

## Aflatoxin M<sub>1</sub> detection in milk in Mashhad-Iran by ELISA method

Malekinezhad Sara, Maleki Maryam, Mohamadi Sanim Ali\*

Department of Food Sci. & Tech., Quchan Branch, Islamic Azad University, Quchan, (IRAN)

E-mail : mohamadisani@yahoo.com

### ABSTRACT

The aim of this study was to evaluate aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) contamination in milk samples in Mashhad in Iran. A total of 61 milk samples were collected from retail stores in June 2013. The occurrence and concentration range of AFM<sub>1</sub> in the samples were investigated by ELISA technique. AFM<sub>1</sub> was found in 53 (86%) of the examined milk samples by average concentration of 118.6ng/L and the contamination level ranging between 0-250ng/L. The concentration of AFM<sub>1</sub> in 30 (49%) samples was lower than the Iranian national standard (100ng/L) but the mycotoxin level in all the samples was lower than Food and Drug Administration limit (500 ng/L), and only in 9 (14.7%) of the samples, the concentration of AFM<sub>1</sub> was lower than the maximum tolerance limit (50ng/L) accepted by European Union and Codex Alimentarius Commission. This situation must be considered as a food safety concern. © 2014 Trade Science Inc. - INDIA

### KEYWORDS

Aflatoxin M<sub>1</sub>;  
Milk;  
ELISA;  
Mashhad.

### INTRODUCTION

Mycotoxins are secondary metabolites of molds, which are associated with certain disorders in animals and humans. In addition to being acutely toxic, some mycotoxins are now linked with the incidence of certain types of cancer, and it is this aspect that has evoked global concern over feed and food safety, especially for milk and milk products<sup>[1]</sup>. Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) is a hepatocarcinogen found in the milk of the animals that have consumed feeds contaminated with aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), the main metabolite produced by the fungi of the genus *Aspergillus*, particularly *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*<sup>[2]</sup>. About 0.3–6.2% of AFB<sub>1</sub> in animal feed is transformed to AFM<sub>1</sub> in milk<sup>[3]</sup>. Since AFM<sub>1</sub> has been evaluated as a possible human carcinogen, the cancer risk arising from AFM<sub>1</sub> contamination in milk is a serious problem in food safety<sup>[4]</sup>.

The occurrence of AFM<sub>1</sub> in milk, especially cow's milk, makes it a particular risk factor for humans because of its importance as a foodstuff for adults and especially for children<sup>[5]</sup>. Due to serious health concerns, many countries have set maximum limits for aflatoxins, which vary from country to country<sup>[6]</sup>. The European Community prescribes that the maximum level of AFM<sub>1</sub> in liquid milk should not exceed 0.05 mg/kg. However, according to the US standard, the level of AFM<sub>1</sub> in liquid milk should not be higher than 0.5 mg/kg<sup>[7]</sup>. The objective of this study was to evaluate the occurrence of AFM<sub>1</sub> using ELISA method in milk distributed in Mashhad.

### MATERIALS AND METHODS

#### Materials

#### Samples

In this study, the levels of AFM<sub>1</sub> in raw milk samples

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in Mashad-Iran were determined in June 2013. A total of 61 milk samples (1000 mL milk sample) collected by simple random sampling method. The samples were transported to the laboratory in an insulated container at about 4\_°C and analyzed upon arrival.

### Reagents

Most of the reagents used to detect AFM<sub>1</sub> were contained in the RIDASCREEN test kit, which included microtiter plate coated with capture antibodies, AFM<sub>1</sub> standard solutions used for the construction of the calibration curve (1.3mL each 0, 5, 10, 20, 40, 80 and 200ppt), peroxidase conjugated AFM<sub>1</sub>, substrate (urea peroxidase), chromogen (tetramethylbenzidine), and stop reagent contains 1N sulphuric acid. Methanol used was of analytical grade and provided by Merck.

### Methods

#### AFM<sub>1</sub> detection

The quantitative analysis of AFM<sub>1</sub> in pasteurized milk samples was performed by competitive ELISA (RIDASCREEN AFM<sub>1</sub>, R-Biopharm) procedure as described by R-biopharm GmbH<sup>[8]</sup>. Prior to analysis of the samples, the ELISA method was validated to ensure data quality. Validation of ELISA was carried out by determination of recoveries and the mean variation coefficient for fresh milk spiked with different concentrations of AFM<sub>1</sub> (5, 10, 20, 40, 80 and 200ppt). The results are expressed in TABLE 1.

Milk samples were centrifuged at 3500 g for 10 min at 10°C. The upper creamy layer was completely removed by aspirating through a Pasteur pipette and from the lower phase (defatted phase) 100 μL was directly used per well in the test. One hundred μL of the AFM<sub>1</sub> standard solutions (100 μL/well) and test samples (100 μL/well) in duplicate were added to the wells of microtiter plate and incubated for 60min at room temperature in the dark. After the washing steps, 100 μL of the enzyme conjugate was added and incubated for 60min at room temperature in the dark. The washing step was repeated three times. Fifty μL of substrate and 50 μL of chromogen were added to each well and mixed thoroughly and incubated for 30min in the dark.

