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Aflatoxin M1 Occurrence in Local Dairy Products in Shiraz, Southern Iran

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ABSTRACT

Background: One of the major problems that threaten milk and other dairy products is aflatoxins contamination. Aflatoxin M1 (AFM1) is the hydroxylated metabolite of aflatoxin B1 (AFB1) which is produced in liver of animals and is further excreted to the milk. Due to the negligible effect of pasteurization, sterilization, and milk processing on aflatoxin, it is eventually transmitted to other dairy products. This study was conducted to evaluate the AFM1 occurrence in local dairy products in Shiraz, southern Iran.
Methods: In this experimental study, from summer 2016 to winter 2016, 76 samples of four dairy products [raw milk (22), cheese (22), ice cream (22), and yogurt samples (10)] were collected from the retailors, and assessed by enzyme-linked immunosorbent assay (ELISA) method.
Results: AFM1 was identified in 92.1% of all samples as the mean contamination was 48.135 ppt. In 23.65% of all samples, AFM1 exceeded the EU limit (50 ppt for milk and dairy products, and 250 ppt for cheese). In 5.26% of all samples, AFM1 was visible more among Iranian national standard limit (100 ppt), and in 1.31% of all samples, AFM1 was more than the US/FDA limit (500ppt).
Conclusion: Due to the adverse effects of aflatoxins, even in low amounts, these toxins make serious health problems for humans. Therefore, continuous control of AFB1 contamination in animal feeding and AFM1 in milk used in the preparation of dairy products is crucial.

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Introduction

Milk and its products are the most important food sources for human beings. Some studies have shown that there is a significant correlation between the consumption of such products and community health. Feeding livestock is considered as one of the most prominent factors influencing the milk quality. Therefore, feeding livestock with unhealthy feeds can adversely influence the health of milk, dairy products and livestock (1). One of the major problems that threaten milk and other dairy products is mycotoxin contamination. Mycotoxins are natural pollutants and secondary toxic metabolites of molds that are produced mainly by specific species such as *Fusarium, Penicillium*, and *Aspergillus* under certain conditions of temperature and humidity (2, 3).

Among these toxins, aflatoxins (AF) are primarily important. Aflatoxins are a class of mycotoxins that are produced by *Aspergillus* species such as *A. nombus, A. parasiticus* and *A. flavus*. Inappropriate conditions of humidity and temperature during the storage, harvesting, and growth bring about mold growth, and consequently, aflatoxin contamination (4). Aflatoxins G1 (AFG1), G2 (AFG2), B1 (AFB1) and B2 (AFB2), and their metabolites, aflatoxin M1 (AFM1), and M2 (AFM2) are the most common types of aflatoxins isolated from contaminated foods (5).

AFB1 is the most toxic type of aflatoxin family. When livestock are fed with contaminated food, AFB1 is converted to AFM1 during the hydroxylation in the liver and subsequently is secreted in the milk of lactating livestock. Due to the negligible effect of pasteurization, sterilization and milk processing on AFM1 toxicity, it is eventually transmitted to various milk products (6, 7). Although the AFM1 toxicity is less than AFB1, the International Agency for Research on Cancer (IARC) classified AFB1 and AFM1 among Groups 1 and 2 carcinogenic agents, respectively (8).

The presence of aflatoxin in the food leads to symptoms such as liver damage, tumor, mutagenesis, and malformation in animals. There are also complications such as weakening of the immune system, reduced growth, reduced milk production, and disturbance in the reproduction of lactating cows such as abortion (9). Aflatoxins in humans cause acute or chronic intoxication with carcinogenic, mutagenic, and teratogenic effects. The main public health issue of dairy products is contamination and the major effect for aflatoxins is creation of various cancers, especially liver cancer (10).

