Journal of
Advances in Environmental Health Research

Summer 2022. Volume 10. Number 3

Research Paper

Aflatoxin M1 Determination in Ice Cream Based on Immunoaffinity Column Clean-up Followed by HPLC-FLD

Soroush Rasolipanah[1], Halaleh Rasolipanah[2], Sirwan Mohammadiazar[3*]

1. Department of Veterinary, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
2. Department of Chemistry, Faculty of Basic Sciences, Tehran South Branch, Islamic Azad University, Tehran, Iran.
3. Department of Chemistry, Faculty of Basic Sciences, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.

Use your device to scan and read the article online

Citation Rasolipanah S, Rasolipanah H, Mohammadiazar S. Aflatoxin M1 Determination in Ice Cream Based on Immunoaffinity Column Clean-up Followed by HPLC-FLD. Journal of Advances in Environmental Health Research. 2022; 10(3):247-252. http://dx.doi.org/10.32598/JAEHR.10.3.1264

http://dx.doi.org/10.32598/JAEHR.10.3.1264

Article info:
Received: 29 Dec 2021
Accepted: 03 Apr 2022
Publish: 01 Jul 2022

Keywords:
Aflatoxin M1, Ice cream, Immunoaffinity

ABSTRACT

Background: Aflatoxin M1 (AFM1) in industrial ice cream was analyzed by immunoaffinity column (IAC) clean-up followed by high-performance liquid chromatography (HPLC) with a fluorescence detector (FLD) as a highly-sensitive method to confirm and quantify AFM1 in the ice cream samples.

Methods: A total of 150 industrial ice cream samples were randomly collected from supermarkets in seven Iranian cities (Tehran, Tabriz, Karaj, Urmia, Sanandaj, Qazvin, and Khoramabad).

Results: AFM1 was detected in more than 80.0% of samples. The average AFM1 concentration in ice cream was 29.79 ng/L. According to the results, in 12.62% of samples, AFM1 levels exceeded the maximum European Union (EU) limits for adults (50 ng/mL). This study recommends regular monitoring of AFM1 concentration in ice cream in Iran.

Conclusion: Results of this study indicate the need to develop a database to assist law enforcement agencies, traders, and policymakers in local governments to minimize or prevent health-related risks caused by AFM1.

*** Corresponding Author:**
Sirwan Mohammadiazar, Assistant Professor.
Address: *Department of Chemistry, Faculty of Basic Sciences, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.*
Phone: *+98 (87) 33288677*
E-mail: *sirwan.mohammadi@yahoo.com*

1. Introduction

Aflatoxins (AFs) are a group of toxic secondary metabolites produced by fungi, including teratogenic, mutagenic, and carcinogenic compounds. They are mainly produced by *Aspergillus parasiticus* and *Aspergillus flavus* [1-6]. *A. parasiticus* produces AFs of G and B types, and *A. flavus* produces only AFs of B type. AFM1 and M2 are the oxidative metabolites of AFB1 and B2 found in milk products or milk from livestock consuming contaminated feed [7]. It is estimated that nearly 1% to 3% of AFB1 appear as AFM1 in milk. However, this transmission rate varies from one milking process to another, animal to animal, and from day to day [8]. Unfortunately, AFM1 is moderately stable during the storage and processing of different dairy products and it is indestructible during pasteurization and sterilization [9]. Cow's milk is one of the main ingredients of ice cream and is added in powder and liquid form. Therefore, AFM1 residues in milk can be transferred into the final product. To protect public health, the European Union (EU) established the highest acceptable levels of AFM1 in dairy products and milk, i.e. 50 ng/L and 25 ng/L for adults and infants, respectively [10]. In addition, the International Agency for Research on Cancer (IARC) classified AFM1 as a possible human carcinogen (class 2B) [11]; therefore, It is essential to identify and measure AFM1 in food products [12].