Following the addition of 100 μL of the stop reagent to each well, the absorbance was measured at 450 nm in ELISA reader (ELX-800, Bio-Tek Instruments, USA). According to the RIDASCREEN kit

guidelines, the lower detection limit is 5 ppt for milk.

### Evaluation of AFM<sub>1</sub>

The absorbance values obtained for the standards and the samples were divided by the absorbance value of the first standard (zero standards) and multiplied by 100 (percentage maximum absorbance). Therefore, the zero standard is thus made equal to 100%, and the absorbance values are quoted in percentages. The values calculated for the standards were entered in a system of coordinates on semilogarithmic graph paper against the AFM<sub>1</sub> concentration in ppt.

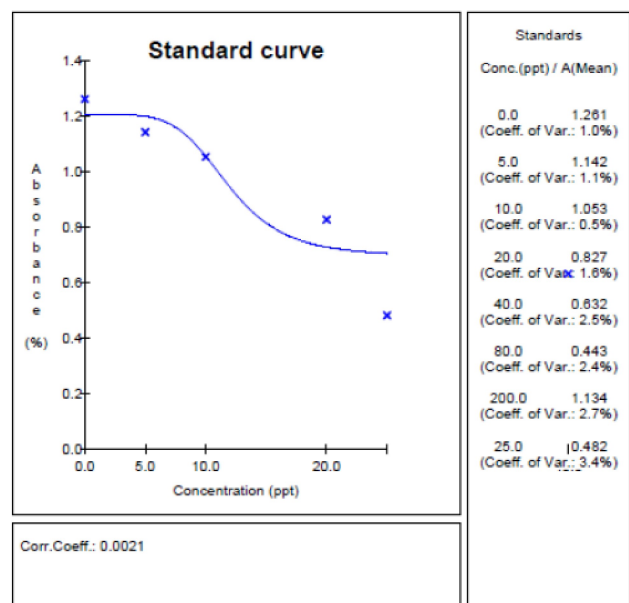
### Statistical analysis

The results were analyzed by Excel 2007 software and results presented as mean±SD.

## RESULTS AND DISCUSSION

The standard solutions of concentrations of 0, 5, 10, 20, 40, 80 and 200ppt AFM<sub>1</sub> were used to find calibration/standard curve. Figure 1 gives the calibration curve of standard solutions of AFM<sub>1</sub> with concentrations of AFM<sub>1</sub> by ELISA analysis.

Analytical results showed that the incidence of



**Figure 1 : Calibration curve of standard solutions of AFM<sub>1</sub> with concentrations of 0, 5, 10, 20, 40, 80 and 200ppt by ELISA method**

AFM<sub>1</sub> contamination in raw milk samples was relatively high. AFM<sub>1</sub> was found in 53 (86%) of the examined milk samples by average concentration of 118.6ng/L

and the contamination level ranging between 0 and 250ng/L. The concentration of AFM<sub>1</sub> in 30 (49%) samples was lower than the Iranian national standard (100ng/L) but the mycotoxin level in all the samples was lower than Food and Drug Administration limit (500 ng/L), and only in 9 (14.7%) of the samples, the concentration of AFM<sub>1</sub> was greater than the maximum tolerance limit (50ng/L) accepted by European Union and Codex Alimentarius Commission.

The mean concentrations of AFM<sub>1</sub> in milk from European, Latin American and Far Eastern diets have been reported by the Joint FAO/WHO Expert Committee on Food Additives<sup>[9]</sup>, to be 23, 22 and 360 ng/L, respectively. Thus, the observed mean concentration of AFM<sub>1</sub> in Mashad milk samples was higher than the European and Latin American and lower than those reported in the Far Eastern diets. Also according to TABLE 1, the incidence of AFM<sub>1</sub> observed in the present study was higher than the incidence of AFM<sub>1</sub> reported by other authors<sup>[10-18]</sup>. The variations may be attributed to the differences in the regions, the shape of animal feeds<sup>[19,20]</sup>, season and 4 special analytical methods. We suggest more researches on determination of this mycotoxin in dairy products to have a strict situation on the contamination.

**TABLE 1 : The incidence of milk contamination in other studies**

Location	Sample size	Percent of contamination	Percent of Contamination >50ng/L
Brazil (Sao Paulo)	125	95.2	26.4
India (Lucknow)	87	87.3	99
Morocco (Rabat)	54	88.8	7.4
Pakistan (Punjab)	168	100	99.4
Syria	126	80	52
Turkey (Anatolia)	129	58.1	47
Iran (Ahwaz)	311	42.1	12.5
Iran (Sarab)	111	76.6	40
Iran (Shiraz)	624	100	17.8
Iran (Tehran)	210	55.2	33.3
Average	194.5	78.33	43.48

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