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Determination of the Aflatoxin M_1 in Ice Cream in Babol City (Northern, Iran)

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Abstract: When animals consume contaminated feed stuff to Aflatoxins, the toxin is metabolized in the liver and execrated as Aflatoxin M_1 (AFM_1) via milk. Due to the carcinogenic and immune-suppressive effects of Aflatoxin and its deteriorating human health, conducting such a study that was survey AFM_1 determination in ice cream necessary.In this study, during the autumn of 2010, ice creams (45 samples) were collected from Supermarkets in Babol city. Samples were tested for Aflatoxin M_1 (AFM_1) contamination by competitive Enzyme Linked Immunosorbent Assay (ELISA). In general, of 45 samples ice cream, 10(22.2%) were positive with above the limit of European community regulations (50 ng/l). Maximum concentration of AFM_1 was 103 ng/l, minimum was 1.2 ng/l and mean was 33.98 ng/l. There is no a significant relationship between AFM_1 contamination level and different months applying statistical test.

Key words: Aflatoxin M_1 · ELISA · Ice Cream

INTRODUCTION

Aflatoxins are generally produced by special strains of *Aspergillus Flavus, A. Parasiticus* and *A. nomius* [1]. The AFM_1 and AFM_2 are oxidative metabolic AFB_1 and AFB_2 which are made by live mycosomal enzymes functions. Theses are discharged via milk, urinary and feces of cattle and some species of mammals that consume feed stuff contaminated to Aflatoxin [2, 3]. Their main target organ for toxicity and carcinogenicity is liver. Although mutagenic and carcinogenic level of AFM_1 is lower than AFB_1, its genotoxic activity is high. Aflatoxin in some human diseases, particularly, liver primary cancer is involved by engaging DNA, mutagen P_{53} gene [4]. European community and Codex Alimentary determined the maximum level of AFM_1 50 ng/kg in raw milk, liquid, powder, heat-treated milk and processed dairy products and it must not exceed the amount [5].

Many countries have conducted inspection program and controlling on mycotoxins for several years to promote public health. *Montagna et al.* [6] surveyed AFM_1 contamination of cheese made at different milks from Italy. The AFM_1 was found in 16.6% of the analyzed samples. In Greece, AFM_1 was measured in pasteurized milk. Of 81 milk samples, 32 had 2-2.5 ng/l, 9 cases above 5 ng/l, 31 cases 0.5-1 ng/l and 9 cases had no AFM_1 [7]. In India AFM_1 was measured in milk and children dairy products. Out of 87 samples 87.3% were contaminated [8]. The AFM_1 was detected in 207 (99.5%) samples out of 208 (0.001-0.24 µg/kg) which was found no significant difference in AFM_1 contamination in various areas in Japan [9].

Some studies have been conducted in some provinces in Iran. *Moktabi et al.* [10] detected 62 samples (34.8%) AFM_1 contamination in UTM, pasteurized and government subsidize (GSM) Milks in Ahvaz area which were over than 50ng/l. The contamination in spring was less than the other seasons. *Habibpour et al.* [11] reported 34.6% AFM_1 contamination in raw milk from Hamedan province, Iran. *Movassagh et al*. [12] detected AFM_1 contamination in cow's raw milk from Miandoab city, west Aazerbaijan province, Iran. AFM_1 was found in 50% of the samples. *Rahimi* [13] reported 81.4% AFM_1 contamination in milk and infant milk products in Iran of ranging from 7 to 476 ng/l. *Gholampour Azizi et al*. [14] from Amol (northern Iran) determination B_1 levels of the feedstuffs in Traditional and semi-industrial cattle farms 44.4% and 43.1%, respectively. *Azarakhsh et al*. [15] reported the Most Common Toxigenic *Aspergillus* Species such as *A. flavus* followed by *A. niger* and *A. fumigatus* in broiler feeds in west Iran. *Gholampour Azizi et al.* [16] in Babol city (northern Iran) detected AFM_1 in 100% milk to the concentration 193-259 ng/l and *Hadizadeh et al.* [17] in northern Iran were found aflatoxin B_1 in animal food between 10.4 to 68.8%. Moreover *Gholampour Azizi et al.* [18] in same area detected of the

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Table 1: The AFM_1 level distribution in ice cream in Babol city (northern Iran) in different months.

Months	Samples tested	Positive sample*	%	Mean±SE	S.D.	Max	Min
Sept	15	3	20	32.8±6.26	24.24	89	2.3
Oct	15	3	20	34.96±6.14	23.77	91	1.2
Nov	15	4	26.7	34.15±7.65	29.63	103	3.2
Sum	45	10	22.2	33.98±3.97	3.79	103	1.2

*>50 ng/l, S.E= Standard Deviation, S.D=Standard Error of Mean

Table 2: The AFM_1 level Frequency in ice cream in Babol city (northern Iran) in different months.

