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A Study of the Occurrence of Aflatoxin M_1 in Dairy Products Marketed in Urmia, Iran

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ABSTRACT

The purpose of this survey was to evaluate natural occurrence and content of Aflatoxin M_1 (AFM_1) in dairy products marketed in Urmia (Iran). During September 2007, 40 samples of pasteurized milk, 40 samples of Ultra High Temperature Treated (UHT) milk, 40 samples of creamy cheese and 40 samples of Iranian Feta cheese were collected from different supermarkets in Urmia city. AFM_1 contents were determined through competitive Enzyme Linked Imunosorbent Assay (ELISA) technique. All milk samples analyzed showed mean AFM_1 concentrations lower than the permissible level of 50 ng L^{-1} (23.22±8.65, and 19.53±7.47 ng L^{-1} in pasteurized milk, and UHT milk, respectively). The mean levels of AFM_1 contamination were 43.31±18.51 ng L^{-1} in Feta cheeses, 21.96±3.23 ng L^{-1} in creamy cheeses. The potential risk of human exposure to aflatoxin M_1 *via* consumption of milk and milk products is well known. Therefore, dairy products must be evaluated for aflatoxin and kept apart from fungal contamination as much as possible.

Keywords: AFM_1, Milk, Cheese, Enzyme Linked Imunosorbent Assay (ELISA).

INTRODUCTION

Aflatoxins are a group of naturally occurring toxins produced mainly by such moulds as *Aspergillus flavus.* When aflatoxin B_1 (AFB_1), the most toxic aflatoxin, is ingested, some animals are able to convert it into AFM_1, which, in turn, is transferred to such food products as milk, eggs or meat (Polan *et al.,* 1974; Trucksess and Stoloff, 1984). The conversion rate of aflatoxin B_1 to aflatoxin M_1 present in milk ranges between 0.5% and 5% for many mammals including dairy cattle and humans, yet values as high as 6% have been reported by Pipet (1998).

AFM_1 exhibits carcinogenic (IARC, 2002), genotoxic (Lafont *et al.,* 1989) and cytotoxic effects (Neal *et al.,* 1998). AFB_1 and AFM_1 have been classified by the International Agency for Research on Cancer as human carcinogens class 1 and 2B, respectively (IARC, 2002). Because of their potential risks and also to minimize their hazard, the World Health Organization recommends the reduction in its consumption to a minimum, because there still is not enough information available to establish a tolerable exposure level (WHO, 2002).

The level of AFM_1 in milk should not exceed 500 pg ml^{-1} according to US regulations but the level is set at 50 pg ml^{-1} in most European countries and in the *Codex Alimentarius* (Van Egmond, 1995). In Iran, the action levels have been officially set at 50 pg ml^{-1} for AFM_1 in milk (FAO, 2004).

As milk is the main nutrient for infants and children who are considered to be more susceptible to adverse effects of mycotoxins, the occurrence of AFM_1 in milk is a concern. On the other hand, milk is not only consumed as liquid milk, but also utilized in the preparation of infant formulas, yogurt,

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cheese, and milk-based confectioneries including chocolates, and pastry. Therefore, it is important and necessary to determine AFM_1 levels in milk and dairy products in order to protect consumers in various age groups, from its potential hazards.

Unfortunately, in Iran, in spite of the fact that the dairy industry has evolved a lot in the last few years, there are very few items of data concerning AFM_1 contamination in milk and milk products. In the present work, some milk and dairy product samples marketed in the city of Urmia, Iran, during the month of September 2007 were collected and analyzed for AFM_1 through direct competitive enzyme-linked imunosorbent assay (ELISA).

MATERIALS AND METHODS

Sample Preparation

A total of 160 samples of commercial pasteurized milk and other dairy products were collected on a weekly basis from supermarkets located in Urmia, during September, 2007. The samples collected included 40 pasteurized, and 40 UHT milk samples, 40 samples of creamy cheeses, and 40 feta cheese. The samples were stored in a cool place and protected against light until the day of analysis. Pasteurized milk samples were collected on the production day and kept freezing cool before being analyzed later. Different brands of feta, creamy cheese and UHT milk samples, had been produced during the summer. Sampling was performed according to Iran standard (ISIRI, 2002); as regards the goal it was an assessment of average AFM_1 contamination in 4 types of dairy products.

