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Contamination of Ice Cream by Aflatoxin M1 in Iran

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Abstract: Aflatoxin M1 (AFM1) may occur in milk and milk products, resulting from the ingestion of aflatoxin B1 in feedstuffs by dairy cows. AFM1 is heat resistant and could be found in UHT and pasteurize milk and other dairies as well as ice-cream. The purpose of this study was to determine the level of the toxin in traditional and commercial ice-cream available in Ahvaz area (South-West of Iran). During January to July 2010, 41 traditional ice-cream and 39 commercial ice-cream samples from different companies were collected from local market and analyzed for AFM1 by ELISA technique. Data showed that from traditional samples, 10 samples (24.37%) exceeded the legal level of AFM1 in milk and dairy products (50 ppt.), 3 samples (7.31%) were contaminated with level more than 200 ppt aflatoxin, contamination of 3 samples (7.31%) were between 100-200 ppt and 4 samples (9.75%) showed contamination level from 50-100 ppt. 31 of samples (75.63%), proud to have legal contamination levels. From commercial samples, 14 samples (35.85%) exceeded the legal level of AFM1 in milk (50 ppt), which 6 samples (15.36%) were contaminated with level more than 200 ppt aflatoxin, contamination of 3 samples (7.68%) were between 100-200 ppt and 5 samples (12.8%) showed contamination level from 50-100 ppt. The rest of samples (64.15%), proud to have legal contamination levels. It can be concluded that AFM1 level in the 30% of ice-cream samples purchased in Ahvaz area appear to be contaminated more that European limits, which 12.5% belongs to traditional ice-creams and 17.5% from commercial samples. To control the problem, dairy cow feeds should be stored in way that they do not became contaminated and milk and milk products have to be under monitoring.

Key words: Aflatoxin M_1 · Ice cream · Milk · ELIZA

INTRODUCTION

Aflatoxins are a group of mycotoxins produced by certain strains of the fungi *Aspergillus flavous*, *Aspergillus parasiticus* and rarely *Aspergillus nomius*. When lactating mammals, such as cows, sheep and goats are fed with feedstuffs containing aflatoxin B1 (AFB1), this metabolite can be converted to Aflatoxin M1 (AFM1). They are both actually and chronically toxic, mutagenic, teratogenic and carcinogenic compounds for animals and humans [1-2]. AFM1 as a main hydroxylate derivative is forming in the liver of human and ruminants and excrete to milk [3-5]. It is noticeable that neither storage of milk nor some processing such as pasteurization or UHT technique do not affect AFM1 concentration because of its heat stability [6-7]. Moreover, as milk and ice cream are the main nutrient for young children whose vulnerability is noteworthy and potentially more sensitive than that of adults, the occurrence of AFM1 in human breast milk or commercially available dairy products is one of the serious problems of food hygiene [6]. According to the European Union and Codex Alimentarius, the maximum level of AFM1 in liquid milk dried or processed milk products such as ice cream should not exceed 50 ng/L [8], but based on the US regulations it should not be higher than 500 ng/L [9]. Institute of Standards and Industrial Research of Iran has been accepted 100 ng/l AFM1 as maximum tolerance level in raw, pasteurized and UHT milk, ice-cream, butter and yogurt [10].

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There is little information about the occurrence of AFM1 in milk and milk products and no information regarding presence and level of AFM1 in ice cream in Iran. Accordingly Alborzi *et al.* [11] reported in 624 samples of pasteurized milk in Shiraz (South of Iran), the amount of AFM1 in 17.8% of the samples was higher than the maximum tolerance limit accepted by European Union. In an early report on 52 UHT milk sample in Tehran it was found that the level of AFM1 in 79.9% of samples was higher than 50ng/L [12]. But subsequent study on 210 UHT milk samples showed that the contamination level has been decreased to 33.3% [13]. In a similar study in Khuzestan province (South-West of Iran) by author (Unpublished data), on 178 UHT, pasteurize and Government subsidize milk it was found that 62 samples (34.8%) were contaminated with AFM1 higher than the maximum tolerance limit (50ng/L) accepted by European countries. Also, the incidence rate of AFM1 in raw cow, water buffalo, camel, sheep and goat milk in this province were 78.7, 38.7, 12.5, 37.3 and 27.1%, respectively [14].

The purpose of this survey was to determine natural occurrence and levels of AFM1 in traditional and commercial ice-cream produced in Iran and available in Ahvaz area.