	Frequency distribution of sample (ng/l)									
	<10		11-25		26-49		50-100		>100	
Months	N	%	N	%	N	%	N	%	N	%
Sept	2	13.3	6	40	4	26.7	3	20	0	0
Oct	1	6.7	4	26.7	7	46.6	3	20	0	0
Nov	3	20	4	26.7	4	26.7	3	20	1	6.7
Sum	6	13.3	14	3.1	15	33.4	9	20	1	2.2

Zeralenone and Ochratoxin in human food 5-8.3 and 5-7.5%, respectively. No research (the AFM_1 in ice cream) is observed to be done in the Northern provinces. The aim of this study was to survey the occurrence of AFM_1 in ice cream in Babol city, Iran. There has been no detection in area.

MATERIALS AND METHODS

A total of 45 ice cream samples randomly collected from supermarkets in Babol (northern Iran) during the autumn of 2010. Samples were tested for Aflatoxin M_1 (AFM_1) contamination by competitive Enzyme Linked Immunosorbent Assay (Tecna, AFLA M_1). The samples were stored in a refrigerator (-20 centigrade) and were centrifuged at 2-8 centigrade for 10 minutes at 3000g, then fat was completely separated from them and added 100 micro-liter them and Aflatoxin M_1 standard solutions in each well and then incubated the plate at 20-25 centigrade for 45 minutes. Each well was washed four times by washing buffer 20X concentration. Then 100 micro-liter conjugated solution (100X) was added to each well. After that, the plate was incubated at 20-25 centigrade for 15 minutes. Next, the wells were washed. Substrate then was added in the wells. Then we incubated the plate at 20-25 centigrade in a dark place for 15 minutes. The reaction was stopped by the stop solution. At most after one hour, light absorption was read at 450 nm by ELISA reader [6].

RESULTS AND DISCUSSIONS

In 10 out of 45 ice cream samples (22.2%) the presence of AFM_1 was detected beyond the limit of European community regulations (50ng/l) by concentration ranging from 1.2 -103 ng/l by the average of 33.98 ng/l. The AFM_1 contamination levels (>50 ng/l) in Sept, Oct and Nov were 20%, 20% and 26.7% respectively. The lowest contamination was observed in Sept and Oct. The highest contamination was observed in Nov (Table 1). The AFM_1 concentration levels >25 ng/l was 55.6 ng/l (Table 2). There is no a significant relationship between AFM_1 contamination level and different months applying statistical test.

To decrease AFM_1 in milk to the lowest point, feed stuff ration should be checked regularly and it should be kept away from fungal contamination. To increase milk quality, it is necessary for feed stuff to be without AFB_1 contamination [19]. *Atanda et al.* [20] reported AFM_1 contamination in human and cow milk and ice cream in Nigeria range of 2.04-4.00 µg/l. High recorded scores of 4.0 2.04 and 2.23 µg/l, respectively. *Moktabi et al.* [21] surveyed AFM_1 in 80 ice cream samples in Ahvaz area (South-West of Iran) that 24 samples(30%) were over than 50 ng/l(Mean=52.79 ng/l). *Fallah* [22] collected 36 ice cream samples from four large Iranian cities and examined AFM_1 that detected 25 (69.4%) AFM_1 (mean: 41 ng/kg; range: 15-132 ng/kg). The contamination of AFM_1 in 27.7% of ice cream samples was higher than Iranian national standard limits (50 ng/l). This present study the contamination of AFM_1 >50 ng/l in ice cream was 22.2% (mean= 33.98 ng/l) but the AFM_1 concentration levels >25 ng/l was 55.6 ng/l which important for children.

The AFM_1 appears in milk by cattle's consuming AFB_1 after 12-24 hours. Milk, ice cream and dairy products consumption is high in human particularly among children; therefore, they are exposed to AFM_1. On the other hand, milk is consumed not only as liquid but as children formulae, ice cream, yoghurt, cheese and milk-based sweets such as chocolate and donuts. Therefore, AFM_1 recognition in milk and dairy products is crucial.

As a result, consumers of various ages are protected from its potential risks [23].The results of the study showed that AFM_1 contamination level in ice cream is high. It is a serious public health problem, because all age groups including babies and children extensively consume the product. As a result, milk and dairy products should be controlled regularly at least twice a year. Beside this, keeping low AFB_1 level in feed stuff is of importance. To reach the goal, feed stuff should be kept away from probable contamination.

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