Occurrence of AFM1 in Other Milk Products

Many other milk products such as cream, butter, ice cream may contain AFM1. Some surveys conducted on the occurrence of AFM1 in milk

products are reported in Table 4. The European Commission (EC) has approved a maximum admissible level of 50ng/l for AFM1 in Doogh, Ice-cream and Butter.

Table 4. AFM1 in Other Milk Products

Year	Region	Technique	Sample	Positive samples	Reference
2009	Tehran, Esfahan, Shiraz, Yazd	TLC	36 Ice-cream	69.4%	[24]
2009	Tehran, Esfahan, Shiraz, Yazd	TLC	31 Butter	25.8	[24]
2010	Tehran	HPLC	225 Doogh	100%	[1]
2011	Guilan	ELISA	90 Ice-cream	68.88%	[48]
2011-2012	Iran market	ELISA	60 Ice-cream	56.7%	[43]
2012	Shahrekord	ELISA	40 Ice-cream	29%	[3]

## CONCLUSION

Dairy products play a significant role in human diet since they are rich sources of bioavailable calcium and proteins. The occurrence of AFM1 in milk and milk products is a public health concern because the International Agency for Research on Cancer has classified it in Group 2, a probable human carcinogen with a high risk of hepatotoxicity and mutagenicity. For this reason, milk and dairy products have to be inspected and controlled continuously for AFM1 contamination and animal feeds should be checked regularly for AFB1 and storage conditions of feeds must be taken under strict control.

## REFERENCES

1. Tabari M., Tabari K., Tabari O. Occurrence of Aflatoxin M<sub>1</sub> in Pasteurized Doogh Commercialized in Tehran, Iran. *Australian Journal of Basic and Applied Sciences*, 2011. 5(12): 1734-1737.
2. Tajkarimi M., Aliabadi-Sh F., Salah Nejad A., Poursoltani H., Motallebi A.A., Mahdavi H., Aflatoxin M<sub>1</sub> contamination in winter and summer milk in 14 states in Iran. *Food Control*, 2008.19: 1033-1036.
3. Nilchian Z., Rahimi E., Aflatoxin M<sub>1</sub> in Yoghurts, Cheese and Ice-Cream in Shahrekord-Iran. *World Applied Sciences Journal*, 2012. 19 (5): 621-624.
4. Khlangwiset P., Shephard G.S., Wu F., Aflatoxins and growth impairment: A review. *Critical Reviews in Toxicol*, 2011. 41: 1-16.
5. Azizollahi Aliabadi M., Issazadeh K., Kazemi Darsanaki R., Laleh Rokhi M., Amini A., Determination of Aflatoxin M<sub>1</sub> Levels in White Cheese Samples by ELISA in Gilan Province, Iran. *Global Veterinaria*, 2012. 8 (7): 707-710.
6. Celik T.H., Sarimehmetoglu B., Kuplulu O., Aflatoxin M<sub>1</sub> contamination in pasteurised milk. *Veterinarski Arhiv*, 2005.75: 57-65.
7. Akkaya L., Birdane Y.O., Oguz H., Cemek M., Occurrence of aflatoxin M<sub>1</sub> in yogurt samples from afyonkarahisar, Turkey. *Bull Vet Inst Pulawy*, 2006. 50: 517-519.
8. European Commission (EC), No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off J Eur Union*, 2006. 364; 5-24.
9. Ghiasian S.A., Maghsood A.H., Neyestani T.R., Mirhendi S.H., Occurrence of aflatoxin M<sub>1</sub> in raw milk during the summer and winter seasons in Hamedan, Iran. *Journal of Food Safety*, 2007. 27: 188-198.
10. Alborzi S., Pourabbas B., Rashidi M., Astaneh B., Aflatoxin M<sub>1</sub> contamination in pasteurized milk in Shiraz (south of Iran). *Food Control*, 2006.17(7): 582-584.
11. Mokhtarian Dalouei H., Mohsenzadeh M., Evaluation of Aflatoxin M<sub>1</sub> in Pasteurized Milk Gonabad city. *Ofoogh-E-Danesh*, 2005. 11(3): 5-9.
12. Gholampour Azizi I., Khoushnevis S.H., Hashemi S.J., Aflatoxin M<sub>1</sub> level in pasteurized and sterilized milk of Babol city. *Tehran University Medical Journal*, 2007. 65: 20-24.
13. Nazari A., Noroozi H., Movahedi M., Khaksarian M., Measurement of Aflatoxin M<sub>1</sub> in Raw and Pasteurized. *Yafte*, 2007. 9(3): 49-57.
14. Tajik H., Razavi Rohani S.M., Moradi M., Detection of Aflatoxin M<sub>1</sub> in Raw and Commercial Pasteurized Milk in Urmia, Iran. *Pakistan Journal of Biological Sciences*, 2007.10: 4103-4107.
15. Sefidgar S.A.A., Mirzae M., Assmar M., Naddaf S.R., Aflatoxin M<sub>1</sub> in Pasteurized Milk in Babol city, Mazandaran Province, Iran. *Iranian J Publ Health*, 2011. 40:115-118.
16. Karimi G., Hassanzadeh M., Teimuri M., Nazari F., Nili A., Aflatoxin M<sub>1</sub> Contamination in