Different methods are proposed to determine mycotoxins in dairy products, including the enzyme-linked immunosorbent assay (ELISA) [13], thin-layer chromatography (TLC) [14], and quantification by high-performance liquid chromatography (HPLC) [15], following sample preparation. Among these methods, HPLC with fluorescence detection (FLD) is one of the crucial techniques for determining AFM1. Pournormohammadi et al. [15] reported the determination of AFM1 in milk in Kerman Province, Iran, using HPLC-FLD. Lee and Lee [16] used HPLC-FLD to determine AFM1 and AFM2 in commercial dairy products (ice cream, yogurt, milk, milk powder, and juice). Chavarría et al. [17] determined AFM1 in sour cream, cheese, and milk samples from Costa Rica using HPLC-FLD. Tajkarimi et al. [18] used HPLC-FLD in a seasonal study of AFM1 contamination in milk in 5 regions in Iran. The same research group studied AFM1 contamination in summer and winter milk in 14 provinces of Iran using HPLC-FLD [19].

Little information is available on the existence of AFM1 in dairy products in Iran. To fill this gap, this study aimed to develop a highly sensitive method to confirm and quantify AFM1 in ice cream samples consumed in Iran. As far as we know, no previous study has investigated the immunoaffinity column (IAC) clean-up method to extract AFM1 from industrial ice cream samples consumed in Iran. IAC leads to effective enrichment and purification of AFM1 before chromatographic analysis. The results of this study can help create a database to assist law enforcement agencies, traders, and policymakers in local governments to minimize or prevent health-related risks caused by AFM1.

2. Materials and Methods

Chemicals

The standard AFM1 (10 mg/L) was purchased from Sigma-Aldrich and Millipore filtered water was used. Acetonitrile and methanol were of HPLC grade (Darmstadt, Germany). A standard curve was constructed by diluting the AFM1 standard with acetonitrile. Other chemicals were at least of analytical reagent grade.

Study sample preparation

A total of 150 (industrial) ice cream samples were randomly collected from supermarkets in seven cities (Tehran, Tabriz, Karaj, Urmia, Sanandaj, Qazvin, and Khoramabad) in Iran during spring 2018 (Table 1). Samples were transferred to the laboratory in ice-packed coolers and stored at -20°C until analysis.

Study tool

An HPLC (Unicam, -Crystal- 200 [UK]) system equipped with FLD (series 200) with λ_{max} excitation and emission at 362 nm and 435 nm, respectively, was used. The mobile phase was a mixture of acetonitrile, methanol, and water (60:23:17 v/v/v) with a flow rate of 1.0 mL/min isocratically. A TSK-gel Super-ODS column (250 mm×4.6 mm and 3 µm) was used at 30°C. The injection volume was 20 µL.

Extraction and clean-up

A total of 40 mL of dichloromethane was added to 5 g of the sample in a conical tube and the mixture was stirred for 15 min. The resulting suspension was filtered using a 45-µm syringe filter and an aliquot of the extract (10 mL) was evaporated at 60°C. Then, the residue was diluted with 0.5 mL of phosphate buffer (pH 7.2), 0.5