Method employed for Analysis of Aflatoxin M_1

Determination of AFM_1 was based on an enzyme-linked immunoassay using the ELISA kit (I'screen AFM_1-Tecna, Italy). This method is quick, reliable and cost effective for an estimation of AFM_1 (Kaniou-Grigoriadou *et al.,* 2005).

Most of the reagents used were available in the I'screen test kit. AFM_1 standard solutions used for the construction of the calibration curve were at levels of 0, 5, 10, 25, 50, 100 and 250 ng L^{-1} and all included in the ELISA test kit. Detection limit in raw milk was 5 ng L^{-1}.

Preparation of samples was conducted according to the instructions of the I'screen kit. Briefly, milk samples were chilled and then centrifuged for 10 min at 4,800 rpm (Heraeus Megafuge 1.0). One hundred µL of the skimmed milk was diluted with 400 µL of the sample diluent. An aliquot (100 µl per well) of this solution was used directly for analysis. Cheese samples (2 g each) were homogenized (Ultraturrax, IKA-Werke) and extractions obtained by shaking the cheese homogenate with 15 mL of dichloromethane for 15 minutes. The suspension was then filtered and 3.75 ml of the extract evaporated under nitrogen stream and at 60°C. The residue was redissolved in 750 µL extraction solution and mixed thoroughly for one minute, then added 750 µL of hexane and mixed for another one minute. After centrifugation for 15 minutes at 3,800 rpm, a 50 µL aliquot of the methanolic/aqueous phase was diluted in a small tube (1.5 ml) with 200µl of dilution buffer and mixed gently. An aliquot of this solution was used in the test (100 µL $well^{-1}$).

Aflatoxin M_1 standards or the prepared sample solutions were added to microtiter wells in duplicate. During incubation for 45 minutes at room temperature and in the dark, the antibody binding sites are occupied in proportion with the aflatoxin M_1 concentration. The liquid was then removed completely from the wells, which were subsequently washed four times with washing buffer. In the next step, any remaining free binding sites are occupied by the enzyme conjugate, which was added (100 µL) and then incubated for 15 minutes at room temperature in the dark. Any unbound enzyme conjugate was then

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Table 1. Occurrence of aflatoxin M_1 (ng L^{-1} or ng kg^{-1}) in dairy products.

Sample	Tested	Average contamination
Pasteurized milk	40	23.22±8.65
UHT milk	40	19.53±7.47
Creamy cheese	40	21.96±3.23
Feta cheese	40	43.31±18.51

Table 2. The prevalence of milk contamination as reported by some studies in Iran.

Survey	Liquid milk samples (Number)	Contaminated milk samples> 50 ng L^{-1}
Tehran (1998)	73	82.2
Hamedan (2000)	186	11.76
Sarab (2003)	111	40
Shiraz (2004)	624	17.8
Tehran (2005)	128	78

removed in a washing step. Developing solution (100 µL) was added to each well and incubated for 15 minnutes at room temperature in the dark. Bound enzyme conjugate converts the colorless chromogen into a blue product. Then the addition of the stop reagent (50 µL per well) led to a color change from blue to yellow. The measurement was made photometrically at 450 nm.

In order to obtain AFM_1 actual sample concentration in ng L^{-1} for milk and ng kg^{-1} for cheese, the concentration read from the calibration curve was further multiplied by a dilution factor of 5 for milk and a dilution factor of 7.5 for cheese.

Data Analysis

The statistical analysis of the data was performed using the MINITAB Statistical Software, Release13.1.

RESULTS AND DISCUSSION

In the present survey, the mean aflatoxins M_1 in pasteurized and UHT milk were 23.22 and 19.53 ng L^{-1}, respectively. The level of AFM_1 contamination in milk was not very high. Mean AFM_1 contamination in milk samples was not over the permissible level of 50 ng L^{-1} as accepted in most European countries (Table1).

Cream separation can affect AFM_1 distribution, since 80% is partitioned in the skim milk portion (Grant and Carlson, 1971) and an amount of 30% is associated with the non-fat milk solids, particularly casein. The behavior of AFM_1 in the processes in which fat separation is involved may be explained by its semi polar character. It is a water soluble compound binding with hydrophobic sides of casein, thus leading to predominance in the non-fat fraction (Van Egmond and Paulsch, 1986). In cheese samples higher level of AFM_1 were detected, mean AFM_1 contaminations being 43.31 and 21.96 ng kg^{-1} for Feta and creamy cheese, respectively (Table1). Mean AFM_1 concentrations among the milk and Feta cheese samples were found to be different.

