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Aflatoxin M_1 Levels of Skim Milk Powders Produced in Turkey

ORGUN DEVECİ* AND EMEL SEZGİN

Dairy Technology Department, Faculty of Agriculture, Ankara University, Dıskapi, Ankara, Turkey

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ABSTRACT

In this study AFM_1 levels of skim milk powders produced in Turkey were determined by immunoaffinity column and HPLC and the results were compared with the values accepted by Turkish Aliment Codex. AFM_1 levels of 21 skim milk powder samples collected from seven firms in four different seasons (March-April-May, June-July-August, September-October-November and December-January-February) ranged between 0 to 0.705 μg/kg. Two samples (0.535 and 0.705 μg/kg) had exceeded the tolerance limit accepted by Turkish Aliment Codex (0.5 μg/kg). It was also recorded that these two samples collected in the second season (June-July-August) had no M_1 content. From the point of seasonal variation, it was realized that AFM_1 contents of the samples collected in summer were lower than that of the samples collected in the winter. Seasonal variations with regard to AFM_1 were statistically significant ($p < 0.01$). As a result, AFM_1 levels in 90.5% of the samples provided throughout the year did not exceed the maximum tolerance limit established by Turkish Aliment Codex.

Key words: Aflatoxin M_1, skim milk powder, contamination

INTRODUCTION

Aflatoxin M_1 (AFM_1) is a hydroxylated metabolite of the potent carcinogen aflatoxin B_1 (AFB_1), which is produced by strains of molds namely *Aspergillus flavus* and *Aspergillus parasiticus* during their growth on feeds, foods and various biological materials[1,2,3]. Secretion of AFM_1 into milk as a percentage of AFB_1 consumed varies from 0.35% to 3%[4,5].

Although, AFM_1 is less mutagenic and carcinogenic than AFB_1, it exhibits high genotoxic activity and has been recently evaluated by the International Agency for Research on Cancer (IARC) as a class 2B, possibly carcinogenic to humans[6,7]. Therefore, clearly there is a concern about potential health effects of AFM_1 in milk. To reduce the risk of exposure, many countries have established maximum levels of permissible AFM_1 in fluid milk and other milk products varying from 0.05 to 1.0 μg/L[8].

Milk has the greatest demonstrated potential for introducing aflatoxin residues from edible animal tissues into the human diet[7,9]. Moreover, as milk is the main dietary component for children who are more sensitive than adults, the presence of AFM_1 in human breast milk, in commercially available milk and milk products is one of the most serious problems of food safety.

The objective of the present study was to investigate the contamination level of Aflatoxin M_1 in spray dried skim milk powders produced in Turkey and to report the compliance of the results with the Turkish Aliment Codex.

* Author for correspondence. Tel: +90-312-3170550 ext. 1739; Fax: +90-312-3182219; E-mail: deveci@agri.ankara.edu.tr

MATERIALS AND METHODS

I. *Samples*

Twenty one samples of spray dried skim milk powder obtained from seven milk powder producers in Turkey were analyzed to determine their AFM_1 levels. All samples were collected during the period of March 2002 to February 2003 for four different seasons (March-April-May, June-July-August, September-October-November, December-January-February). Samples were analyzed in duplicate.

(I) *Methods*

AFM_1 in milk powder was extracted using the immunoaffinity column method, as reported in ISO 14501[10]. Briefly, 10 g of skim milk powder was reconstitued in 50 mL of water. Prepared reconstitued milk was quantitatively transferred to a 100 mL volumetric flask and was diluted to the 100 mL mark with water. After reconstitued milk was warmed to 37°C and centrifuged at 4000 rpm for 10 min, the upper thin fat layer was discarded. The residue was filtered through Whatman No. 4 filter paper. The filtrate (at least 50 mL) was then passed through an immunoaffinity column (Vicam AFLA M_1) containing specific monoclonal antibodies bound to a solid support material. As the sample passed through the column, the antibodies selectively bound with present AFM_1 and formed an antibody-antigen complex. The toxin was slowly eluted from the column using 3 mL of acetonitrile and the final extract was evaporated to 300 μL under the nitrogen steam and finally re-dissolved in the HPLC mobile phase to 3 mL again. The volume of injection into HPLC was 100 μL.

Determination of AFM_1 was carried out by a high performance liquid chromatographic system (HPLC) (Hewlett Packard, 1100) equipped with a C18 Hicrom column, 5 μm, 250 × 4.6 mm. The mobile phase that consisted of acetonitrile-water (25:75, v/v) was delivered to the column at a rate of 1 mL/min. Under these conditions AFM_1 was eluted from the column at about 10.5 min (Figure 1). Fluorescence detector was used (Hewlett Packard, G-1321A) with excitation wavelength of 360 nm and emission wavelength of 430 nm.