Due to the destructive effects of AM1 on the human body and the per capita consumption of milk and other dairy products, most countries have set up a maximum admissible level of AFM1 in milk and dairy products, which vary from the 50 ppt established by the EU (250 ppt for cheese) to the 500 ppt established by the US/FDA (11). In the national standard of Iran, the highest levels of AFM1 in raw and heat-treated milk, yogurt, dough, ice cream, cream, and whey were reported 100 ppt (12). Since milk and dairy products are important nutrients in the daily human diet, this study was undertaken to assess the AFM1 level in these products in Shiraz city in 2016.

Materials and Methods

In this experimental study, determination of AFM1 was performed applying EuroProxima kit. The AFM1 kit had AFM1 standard solutions (0 ppt, 5 ppt, 10 ppt, and 20 ppt), peroxidase-conjugated AFM1, micro-titer plates coated with capture antibodies, substrate (urea peroxidase), chromogen (tetramethylbenzidine) and stop reagent containing sulfuric acid 1 N. Methanol, n-hexane, and dichloromethane were provided from Merck. In this study in 2016, a total of 76 samples of local dairy products (10 yogurt, 22 cheese, 22 ice cream and 22 raw milk samples) were randomly prepared from retailors and transferred to the laboratory in sterile containers on ice which was further stored at -20°C until use.

Sample preparation was according to the kit guideline. Cold milk samples were centrifuged at 3000 g and 4°C for 10 minutes. The upper-fat layer was taken out and 100 µL of the defatted part was used for ELISA test. Ice cream samples were prepared a two-fold dilution and then were centrifuged at 5000 g and 4°C for 10 minutes. The upper-fat layer was taken out and 100 µL of the defatted milk samples were used for ELISA test. For cheese and yogurt samples, 2 g of each homogenized sample was transferred in a 15 mL tube separately and later, 4 mL HCl (0.1 M) and 8 mL dichloromethane were added and mixed for 15 minutes (head over head).

Samples were centrifuged for 10 minutes at 5000 g and 20-25°C, and the upper layer was taken out. The extract was filtered and the filtrate (4 mL) was placed in a 60°C oven to evaporate and to be dried. The residue was dissolved in one milliliter of sample dilution buffer and then one milliliter of n-hexane was added (for defatting) and finally was vortexed. Thereafter, the prepared samples were centrifuged for 10 minutes at 3000 g and 100 µL of the samples was taken for the ELISA test. All data were analyzed by SPSS software (version 21; SPSS Inc., Chicago, IL, USA). ANOVA test was used to evaluate the proportion of contamination in dairy products. A $P<0.05$ was considered statistically significant.

Results

The contamination level of AFM1 in dairy products was presented in Table 1. Based on the results of ELISA analysis, AFM1 was noted in 92.1% of all samples, and the mean contamination was 48.135 ppt. In 23.65% of all samples, AFM1 was more than the EU limit (250 ppt for cheese and 50 ppt for milk and dairy products); in 5.26% of total samples, AFM1 was more than Iranian national standard limit (100 ppt) and in 1.31% of all samples, AFM1 was more than the US/FDA limit (500 ppt) (Figure 1).

Also, statistical analysis (ANOVA test) showed no significant difference between dairy product in the experimental groups ($P>0.05$). A total of 22 milk samples were tested for AFM1 using an ELISA method, while all samples showed contamination. The contamination range was from 0.1 to 90.6 ppt, mean contamination was 36.16 ppt and in 36.36% of milk samples, AFM1 was more than the EU limit (50 ppt) (Table 1, Figure 2). Totally,

Table 1: AFM1 levels in non-pasteurized dairy products samples

Product type	Total number	Contamination level (%)	Range (ppt)	Mean (ppt)	>EU limit (%)	>Standard limit (%)	>US FDA limit (%)
Milk	22	100	0.1-90.6	36.16	36.36	0	0
Cheese	22	100	5.8-528	82.81	9.09	9.09	4.54
Ice- cream	22	100	0.3-71.1	26.88	22.72	0	0
Yogurt	10	40	60-220	49	40	20	0
Total	76	92.1	0.1-528	48.67	23.68	5.26	1.31