MATERIALS AND METHODS

During January to July 2010, 41 traditional and 39 commercial ice-cream samples from 6 companies were purchased from local markets in Ahvaz city and analyzed for AFM1 by ELIZA kite (Euro Proxima, Netherlands). Based on company instruction, 2 g of ice cream was weight in a screw cap glass test tube. Then 15 ml of dichloromethane was added and extract by shaking the vial for 30 min. the suspension was filtered and 4 ml of the extract was transferred into a glass tube and evaporate at 50 °C under a nitrogen stream. To each glass tube, 0.5 ml dilution buffer and 0.5 ml heptane was added and vortex for 1 min. The samples were centrifuged at 2000 g for 5 min. the upper layer was removed and again 0.5 ml heptane was added and centrifuged. Then 100 il of aliquot was taken for next stage. Hundred microliters of the AFM1 standard solutions and test samples were added to the wells of micro-titer plate and shake the plate gently with rotatory motion for few seconds and then incubated for 60 min at room temperature (20-25 °C) in the dark. The liquid was poured out of the wells and the wells were filed completely with working wash solution and poured out the liquid again. The remaining droplets were removed by tapping the microplate upside down vigorously against absorbent paper. The washing sequence was repeated 3 times. In the next stage, 100 µl of enzyme conjugate solution was added to the wells using multichannel pipette and shake the plate gently for few seconds and incubate for 30 minutes in the dark. 100 µl of developing solution was added to each well and mix for few seconds and incubate for 15 minutes. Then 100ul of stop solution was added to each well and mix for few seconds. Finally, absorbance was measured at 450 nm and calibration curve was made.

The results were analyzed by one way analysis of variance (ANOVA) and independent sample T-test. Means were considered statistically different at 95% confidence levels.

RESULTS AND DICUSSION

Aflatoxin M1 was found in almost 100% of the examined ice cream samples. As it is shown in table 1, 10 (24.37%) sample of traditional ice cream and 14 (35.85%) sample of commercial ice cream were exceeded the legal level of AFM1 accepted by European Union (50 ng/L).

One way analysis of variance showed that the mean and the standard error of the mean of AFM1 level in commercial and traditional samples were 64.36±11.58 and 41.78±9.35, respectively. Independent sample t test showed that although the mean of AFM1 level in traditional samples is lower than commercial samples, but this differences was not significant. Also, no significant difference between AFM1 level in traditional and commercial ice cream tested and the maximum level of AFM1 in milk products accepted by European Union was observed. Institute of Standards and Industrial Research of Iran currently reduced the maximum tolerance level of AFM1 from 500ng/L to 100ng/L in milk, ice-cream, butter and yogurt to improve the public health standards. Our data showed that out of 80 samples, the AFM1 level in 24 samples (30%) was higher than the maximum tolerance level accepted by European Union, but base on Iranian standards 15 samples (18.75%) were exceeded the legal level. Table 2 shows few literature data on the occurrence of AFM1 levels in milk and milk products in Iran and some neighboring countries. These studies have shown that the contamination of milk and dairy products and variations in the concentrations were related to geographic position, national development level and season. It seems that the magnitude of concentration of AFM1 in Iran milk and milk product samples is still high.

Table 1: Aflatoxin M_1 concentration of commercial and traditional ice-cream samples

		Aflatoxin levels				
Type of sample	No of sample	0-50ng/L	50-100ng/L	>100ng/L	Mean ng/L	St. Error
Commercial	39	25	5	9	64.36	11.58
Traditional	41	31	4	6	41.78	9.35
Total	80	56	9	15	52.79	7.46

Table 2: The prevalence of milk contamination in other studies

Location	Type of milk samples	No of samples	Number of contaminated milk samples (>50ng/L)	percent	References
Iran (Shiraz)	Pasteurized	624	101	17.8	[11]
Iran (14 state)	Raw cow milk	319	217	68	[15]
Iran (Tabriz)	Pasteurized	50	31	62	[2]
Iran (Ahvaz)	Raw cow milk	75	27	36	[14]
Iran (Sanandaj)	pasteurized	272	12	4.4	[16]
Pakistan	Raw cow milk	168	167	99.4	[17]
Turkey	UHT milk	100	31	31	[18]
Taiwan	Pasteurized	144	1	0.7	[19]
Egypt	Raw cow milk	50	17	34	[20]
Syria	Raw, Pasteurized and powdered milk	126	66	52	[21]

Milk production in Iran is mainly done by two types of dairy farms, industrial and traditional. Also, Iran is an arid country whereas the pasture is not widely available and cows are fed with great amounts of concentrated feed and hay containing fungi. Therefore, it is important to constantly monitor the level of AFM1 in milk and AFB1 in feedstuffs of dairy animals especially in traditional dairy farms. Education of dairy farmers by government authorities on potential health consequences of aflatoxin and creating stringent regulations regarding reducing of AFB1 in feedstuffs is recommended.

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