Pure AFM_1 standard in the form of cyrstal (Sigma-Aldrich) was dissolved in chloroform to prepare a stock solution. Using this stock solution a series of calibration solutions were prepared in the concentrations of 0.5, 1.0, 2.5, 5.0 and 7.5 AFM_1 ng/mL, respectively. Calibration curves were constructed by plotting the peak area for each calibration solution against the mass of AFM_1 injected (Figure 2). The detection limit was 0.01 μg/kg. To determine the recovery of AFM_1, pure M_1 stock solution was added to three blank skim milk powder samples (BCR-Information Reference Materials, England) at the rate of 0.5 μg/kg. Samples were prepared for analysis as mentioned above. Totally six injections to HPLC were carried out (two injections for each three spiked samples). Their AFM_1 contents were calculated and recovery of AFM_1 was found 99.24%. Analytical results were not corrected for recovery.

Statistical analysis was carried out to determine differences of AFM_1 contents among milk powder samples collected in different seasons. All data were subjected to analysis of variance (ANOVA), whereas differences between means were tested for significance by Duncan's multiple range test in the general linear model of SPSS statistical programme (SPSS ver10.0, SPSS Ltd., Working, UK). Differences between means were considered significant at $p < 0.01$.

RESULTS AND DISCUSSION

The incidence of AFM_1 contamination in skim milk powders were quite high, given that 90.5% of samples were positive (Table 1). In other words, only two samples collected in the second season had no AFM_1 content. On the other hand, AFM_1 content of two samples provided in the first season (March-April-May) from firm 4 and in the

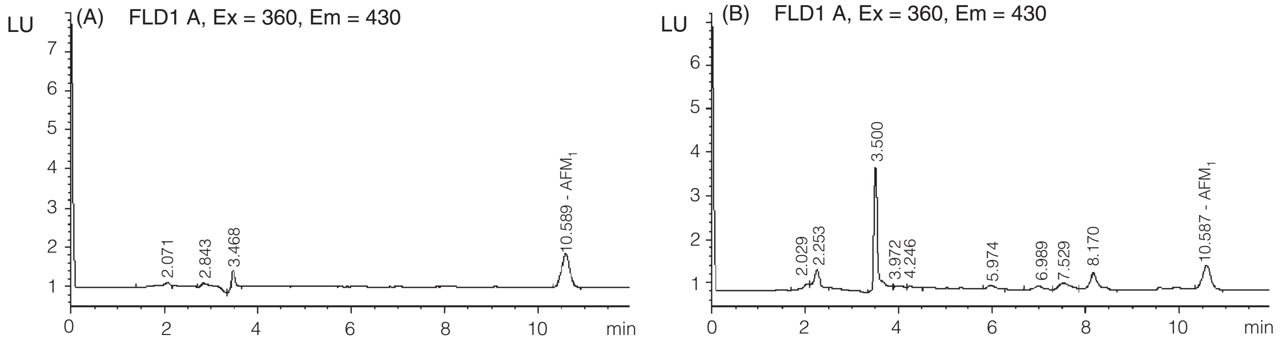

Figure 1. Chromatograms of AFM_1. A: 0.5 ng/mL AFM_1 standard solution, B: representative milk powder sample containing 0.303 μg/kg AFM_1.

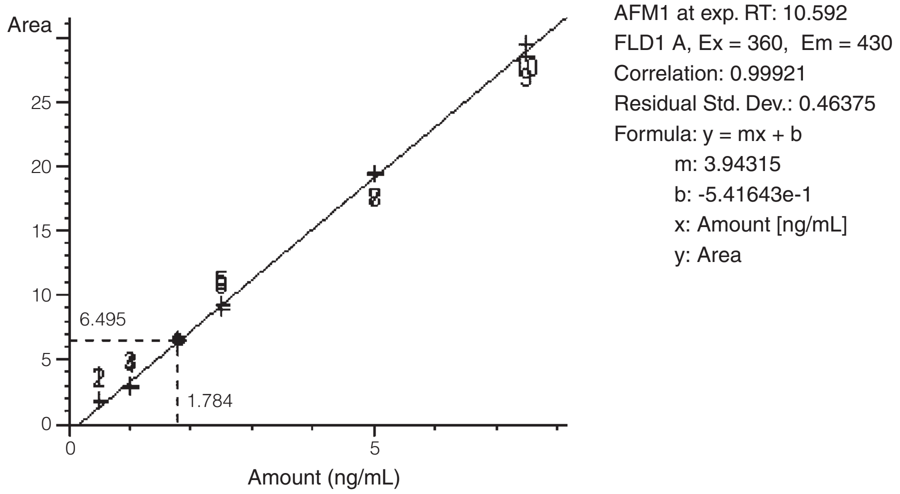

Figure 2. Calibration curve of AFM_1.

