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Aflatoxin M1 in Pasteurized Milk and White Cheese in Ahvaz, Iran

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Abstract: In the present study, 121 dairy product samples consisting of pasteurized milk (60 samples) and white cheese (61 samples), collected from retail markets during August 2010 to September 2011 in Ahvaz, Iran, were examined for AFM1 by enzyme-linked immunosorbent assay (ELISA) technique. Analytical results showed that 61 (50.4%) samples were contaminated with AFM1 ranging from 11 to 209 ng/l and consisted of 24 (40%) pasteurized milk samples (range: 11-94 ng/l and mean±SD: 34.7±19.9 ng/l) and 37 (60.7%) white cheese samples (range: 32-209 ng/l and mean±SD: 103.6±54.1 ng/l). Considering the US FDA and Iranian national standard limits for AFM1 in milk (500 ng/l), any of samples had levels above the maximum tolerance limit. However, the concentration level of AFM1 in 3 (5%) samples of pasteurized milk was higher than European Commission limit (50 ng/l). No significant differences in the concentration of AFM1 were observed for pasteurized milk samples taken in different seasons in Ahvaz; however, significantly higher concentration of AFM1 ($P < 0.05$) were found in white cheese samples taken in cold seasons. Although the concentration of AFM1 in none of the examined samples exceeded the Iran regulation, but by attention to high consumption and significance of milk and its products in Iranian dietary, survey and control of milk and its products continuously for measuring of AFM1 has high significance.

Key words: Pasteurized Milk · Cheese · Aflatoxin M1 · ELISA

INTRODUCTION

Aflatoxins are a group of structurally-related toxic compounds produced by certain strains of the fungi *Aspergillus flavus, A. parasiticus* and the rare *A. nomius* [1], which contaminate plant and plant products particularly under conditions that favor the growth of moulds. The toxins have been implicated as causative agents in human hepatic and extrahepatic carcinogenesis [2-4].

The best known B1, B2, G1& G2, that are ingested by animals in contaminated pellets are forage; aflatoxin B1 (AFB1) is notoriously the most toxic of these metabolites [5]. When ingested by dairy animals, the metabolite is bio transformed at the hepatic level by microsomial cytochrome P450 into aflatoxin M1 (AFM1) [1]. The amount of AFM1 excreted as a percentage of AFB1 in feed is usually 1-3%. 12-24h after the first AFB1 ingestion, the toxin can be detected in the milk. When the intake of AFB1 is stopped, the AFM1 concentration in the milk decreases to an undetectable level after 72 hours [1].

Milk has the greatest demonstrated potential for introducing AFM1 into the human diet. Evidence of potential hazardous human exposure to AFM1 through milk and milk products has been shown in several studies. The consumption of milk and milk products by human is

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quite high, particularly among infants and young children, thereby increasing the risk of exposure to AFM1 [6, 7]. AFM1 is relatively stable during pasteurization, sterilization, preparation and storage of various dairy products [8, 9]. Milk is not only consumed in liquid form but also used for the preparation of infant formulas, yogurt and cheese. Therefore, it is important to determine AFM1 level in milk and dairy products in order to inform consumers of its potential hazard. The aim of this work was to determine concentrations of AFM1 in pasteurized milk and white cheese marketed in Ahvaz, Iran.

MATERIALS AND METHODS

Collection of Samples: In the present study, 121 dairy product samples consisting of pasteurized milk (60 samples) and white cheese (61 samples), collected from retail markets during August 2010 to September 2011 in Ahvaz, Iran. The samples were immediately transported to the laboratory in a cooler with ice packs and stored at -20°C until analysis.

Method of Analysis: The quantitative analysis of AFM_1 was performed using enzyme immunoassay: Ridascreen® aflatoxin M_1 kit (R-Bipharm AG, Germany). The test is based on the antigen-antibody reaction. The assay was performed according to the manufacturer's recommendation and as described elsewhere [10]. The mean lower detection limit of the assay was 5 ng/l.

Statistical Analysis: Statistical analysis of results was performed with SPSS (version 16) software (SPSS Chicago, IL, USA). The mean AFM1 concentration in milk and cheese samples and the mean of the AFM1 concentration of different seasons in milk samples was compared by one way analysis of variance (ANOVA) and Tukey's (HSD) tests.

RESULTS AND DISCUSSION

In the present study, 121 dairy product samples consisting of pasteurized milk (60 samples) and white cheese (61 samples), collected from retail markets in Ahvaz, Iran and examined for AFM1 by enzyme-linked immunosorbent assay (ELISA) technique. Analytical results showed that 61 (50.4%) samples were contaminated with AFM1 ranging from 11 to 209 ng/l, consisted of 24 (40%) pasteurized milk samples (range: 11-94 ng/l and mean ± SD: 34.7±19.9 ng/l) and 37 (60.7%) white cheese samples (range: 32-209 ng/l and mean ± SD: 103.6±54.1 ng/l). Considering the US FDA [11] limits for AFM1 in milk (500 ng/l), any of samples had levels above the maximum tolerance limit. However, the concentration level of AFM1 in 3 samples of pasteurized milk was higher than European Commission limit (50 ng/l) [12].