- Pasteurized Milk in Mashhad, Iran. *Iranian Journal of Pharmaceutical Sciences*, 2007. 3(3): 153-156.
17. Sefidgar S.A., Azizi G., Khosravi A.R., Roudbar-Mohammadi S., Presence of Aflatoxin M1 in raw Milk at Cattle Farms in Babol, Iran. *Pakistan Journal of Biological Sciences*, 2008. 11(3):484-486.
18. Mohammadian B., Khezri M., Ghasemipour N., Mafakheri Sh., Poorghafour Langroudi P., Aflatoxin M1 contamination of raw and pasteurized milk produced in Sanandaj, Iran. *Archives of Razi Institute*, 2010. 65(2): 99-104.
19. Kamkar A., Detection of aflatoxin M1 in powdered milk samples by ELISA. *Pajouhesh & Sazandegi*, 2007. 79: 174-180.
20. Oveisi M.R., Jannat B., Sadeghi N., Hajimahmoodi M., Nikzad A., Presence of aflatoxin M1 in milk and infant milk products in Tehran, Iran. *Food Control*, 2007.18: 1216–1218.
21. Movassagh M.H., Presence of aflatoxin in UHT milk in Tabriz (Northwest of Iran). *Journal of Food Safety*, 2011. 31(2): 238–241.
22. Mohamadi Sani A., Nikpooyan H., Moshiri R., Aflatoxin M 1 contamination and antibiotic residue in milk in Khorasan province, Iran. *Food and Chemical Toxicology*, 2010. 48(8): 2130-2132.
23. Riazipour M., Tavakkoli H.R., Razzaghi Abyane M., Rafati H., Sadr Momtaz S.M., Measuring the amount of M1 Aflatoxin in pasteurized milks. *Kowsar Medical Journal*, 2010. 15(2): 89-93.
24. Fallah A.A., Aflatoxin M1 contamination in dairy products marketed in Iran during winter and summer. *Food Control*, 2010. 21: 1478-1481.
25. Movassagh M.H., Presence of Aflatoxin M1 Ewe's Milk in the Northwest Region of Iran. *Journal of Applied Biological Sciences*, 2009. 3(3):17-19.
26. Behfar A., Nazari Khorasgani Z., Alemzadeh Z., Gudarzi M., Ebrahimi R., Tarhani N., Determination of Aflatoxin M1 Levels in Produced Pasteurized Milk in Ahvaz City by Using HPLC. *Jundishapur J Nat Pharm Prod*, 2012. 7(2):80-84.
27. Behnamipour S., Arast Y., Mohammadian M., Occurrence of aflatoxin M1 in two dairy products by ELISA in central part of Iran. *Life Science Journal*, 2012. 9(3): 1831-1833.
28. Fallah A., Assessment of aflatoxin M1 contamination in pasteurized and UHT milk marketed in central part of Iran. *Food and Chemical Toxicology, Food Chem Toxicol*, 2010. 48(3): 988-991.
29. Daraei Garmakhany A., Zighamian H., Sarhangpour R., Rasti M., Aghajani N., Occurrence of Aflatoxin M1 in Raw and pasteurized milk in Esfahan province of Iran. *Minerva Biotechnologica*, 2011. 23: 53-7.
30. Kamkar A., Jahed Khaniki G.R., Alavi S.A., Occurrence of aflatoxin M1 in raw milk produced in Ardebil of Iran. *Iran. J. Environ. Health. Sci. Eng*, 2011. 8(2): 123-128.
31. Kamkar A., The study of aflatoxin M1 in UHT milk samples by ELISA. *J.Vet.Res*, 2008. 63(2): 7-12.
32. Sadeghi E., Almasi A., Bohloli-Oskoi S., Mohamadi M., The Evaluation of Aflatoxin M1Level in Collected Raw Milk for Pasteurized Dairy. *Zahedan J Res Med Sci*, 2013. 15(3): 26-29.
33. Rahimi E., Nilchian Z., Behzadnia A., Presence of Aflatoxin M1 in Pasteurized and UHT Milk Commercialized in Shiraz, Khuzestan and Yazd, Iran. *Journal of Chemical Health Risks*, 2011.1(1): 7-10.
34. Panahi P., Kasaei S., Mokhtari A., Sharifi A., Jangjou A., Assessment of Aflatoxin M1Contamination in Raw Milk by ELISA in Urmia, Iran. *American-Eurasian Journal of Toxicological Sciences*, 2011. 3(4): 231-233.

