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Aflatoxin M1 Contamination in Ice-Cream

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

Department of Microbiology, Faculty of Science, Lahijan Branch, Islamic Azad University, Lahijan, Iran
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Abstract: Aflatoxin M1 (AFM1) is the hydroxylated metabolite of aflatoxin B1 (AFB1) that it can be found in milk and dairy products. In this study, ELISA (Enzyme Linked Immunosorbent Assay) technique was used for detection of AFM1 in ice-cream in Guilan province (Northern Iran). A total of 90 ice-cream samples was randomly obtained from different supermarkets. In 62 of the 90 ice-cream samples examined (68.88%), the presence of AFM1 was detected in concentrations between 8.4 -147.7 ng/l. The mean level of AFM1 in positive samples was 40.36 ng/l. AFM1 levels in 11 samples (12.22%) were higher than the maximum tolerance limit (50 ng/l) accepted by ISIRI, European Community and Codex Alimentarius.

Keywords*: Aflatoxin* M1, ELISA, Ice-Cream

INTRODUCTION

Aflatoxins are toxic metabolites produced by fungi, e.g., *Aspergillus flavus* and *Aspergillus parasiticus*, growing on cereals, nuts, legumes, fruits and other susceptible crops. The presence of mycotoxins in food and feed depends on many biological factors, such as region, season, humidity and temperature, as well as the conditions under which crops are harvested, stored and processed. When not controlled, these toxins can be transferred to animals and humans through the ingestion of contaminated feed and food [1]. Aflatoxin M1 is the hydroxlated metabolites of aflatoxin B1 found in milk and milk products obtained from livestock that have ingested contaminated feed [2]. Milk and milk products are a good source of many nutrients such as proteins and calcium and are mainly consumed by children [3]. AFM1 is not destroyed during the pasteurization process or in yoghurt and cheese making. AFM1 could be detected in milk 12-24 h after the AFB1 ingestion, reaching a high level after a few days. When AFB1 intake is stopped, the AFM1 concentration in milk decreases to an undetectable level after 72 h [4]. Thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and enzyme linked immunosorbent assay (ELISA) are the most common techniques for detecting AFM1 in milk and dairy products [5]. Many countries have established regulations to control the levels of AFB1in feeds and to have maximum permissible levels of AFM1 in milk to reduce this hazard. The European Community and Codex Alimentarius Commission prescribed that the maximum level of AFM1 in milk and milk products should not exceed 50 ng/L [6, 7]. Therefore, the aim of this study was to investigate the presence of AFM in ice-cream samples consumed in Guilan province (Northern Iran) by ELISA method.

Corresponding Author: R. Kazemi Darsanaki, Young Researchers Club, Lahijan Branch, Islamic Azad University, Lahijan, Iran. Reza_kazemi_d@yahoo.com. Tel: +989366201665

MATERIALS AND METHODS

Preparation of samples

A total of 90 ice-cream (industrial) samples was collected randomly during, 2011 from different supermarkets in Guilan province (Northern Iran). The samples were stored in a refrigerator (-20 centigrade) and were centrifuged at 2-8 centigrade for 10 minutes at 3500g and fat was completely separated from them.

ELISA Test Procedure

The analysis of AFM1 was performed using Ridascreen aflatoxin M1 kit (R- Bipharm, Germany). Before starting the test, the reagents were brought up to room temperature. The AFM1 standards and test samples (100 µl per well) in duplicate were added to the wells of a micro-titer plate pre-coated with antibodies for AFM1 and incubated at room temperature in dark for 60 min. After the washing step, 100µl of peroxidase conjugate was added to the wells and plate was incubated again for 60 min at room temperature in dark. After the washing step, the unbound conjugate was removed during washing. Subsequently, 50 µl each substrate (urea peroxide) and chromogen (tetramethyl-benzidine) were added to the wells and incubated for 30 min in dark. Finally, 100 µl of stop solution were added to each well. The optical absorbance of each well was read at 450 nm with ELISA plate reader. Absorbance percentages were taken to the calibration curve performed with standards at different concentrations [8, 9]. Statistical analyses were performed using SPSS software.

RESULTS AND DISCUSSION

The distribution and evaluation of AFM1 levels in ice-cream samples are given in Tables 1. A total of 90 ice-cream samples was analyzed with competitive ELISA. Of the 90 samples analyzed, 62 samples (68.88%) were found to be contaminated with AFM1. 11 samples (12.22%) failed to reach the desired level of the European Communities and Codex, defined as 50ng/l. The aflatoxin M1 contamination levels were between 8.4 -147.7 ng/l. with the mean of 40.36 ng/l.

Table 1. Occurrence of AFM1 in ice-cream samples from Northern Iran

AFM1 levels ng/l	Sample No.	%	Average	Range
Not detected	28	31.11%	-	-
< 10	2	2.22%	8.85	8.4-9.3
11-25	17	18.88%	17.17	10.2-24.8
26-50	32	35.55%	38.16	25.4-49.5
≥ 50	11	12.22%	96.53	55.4-147.7
Total Sample	90	68.88%	40.36	8.4-147.7

Several surveys were performed in order to determine the AFM1 levels in ice-cream. Atanda and his colleagues [10] reported AFM1contamination in human and cow milk and ice cream in Nigeria range of 2.04-4.00 µg/l. High recorded scores of 4.02.04 and 2.23 µg/l, respectively. In study Gholampour Azizi and his colleagues [9]. In general, of 45 ice cream samples, 10(22.2%) were positive with above the limit of European community regulations (50 ng/l). Maximum concentration of AFM1 was 103 ng/l, minimum was 1.2 ng/l and mean was 33.98 ng/l. In Iran, a study done by Moktabi and his colleagues [11], 24 ice cream samples (30%) were over than 50 ng/l with the mean of 52.79 ng/l.

Cadirci and his colleagues [12], detected AFM1 contamination in 30 (26.08%) of 115 ice cream samples. In another study 36 ice cream samples were analyzed by Fallah [13], AFM1 was found in 69.4% of the samples (mean: 0.041 µg/kg: range: 0.015-0.132 µg/kg). Nilchian and Rahimi [14], analyzed 40 ice cream samples and in 29%, AFM1

was detected in concentrations between 20.1-197.4 ng/l. In study, Rahimi [15], 60 ice cream samples were analyzed for AFM1, 56.7% were contaminated with AFM1 (ranging 14.9-147.4 ng/l). In our study, of the 90 samples analyzed, 62 samples (68.88%) were found to be contaminated with AFM1. levels in 11 samples (12.22%) were higher than the maximum tolerance limit (50 ng/l) accepted by ISIRI and of European community regulations.

CONCLUSION

Dairy products play a significant role in human diet since they are rich sources of bioavailable calcium and proteins. However, many of previous studies have indicated the presence of AFM1 at high concentrations in dairy products. 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 a major nutrient for humans, especially children. According to results obtained, incidence and contamination levels of AFM1, seem to be a serious problem for public health. 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.

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