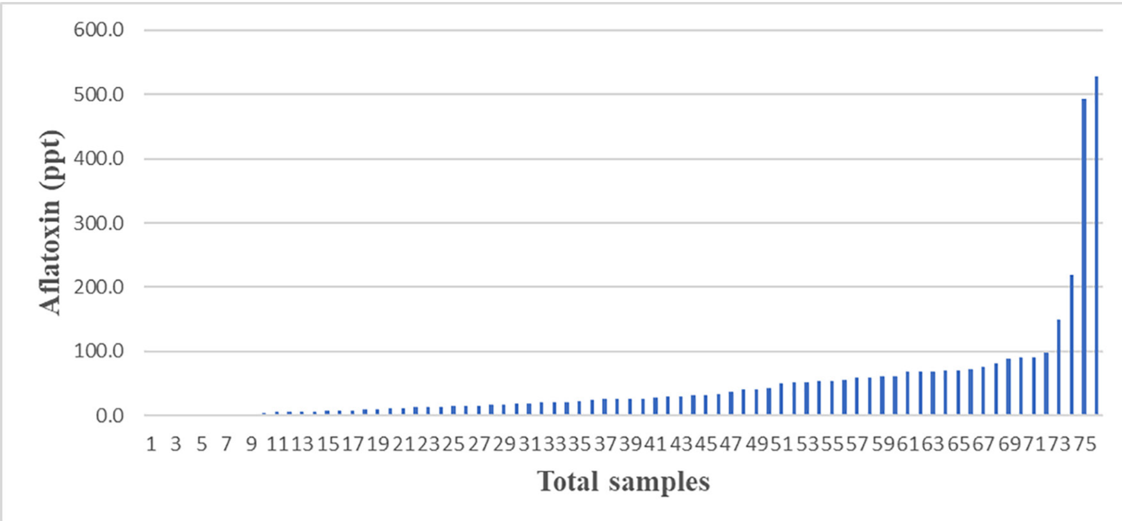

Figure 1: Comparison of aflatoxin levels of non-pasteurized dairy product samples with US/FDA limits (500 ppt).

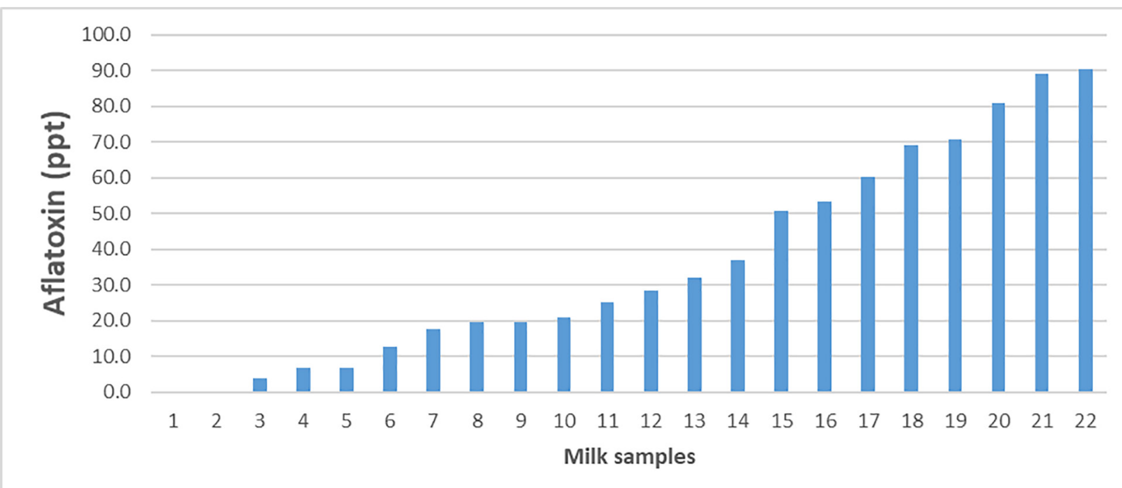

Figure 2: Comparison of aflatoxin levels of milk samples with Iran standard limit (100 ppt).