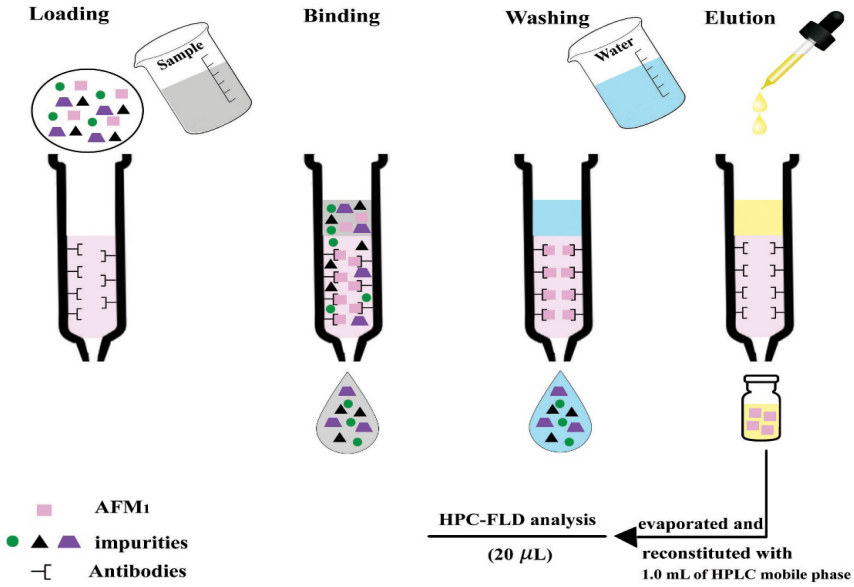

Figure 1. Illustration of the procedure for IAC-HPLC-FLD

mL of methanol, and 1 mL of heptane. The mixture was then centrifuged at 10°C (2700 rpm for 10 minutes). The supernatant (heptane layer) was completely removed and then 100 µL of the sub-phase (methanol layer) was diluted with 400 µL of phosphate buffer. For IAC, 20 mL of phosphate-buffered saline (PBS) passed through the immunoaffinity column. Then, 20 mL of milk passed through the column at about 1 to 2 drops per minute. The column was washed with 10 mL of water at the same flow rate. The AFM1 was eluted with 1.0 mL of acetonitrile at a flow rate of 2 to 3 drops per minute and collected in a 4-mL glass vial. The acetonitrile was evaporated at 40°C, and the residue was dissolved in 1 mL of the mobile phase. Finally, an aliquot of 20 µL was injected directly into the HPLC system (Figure 1).

Study statistical data analysis

SPSS software version 24 was used for the statistical analysis of data. Mean AFM1 concentration between different cities was compared using 1-way analysis of variance (ANOVA) tests.

3. Results and Discussion

Table 2 presents the levels of AFM1 contamination in ice cream. Based on the results of the IAC-HPLC-FLD analysis, AFM1 was detected in more than 80.0% of samples which ranged between 0 ng/L and 87.8 ng/L. The average AFM1 in ice cream was 29.79 ng/L and 12.62% of samples exceeded the maximum EU limits for adults (50 ng/mL). Also, the average AFM1 level exceeded the EU

Table 1. Ice Cream samples analyzed in this study

Cities	Number of Samples
Tehran	20
Tabriz	45
Karaj	15
Urmia	20
Sanandaj	25
Qazvin	10
Khoramabad	15
Total	150

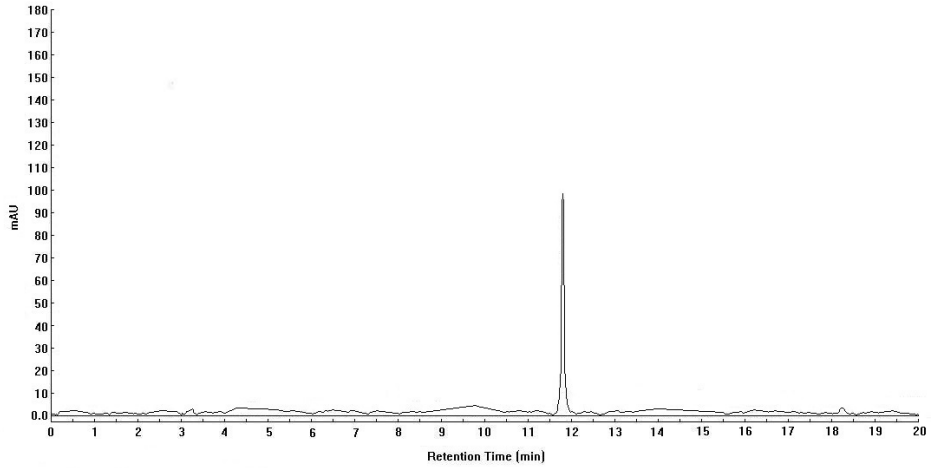

Figure 2. Typical chromatogram of a real sample after IAC-HPLC-UV

limit for infants (25 ng/L) in all samples except in Tabriz City. According to Table 2, Karaj City and Qazvin City, Iran showed higher toxin levels than other cities and the lowest mean level of AFM1 was in the samples of Tabriz (10.52±1.83 ng/L). Figure 2 shows the chromatogram of the real sample after IAC-HPLC-UV.