35. Rahimi E., Mohammadhosseini M., Alimoradi M., Rezaei P., Arab M., Goudarzi M.A., Tarkesh Esfahani M., Torki Z., Aflatoxin M1 in Pasteurized Milk and White Cheese in Ahvaz, Iran. *Global Veterinaria*, 2012. 9(4): 384-387.
36. Mohamadi Sani A., Khezri M., Moradnia H., Determination of AflatoxinM1 in Milk by ELISA Technique in Mashad (Northeast of Iran). *ISRN Toxicology*, 2012.1-4.
37. Gholi pour M., Karim zadeh L., Ali Nia F., Babaee Z., Determination of Aflatoxin M1 in Mazandaran Province at the First Half of 1390. *J Mazand Univ Med Sci*, 2012. 22(93): 40-46.
38. Laleh rokhi M., Kazemi Darsanaki R., Mohammadi M., Hassani Kolavani M., Issazadeh K., Azizollahi Aliabadi M., Determination of Aflatoxin M1 Levels in Raw Milk Samples in Gilan, Iran. *Advanced Studies in Biology*, 2013. 5: 151-156.
39. Mohamadi Sani A., Nikpoyan H., Determination of aflatoxin M1 in milk by high-performance liquid chromatography in Mashhad (north east of Iran). *Toxicol Ind Health*, 2013. 29(4): 334-338.
40. Fallah A.A., jafari T., Fallah A., Rahnama M., Determination of aflatoxin M1 levels in Iranian white and cream cheese. *Food and Chemical Toxicology*, 2009. 47: 1872-1875.
41. Rahimi E., Jafarian Dehkordi M., Iranpoor A., A Survey of Aflatoxin M1 Contamination in Iranian White Cheese. *Food Technology & Nutrition*, 2011. 8(4): 51-56.
42. Rahimi E., Mohammadhosseini M., Alimoradi M., Rezaei P., Arab M., Goudarzi M.A., Tarkesh Esfahani M., Torki Z., Aflatoxin M1 in Pasteurized Milk and White Cheese in Ahvaz, Iran. *Global Veterinaria*, 2012. 9(4): 384-387.
43. RahimiE., Survey of the occurrence of aflatoxin M1 in dairy products marketed in Iran. *Toxicol Ind Health*, 2012. doi: 10.1177/0748233712462476.
44. Akrami Mohajeri F., Ghalebi S.R., Rezaeian M., Gheisari H.R., Khorramdel Azad H., Zolfaghari A., Fallah A., Aflatoxin M1 contamination in white and Lighvan cheese marketed in Rafsanjan, Iran. *Food Control*, 2013. 33:525-527.
45. Barjesteh M.H., Gholampour I., Noshfar E., Occurrence of aflatoxin M1 in pasteurized and local yogurt in mazandaran province (Northern of Iran) using ELISA. *Global Veterinaria*, 2010. 4(5): 459-462.
46. Issazadeh K., kazemi Darsanaki R., Majid Khoshkholgh Pahlaviani M. R., Occurrence of aflatoxin M1 levels in local yogurt samples in Gilan Province, Iran. *Annals of Biological Research*, 2012. 3 (8): 3853-3855.
47. Tabari M., Tabari K., Tabari O., Aflatoxin M1 determination in yoghurt produced in Guilan province of Iran using immunoaffinity column and high-performance liquid chromatography. *Toxicol Ind Health*, 2013. 29(1):72-76.
48. Kazemi Darsanaki R., Azizollahi Aliabadi M., Mohammad Doost Chakoosari M., Aflatoxin M1 Contamination in Ice-Cream. *Journal of Chemical Health Risks*, 2013. 3(1): 43-46.
49. Khosravi A., Shokri H., Eshghi S., Darvishi S., Global occurrence of aflatoxin M1 in milk with particular reference to Iran, *Food Security*. 2013. 10.1007/s12571-013-0281-9.
50. Davoudi Y., Garedaghi Y., Survey on Contaminated Raw Milks with Aflatoxin M1 in the Sarab Region, Iran. *Research Journal of Biological Sciences*, 2011. 6: 89-91.
51. Vagef R., Mahmoudi R., Occurrence of Aflatoxin M1 in raw and pasteurized milk produced in west region of Iran (during summer and winter). *International Food Research Journal*, 2013. 20(3): 1421-1425.
52. Heshmati A., Occurrence of aflatoxin M1 in Iranian white cheese. *JFST*, 2010. 7(2):117-122.