22 cheese samples were tested for AFM1 using an ELISA method, while all samples illustrated contamination. The contamination range was from 5.8 to 528 ppt and the mean contamination was 79.72 ppt in 9.09% of cheese samples. AFM1 was more than the EU limit (250 ppt), and in 9.09% of cheese samples, AFM1 was also more than the Iranian national standard limit and in 4.54% of all samples, this was more than the US/FDA limit (Table 1, Figure 3).

A total of 22 ice cream samples were tested for AFM1 using an ELISA method, while all samples demonstrated contamination. The contamination range was fom 0.3 to 71.1 ppt, mean contamination was 27.66 ppt in 22.72% of cheese samples. AFM1 was more than the EU limit (Table 1, Figure 4). Totally, 10 yogurt samples were assessed for AFM1 using ELISA, while all samples revealed contamination. The contamination range was from 60 to 220 ppt, and the mean contamination was 49 ppt in 40% of yogurt samples. AFM1 level was more than the EU limit and in 20% of cheese samples, AFM1 level was more than the Iranian national standard limit (Table 1, Figure 4).

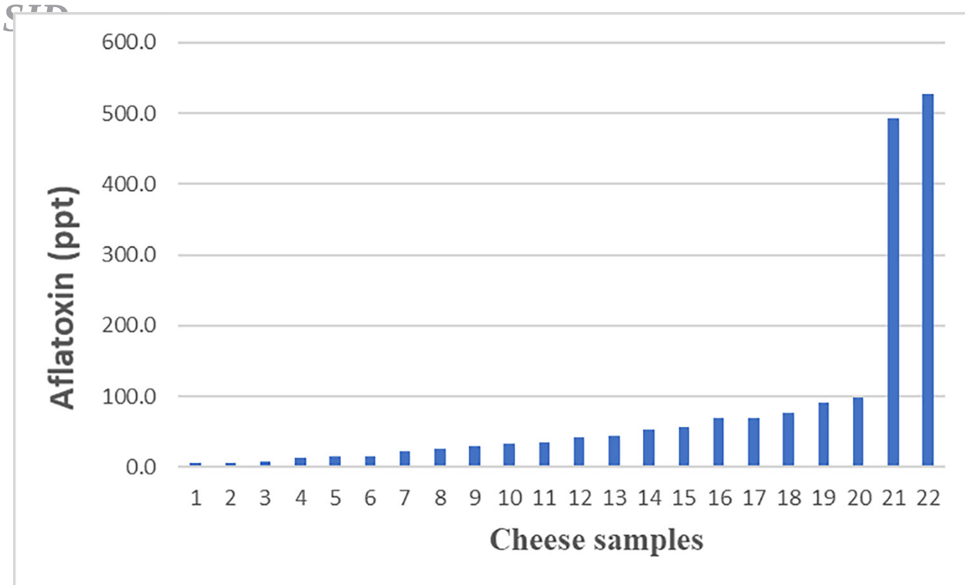

Figure 3: Comparison of aflatoxin levels of cheese samples with EU limit (250 ppt).