One-way analysis of variance (ANOVA) test (statistical analysis) showed no significant difference between ice cream samples in Qazvin and Karaj (P>0.05) (Table 2). In addition, no significant difference was observed in the mean AFM1 concentration in ice cream samples marketed in Tehran, Sanandaj, Urmia, and Khoramabad Cities, Iran (P>0.05). However, average AFM1 levels in ice cream samples marketed in Tabriz were significantly different from samples from other cities.

Table 2. Aflatoxin M1 (AFM1) levels in ice cream samples marketed in seven Iranian cities

Samples	Number of Samples	Mean±SD (ng/mL)	Min (ng/mL)	Max (ng/mL)	>EU Limit, No. (%) (50 ng/mL)
Karaj	15	53.16±4.06	32.40	87.80	46.66
Tehran	20	29.16±1.72	16.60	43.50	0
Urmia	20	36.55±2.12	12.10	53.60	5
Sanandaj	25	31.54±3.83	0.00	59.10	20
Qazvin	10	52.21±5.59	31.50	75.60	50
Khoramabad	15	38.21±2.45	23.40	54.60	6.66
Tabriz	45	10.52±1.83	0.00	31.40	0
Total	150	29.79±1.60	0.00	87.80	12.66

Table 3. Previous studies of AFM1 determination in ice cream samples in Iran

Locations	Techniques	AFM1 Average Concentration (ng/L)	Matrix	Reference
Shiraz, Iran	ELISA	26.88	Ice cream	20
Isfahan, Iran	ELISA	65.1	Ice cream	11
Gilan, Iran	ELISA	40.36	Ice cream	21
Four large Iranian cities	TLC	41.0	Ice cream	9

ELISA: enzyme-linked immunosorbent assay; TLC: thin-layer chromatography

The main reasons for different levels of AFM1 contamination in ice cream in different cities can be ascribed to the various levels of AFB1 contamination in livestock diets and geographical areas. Table 3 presents the recent studies of AFM1 quantification in ice cream samples in different cities of Iran. In a recent study conducted in Shiraz City, Iran, Abdali et al. reported the detection of AFM1 in ice cream based on the ELISA method [20]. The level of AFM1 in all samples ranged from 0.3 ng/mL to 71.1 ng/mL. The mean AFM1 level was 26.88 ng/mL and 22.72% of samples exceeded the maximum EU maximum limits for infants. In previous studies conducted in Isfahan City, Iran, Rahimi used the ELISA technique to determine AFM1 in ice cream. Results showed that 56.7% of 34 ice cream samples were contaminated with AFM1 in various concentrations ranging from 14.9 ng/L to 147.4 ng/L with a mean and SD value of 65.1±31.4 ng/L [11]. Darsanaki et al. used ELISA to analyze AFM1 in ice cream in Gilan City, Iran [21]. In 68.9% of ice cream samples, the concentration level of AFM1 ranged between 8.4 ng/L and 147.7 ng/L. Mean AFM1 levels in positive samples were 40.36 ng/L, and in 11 samples, the AFM1 levels exceeded the maximum EU limits for adults. Fallah used TLC to report the AFM1 contamination of ice cream samples in four large Iranian cities [9]. AFM1 was detected in 25 ice cream samples (69.4%) with a mean level of 0.041 ng/L.