Some results about aflatoxin M_1 contamination in milk products have been reported in Iran (Table 2). In Tehran, 1982, the occurrence of AFM_1 in 52 liquid milk samples was studied. Contamination with AFM_1 was reported at concentrations between 23 and 3000 ng L^{-1} (Karim *et al.,* 1982). In another study 73 milk samples delivered to Tehran milk pasteurization plants were analyzed for AFM_1. All the contaminated samples had a level of AFM_1 above the European countries' standard, which is 50 ng L^{-1} (Karim *et al.,* 1998).

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AFM_1 contamination of 624 pasteurized milk samples was studied in Shiraz (Alborzi *et al.,* 2006). AFM_1 was found in 100% of the samples and in some cases, it was greater than the maximum tolerance limit (50 ng L^{-1}) accepted by European Union. Recently, Oveisi *et al.* (2007) reported that the occurrence of contamination of aflatoxin M_1 in pasteurized liquid milk was 72.2 ng L^{-1} in Tehran. There exists only one study about AFM_1 contamination in cheese in Iran. Kamkar found AFM_1 in 82.5% of 80 samples of Feta cheese examined. The range of contamination levels varied among different months, the mean value being was 0.41 μg kg^{-1}.

In many studies, the level of cheese contamination by AFM_1 seems to vary. These variations may be explained in part by different reasonings such as cheese manufacturing procedures, variation in the original milk contamination, type of cheese, condition of cheese ripening, and the analytical methods employed (Galvano *et al.,* 1996). In addition, AFM_1 level in milk was significantly affected by the geographical region, the country, and as well by the season. It is demonstrated that summer milk is less contaminated than milk produced in the winter.

CONCLUSIONS

The results of this study indicate that the occurrence of aflatoxin M_1 contamination in the samples were low in the study region, most probably because of uncontaminated food collections consumed by the milking cows. It can be concluded that the contamination of aflatoxin M_1 in dairy products produced and commercialized in Urmia does not appear to be a serious public health problem at the moment. However, more samples of the dairy products will have to be taken for analysis, and more surveys conducted over a wider and more extended period.

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بررسی وقوع آفلاتوکسین M_1 در محصولات لبنی مورد فروش در ارومیه ، ایران

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چکیده

هدف از این مطالعه ارزیابی وقوع طبیعی و اندازه گیری محتوای آفلاتوکسین M_1 در محصولات لبنی واقع در شهر ارومیه بود. در طول سپتامبر ۲۰۰۷ ، ۴۰ نمونه از شیر پاستوریزه ، ۴۰ نمونه از شیر استریلیزه (UHT)، ۴۰ نمونه پنیر خامه‌ای و ۴۰ نمونه از پنیر ایرانی فتا از سوپرمارکت‌های واقع در سطح شهر ارومیه جمع آوری شد. مقدار آفلاتوکسین M_1 نمونه‌ها با استفاده از روش ELISA مورد ارزیابی قرار گرفت. آنالیز نمونه‌های شیر نشان داد که میانگین آلودگی به AFM_1 در آنها کمتر از میزان مجاز مطابق قوانین اتحادیه اروپا (حداکثر ۵۰ نانوگرم در لیتر) است. میانگین آلودگی در نمونه‌های شیر پاستوریزه و استریلیزه به ترتیب ۸/۶۵±۲۳/۲۲ و ۷/۴۷± ۱۹/۵۳ نانوگرم در لیتر بود. میانگین آلودگی در پنیر فتا برابر ۱۸/۵۱±۴۳/۳۱ و در پنیر خامه‌ای برابر ۳/۲۳±۲۱/۹۶ نانوگرم بر کیلوگرم بود. خطر بالقوه مصرف آفلاتوکسین از طریق محصولات لبنی شناخته شده است. بنابراین ، محصولات لبنی باید برای آفلاتوکسین مورد ارزیابی قرار بگیرند و در حد امکان از آلودگی‌های قارچی در آنها جلوگیری شود.

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