Concentrations of AFM1 in milk samples found in the present study are similar to those reported in other countries [13-15] although higher than those in many European countries were reported [16, 17]. When compared with those found in other Iranian cities they are lower than those found in Gorgan (76 ng/l), Rasht (44 ng/l), Shiraz (52 ng/l) and Tehran (61 ng/l) and higher than those found in Hamedan (8 ng/l), reported by Tajkarimi *et al*. [3]. Also, our results showed that the contaminations of pasteurized milk with AFM_1 are in agreement with those of Alborzi *et al*. [18] in Shiraz and Rahimi *et al*. [19] in Esfahan and Shahrekord.

AFM1 contamination of milk is the result of cows feeding on material containing aflatoxin B1 [20]. The concentration of this mycotoxin in animal feedstuffs is influenced by the type, time and method of harvesting and temperature and relative humidity of storage facilities, all of which vary among different regions of Iran [3].

The results showed that 60.7% of white cheese samples tested positive for AFM1 (37 out of 61) with levels ranging from 32 to 209 ng/l. Sixteen of the positive samples had AFM1 levels exceeding 100 ng/l and the AFM1 level in any of samples had levels above the maximum tolerance limit in cheese (250 ng/l) [21]. In a study in Iran by Kamkar [22] the presence of AFM1 was detected in 82.5% of 80 feta cheese samples tested. In another study AFM1 was found in 53.4% of 88 traditional cheese samples in Esfahan, Iran, at concentrations between 82 and 1254 ng/kg [23]. In comparison with other studies conducted in the nearby region, AFM1 levels in 18% of 100 cream cheese samples in Turkey exceeded the action limit of 250 ng/l [24]. In another study in Turkey, 80% of cheese samples were shown to be contaminated with AFM1 using ELISA, with a range 23.8-452 and mean of 87.6 ng/l, with one sample being above the regulatory limit (250 ng/l) [25]. In USA, a report indicated that one in 399 Cheddar and Cottage cheese samples was contaminated with AFM1 levels equivalent to 0.08 mg/kg [26]. In North Africa, of 20 samples of fresh white soft cheese, 15 samples (75%) were contaminated with AFM1 in concentrations between 110 and 520 mg/kg [27]. The differences between results obtained from various countries may have several causes. The method of cheese production, rate of milk concentration used in cheese making, conditions of cheese ripening and the analysis method, are the most

Table 1: Distribution by season of pasteurized milk samples and aflatoxin M_1 concentration (ng/l) in Ahvaz, Iran

Season	Analyzed (n)	Positive n (%)*	Minimum	Maximum	Mean	SD
Winter	15	9 (60.0)	19	94	43.2	22.8
Spring	15	6 (40.0)	11	36	21.3	9.6
Summer	15	5 (33.3)	18	41	30.2	9.2
Autumn	15	4 (26.7)	20	80	41.3	27.2

* < 5 ng/l - negative for aflatoxin M_1

Table 2: Distribution by season of cheese samples and aflatoxin M_1 concentration (ng/l) in Ahvaz, Iran

Season	Analyzed (n)	Positive n (%)*	Minimum	Maximum	Mean	SD
Winter	15	10 (66.7)	59	209	148.5	52.1
Spring	16	12 (75.0)	32	177	78.9	48.6
Summer	15	10 (66.7)	39	108	70.8	22.2
Autumn	15	5 (33.3)	99	206	138.4	42.4

* < 5 ng/l - negative for aflatoxin M_1

important ones. Table 1 and 2 shows distribution by season of pasteurized milk and cheese samples and their AFM_1 concentrations, respectively.

No significant differences in the concentration of AFM1 were observed for pasteurized milk samples taken in different seasons in Ahvaz; however, significantly higher concentration of AFM1 ($P < 0.05$) were found in white cheese samples taken in cold seasons in agreement with the results of other authors. One reason for this is that milking animals are fed with compound feeds in winter that are prone to aflatoxin B1 concentration [7, 22, 28-30].

AFM1 concentration of milk and milk products is potentially a serious public health problem as all age groups, including infants and children, who consume these products world wide. For this reason, milk and dairy products have to be inspected and controlled continuously for AFM1 contamination. Frequent analytical surveillance by food control agencies is highly recommended to control the incidence of mycotoxin contamination in Iran especially in dairy products and feed. Implementing a food control system, such as the HACCP system, in the food industries is suggested as an efficient means for limiting mycotoxin contamination in Iran's food supply.

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