AFM1 in ice cream is a serious hazard for children who are more sensitive to the side effects of AFs than adults. Therefore, due to the adverse effects of AFs on human health even in low concentrations, continuous AFM1 monitoring in milk used for ice cream and AFB1 monitoring in livestock diet is essential.

4. Conclusion

The IAC-HPLC-FID technique was successfully used to determine AFM1 in ice cream samples. Results of this study showed that the ice cream market in some Iranian cities can be a serious hazard to public health, particularly to children and infants. Therefore, continuous monitoring of AFM1 in milk used for ice cream has a great importance. It is also essential to address this problem by reducing the AFB1 levels in livestock diet by improving storage and processing methods. Furthermore, banning the consumption of ice cream containing a high concentration of AFM1 is essential to prevent its adverse effects on human health.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Conceptualization and Supervision: Sirwan Mohammadiazar; Methodology: Soroush Rasolipanah; Investigation, Writing–original draft, and Writing–review & editing: All authors; Data collection: Halaleh Rasolipanah; Data analysis: Soroush Rasolipanah; Funding acquisition and Resources: Soroush Rasolipanah, Halaleh Rasolipanah.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors would like to thank Dr. Mehdi Maham, for his assistance in editing the manuscript.

References

[1] Gao L, Sun Y, He L, Zhao W, Xiang G, Jiang X, et al. A polyhedral oligomeric silsesquioxanes/dual ligands-based magnetic adsorbent for effective extraction of aflatoxins in cereals via multiple interactions. Microchem J. 2021; 160:105626. [DOI:10.1016/j.microc.2020.105626]

[2] Pellicer-Castell E, Belenguer-Sapiña C, Amorós P, Herrero-Martínez JM, Mauri-Aucejo AR. Bimodal porous silica nanomaterials as sorbents for an efficient and inexpensive determination of aflatoxin M1 in milk and dairy products. Food Chem. 2020; 333:127421. [DOI:10.1016/j.foodchem.2020.127421] [PMID]

[3] Miklós G, Angeli C, Ambrus Á, Nagy A, Kardos V, Zentai A, et al. Detection of aflatoxins in different matrices and food-chain positions. Front Microbiol. 2020; 11:1916. [DOI:10.3389/fmicb.2020.01916] [PMID] [PMCID]

[4] Karami-Osboo R, Maham M, Nasrollahzadeh M. Rapid and sensitive extraction of aflatoxins by Fe_3O_4/zeolite nanocomposite adsorbent in rice samples. Microchem J. 2020; 158:105206. [DOI:10.1016/j.microc.2020.105206]

[5] Altunay N, Katin KP, Gürsoy N, Elik A, Şimşek S, Kaya S. Spectrophotometric determination of aflatoxin B1 in food sample: Chemometric optimization and theoretical supports for reaction mechanisms and binding regions. J Food Compost Anal. 2020; 94:103646. [DOI:10.1016/j.jfca.2020.103646]

[6] Manoochehri M, Asgharinezhad AA, Safaei M. Determination of aflatoxin M1 in milk powder by ultrasonic-assisted extraction and dispersive solid-phase clean-up. J Chromatogr Sci. 2015; 53(6):1000-6. [DOI:10.1093/chromsci/bmu131] [PMID]

[7] Ossa DEH, Hincapié DA, Peñuela GA. Determination of aflatoxin M1 in ice cream samples using immunoaffinity columns and ultra-high performance liquid chromatography coupled to tandem mass spectrometry. Food Control. 2015; 56:34-40. [DOI:10.1016/j.foodcont.2015.03.011]

[8] Atanda O, Oguntubo A, Adejumo O, Ikeorah J, Akpan I. Aflatoxin M1 contamination of milk and ice cream in Abeokuta and Odeda local governments of Ogun State, Nigeria. Chemosphere. 2007; 68(8):1455-8. [DOI:10.1016/j.chemosphere.2007.03.038] [PMID]