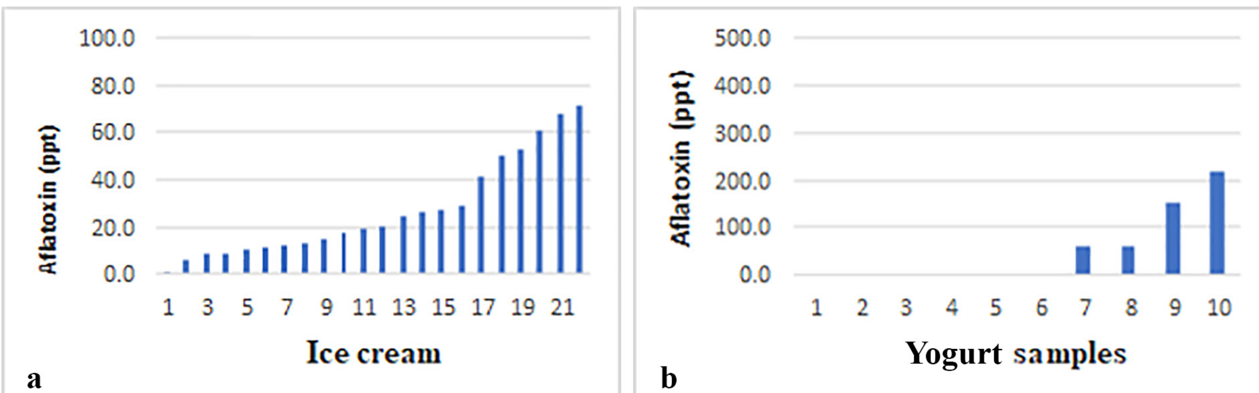

Figure 4: Comparison of aflatoxin levels of ice cream (a) and yogurt (b) samples with Iran standard limit (100 ppt).

Discussion

In this study, non-pasteurized dairy products were collected and evaluated using the ELISA method. AFM1 was observed in 92.1% of the total samples, also the results showed that 5.26% of samples were more than the Iranian standard limit and 23.65% of all samples. AFM1 was more than the EU limit too. Also, there was no statistically significant difference between dairy product groups (P>0.05). Due to the fact that thermal processes such as sterilization and pasteurization did not affect the reduction in the amount of milk aflatoxin, so if the products in the province were provided from less polluted centers, they would be more consistent with international standards (13).

In a study of 44 lactating dairy products in Taiwan (2002), 90.9% of samples were contaminated with AFM1 (14). According to a study conducted in Lahore, Pakistan (2010), of 84 raw milk samples, 81% were higher than the EU and the US limits (15). In south China (2019), AFM1 was seen in 64 (74.4%) samples of dairy products, and the content ranged from 4 to 235 ppt. None of the raw milk and dairy products of AFM1 content was more than China standard (500 ppt) (16).

In a study conducted in Tabriz (2009), AFM1 was present in all milk samples. Totally, 62% of the samples showed AFM1 more than the maximum tolerance limit acceptable by the EU (50 ppt) (17). In a study by Mohammadi Sani et al. (2012), the findings revealed that aflatoxin was present in 97.6% of 42 dairy samples. The mean contamination of the samples was 23 ppt, and in 3 samples (7.1%) the contamination was more than 50 ppt (18). According to a study conducted in four large Iranian cities in 2010, the AFM1 was detected in 72.5% dairy product samples. The concentration of AFM1 in 36.2%, 30.5%, 27.7%, 20.6%, and 9.6% of pasteurized milk, white cheese, ice cream, yogurt and butter samples, respectively, were more than Iranian national standard limits (19).

The most important reasons for the difference in levels of contamination of AFM1 in dairy products in different parts of the world can be attributed to the geographical area, the type of survey, the type and number of samples, the season and the difference in the level of livestock feed contamination with AFB1. The findings of the current study revealed that the level of contamination with aflatoxin in most samples was lower than the Iranian national standard limit and these samples could be consumed. But due to the harmful effects of aflatoxins and the fact that contamination of these toxins, even in low amounts, can be associated with a serious health problem for humans; therefore, continuous control of animal feeding in terms of AFB1 and milk used for preparation of dairy products in terms of AFM1 is necessary.

Conclusion

Since the carcinogenic role of aflatoxins has been repeatedly approved, the reduction of food contamination with these toxins, as well as the control and neutralization of their effects, should be accentuated. In this regard, the role of research and educational centers is very important in providing appropriate solutions for solving this problem and training to increase health services in the field of healthy food. Another notable point is the effect of cumulative accumulation of this toxin in the body, which needs further investigation.

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Conflicts of Interest

None declared.

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