53. Hazhir M., Sanoubar Tahaiee N., Rashidi K., Rezaie R., Shaykhi H., Determination of the amount of aflatoxin in milk samples delivered to Sanandaj pasteurized Milk Corporation. *SJKU*, 2008; 13(1): 44-50.
54. Rahimi E., Karim G., Determination of AFM1 in the Raw Milk Producing in Dairy Farms in Chahar-Mahal-Bakhtiari Province Using ELISA. *JFST*, 2008. 5(1): 51-58.
55. Afshar P., Shokrzadeh M., Kalhori S., Babae Z., Saeedi Saravi S.S., Occurrence of Ochratoxin A and Aflatoxin M1 in human breast milk in Sari, Iran. *Food Control*, 2013. 31: 525-529.
56. Kamkar A., Yazdankhah S., Mohammadi Nafchi A., Mozaffari Nejad A., Aflatoxin M<sub>1</sub> in raw cow and buffalo milk in Shush city of Iran. *Food Additives & Contaminants: Part B*, 2013. doi:10.1080/19393210.2013.830277.
57. Tavakoli HR., Riazipour M., Rafati Shaldehi H., Shahriari M., Naghavi S., Rahmati Najarkolaei F., AFM1 Contamination in used yogurt in one the of universities of medical sciences in Tehran by ELISA. *Quarterly of the Horizon of Medical Sciences*, 2013. 5S: 261-268