[9] Fallah AA. Aflatoxin M1 contamination in dairy products marketed in Iran during winter and summer. Food control. 2010; 21(11):1478-81. [DOI:10.1016/j.foodcont.2010.04.017]

[10] Iqbal SZ, Asi MR, Jinap S. Variation of aflatoxin M1 contamination in milk and milk products collected during winter and summer seasons. Food Control. 2013; 34(2):714-8. [DOI:10.1016/j.foodcont.2013.06.009]

[11] Rahimi E. Survey of the occurrence of aflatoxin M1 in dairy products marketed in Iran. Toxicol Ind Health. 2014; 30(8):750-4. [DOI:10.1177/0748233712462476] [PMID]

[12] Shokri R, Almasi A, Rabihavi J, Ganjali Dashti S, Hajiveisi H, Valipour AA, et al. The assessment of environmental health status in the route of Arbaeen pilgrims at Shalamcheh border in southwestern Iran. J Adv Environ Health Res. 2020; 8(2):133-42. [DOI:10.22102/JAEHR.2020.222976.1163]

[13] Kohzadi S, Loqmani H, Reshadmanesh N, Babaei E, Nadimi H, Salehzadeh H, et al. Aflatoxin M1 levels in the raw milk produced by a dairy factory and the milk distribution centers in Sanandaj, Iran (2015). J Adv Environ Health Res. 2019; 7(2):101-5. [DOI:10.22102/JAEHR.2019.155114.1108]

[14] Zhang HX, Zhang P, Fu XF, Zhou YX, Peng XT. Rapid and sensitive detection of aflatoxin B1, B2, G1 and G2 in vegetable oils using bare Fe_3O_4 as magnetic sorbents coupled with high-performance liquid chromatography with fluorescence detection. J Chromatogr Sci. 2020; 58(7):678-85. [DOI:10.1093/chromsci/bmaa026] [PMID]

[15] Pournormohammadi S, Ansari M, Nezafati Olfati L, Kazemipour M, Hasibi M. Determination of aflatoxin M1 in pasteurized milk consumed in Kerman Province. J Kerman Univ Med Sci. 2009; 16(3):271-80. [Link]

[16] Lee D, Lee KG. Analysis of aflatoxin M1 and M2 in commercial dairy products using high-performance liquid chromatography with a fluorescence detector. Food Control. 2015; 50:467-71. [DOI:10.1016/j.foodcont.2014.09.020]

[17] Chavarría G, Granados-Chinchilla F, Alfaro-Cascante M, Molina A. Detection of aflatoxin M1 in milk, cheese and sour cream samples from Costa Rica using enzyme-assisted extraction and HPLC. Food Addit Contam Part B Surveill. 2015; 8(2):128-35. [PMID]

[18] Tajkarimi M, Aliabadi FS, Nejad MS, Pursoltani H, Motallebi A, Mahdavi H. Seasonal study of aflatoxin M1 contamination in milk in five regions in Iran. Int J Food Microbiol. 2007; 116(3):346-9. [DOI:10.1016/j.ijfoodmicro.2007.02.008] [PMID]

[19] Tajkarimi M, Aliabadi-SH F, Nejad AS, Poursoltani H, Motallebi AA, Mahdavi H. Aflatoxin M1 contamination in winter and summer milk in 14 states in Iran. Food Control. 2008; 19(11):1033-6. [DOI:10.1016/j.foodcont.2007.10.011]

[20] Abdali F, Zare M, Abbasi A, Berizi E. Aflatoxin M1 occurrence in local dairy products in Shiraz, Southern Iran. Int J Nutr Sci. 2020; 5(3):146-7. [DOI:10.30476/IJNS.2020.87377.1080]

[21] Kazemi Darsanaki R, Azizollahi Aliabadi M, Mohammad Doost Chakoosari M. Aflatoxin M1 contamination in ice-cream. J Chem Health Risks. 2013; 3(1):43-6. [Link]