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Table 1. AFM_1 contents of skim milk powders collected throughout one year period (μg/kg)

	Season			
Firm	First season (Mar - Apr - May)	Second season (Jun - Jul - Aug)	Third season (Sept - Oct - Nov)	Fourth season (Dec - Jan - Feb)
1	0.303 ± 0.000	BD*	0.705 ± 0.001	0.356 ± 0.009
2	0.202 ± 0.010	BD	NP**	0.372 ± 0.017
3	0.392 ± 0.005	0.309 ± 0.000	0.298 ± 0.005	0.353 ± 0.012
4	0.535 ± 0.000	NP	NP	NP
5	0.483 ± 0.015	0.324 ± 0.001	0.262 ± 0.016	NP
6	0.193 ± 0.009	0.231 ± 0.036	0.494 ± 0.018	NP
7	0.238 ± 0.002	0.273 ± 0.014	NP	0.350 ± 0.080
Minimum	0.193	0.000	0.262	0.350
Maximum	0.535	0.324	0.705	0.372
Average*	0.335^a	0.190^b	0.440^c	0.358^d
Std. Dev.	± 0.138	± 0.150	± 0.204	± 0.010

*BD: Below detection limit.
**NP: No production of skim milk powder.
***Differences between averages of sample with different symbols in same row was found to be significant ($p < 0.01$).

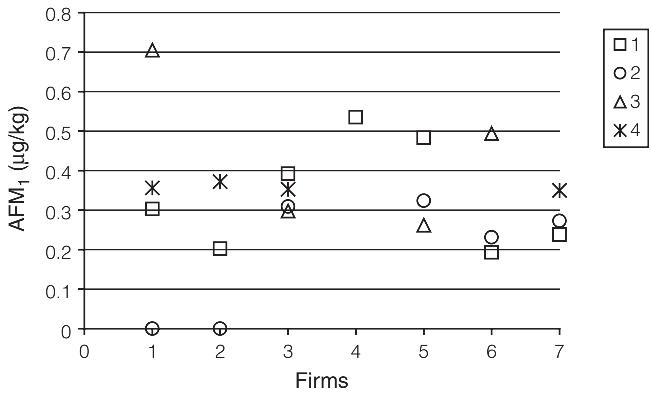

Figure 3. AFM_1 values of skim milk powders against firms.
1: First season (March-April-May).
2: Second season (June-July-August).
3: Third season (September-October-November).
4: Fourth season (December-Janury-February).

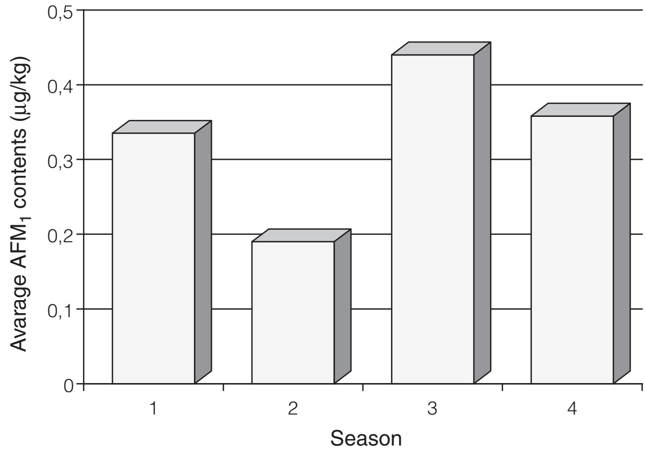

Figure 4. Distribution of AFM_1 levels of skim milk powder samples according to seasons.
1: First season (March-April-May).
2: Second season (June-July-August).
3: Third season (September-October-November).
4: Fourth season (December-Janury-February).

thirth season (September-October-November) from firm 1 were over the legal limit (0.5 μg/kg) according to Turkish Aliment Codex with the level of 0.535 and 0.705 μg/kg, respectively. It was also shown that AFM_1 contents of two samples provided in first season from firm 5 and in third season from firm 6 were found very close to legal limit with the levels of 0.483 and 0.494 μg/kg, respectively (Table 1). AFM_1 levels of skim milk powder samples generally ranged between 0.2 - 0.4 μg/kg (Figure 3).

The season also had an effect on levels of aflatoxin M_1 in the samples. Average AFM_1 levels of skim milk powders collected in the summer were found to be low (0.190 μg/kg) while the highest level of AFM_1 was 0.440 μg/kg collected in the fall. AFM_1 contents of milk powder samples provided by firm 1 and 2 were found 0.303 and 0.202 μg/kg respectively in the spring whereas AFM_1 was not detected in the samples of the same two firms in the summer (Table 1 and Figure 3). In addition, although the sample provided by firm 1 in the summer had no AFM_1 content, the sample from the same firm in autumn had 0.705 μg/kg AFM_1 level (Table 1). Seasonal variations observed in the AFM_1 contents were significant ($p < 0.01$).

The low incidence of AFM_1 in the summer months is likely to be due to the increased use of pasture feeding. These results are in agreement with those of Veringa *et al.*[11], Fritz *et al.*[12] and Galvano *et al.*[13]. They also found that dried milk produced in the grazing season contained much less AFM_1 than that produced in the indoor feeding winter period.

In conclusion, the results of the study showed that AFM_1 contents of 90.5% of skim milk powder collected throughout the year did not exceed the tolerance limit suggested by Turkish Aliment Codex (0.5 μg/kg). However, milk powder has many common uses, such as to increase total dry matter, to enrich protein content and to produce infant formula. Therefore, attention should be given to routine inspection of these products in Turkey.

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