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RESEARCH ARTICLE

Determination of Aflatoxin M_1 Level in Butter Samples Consumed in Erzurum, Turkey

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Summary

In this study; the levels of aflatoxin M_1 (AFM_1) in 80 butter samples were determined. They were obtained from supermarkets in city center of Erzurum. The presence and concentration range of AFM_1 in the samples were investigated by competitive enzyme-linked immunoabsorbent assay (ELISA) method. AFM_1 was found in 66 (82.5%) samples at levels ranging from 10 to 121 ng/kg with mean concentration of 30.4±23.9 ng/kg. The levels of AFM_1 in 13 (16.3%) samples were higher than the maximum legal limit accepted by Codex Alimentarious Commission (CAC). None of the contaminated butter sample exceeded the legal limit regulated by Turkish Food Codex (TFC) for AFM_1. The results indicated that contamination of the butter samples with AFM_1 in high level could be a potential hazard for public health.

Keywords: *Aflatoxin M_1, Butter, ELISA*

Erzurum'da Tüketime Sunulan Tereyağlarında Aflatoxin M_1 Düzeyinin Belirlenmesi

Özet

Bu çalışmada 80 tereyağ örneğinde Aflatoksin M_1 (AFM_1) seviyesi belirlendi. Örnekler Erzurum şehir merkezindeki marketlerden temin edildi. Örneklerin AFM_1 içeriği ve konsantrasyonu kompetitiv ELISA metoduyla araştırıldı. AFM_1 seviyesi 66 örnekte 10 ile 121 ng/kg arasında değişmekte olup ortalama 30.4±23.9 ng/kg olarak bulundu. 13 numunede (16.3%) belirlenen AFM_1 düzeyinin Kodeks Alimentarius Komisyonu (CAC) tarafından düzenlenen yasal limitleri aştığı belirlendi. Kontamine tereyağı örneklerinin hiç birinde Türk Gıda kodeksi limitlerini aşan numuneye rastlanmadı. Bu sonuçlar, tereyağındaki yüksek AFM_1 düzeyinin halk sağlığı açısından risk oluşturabileceğini göstermektedir.

Anahtar sözcükler: *Aflatoksin M_1,Tereyağ, ELISA*

INTRODUCTION

Aflatoxin is a family of highly toxic and carcinogenic fungal metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* and the rare *A. nomius fungi* [1,2]. *A. flavus* produces only B aflatoxins, while the other two species produce both B and G aflatoxins. Aflatoxin M_1 (AFM_1) is the principal hydroxylated aflatoxin metabolite in the milk of dairy cows fed contaminated feed with aflatoxin B_1 (AFB_1). These toxins show a serious risk for animal and human health, particularly for children, who are the major milk consumers. It has been reported several effects of aflatoxins on health such as hepato-toxic, genotoxic, carcinogenic, teratogenic, immuno-

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suppressive and antinutritional [1].

AFM_1 is mainly soluble in the aqueous phase of milk or adsorbed to casein particles; information of several studies show that a small ratio of AFM_1 in milk is carried-over to cream, and yet a smaller proportion to butter. The remainder of AFM_1 in milk, however, remains in skim milk and buttermilk [3].

To protect consumers, several countries have established legislation to regulate the levels of mycotoxins [4,5]. Several factors may influence the establishment of mycotoxin limits and regulations. These include scientific factors such as the availability of toxicological data and survey data, knowledge about the distribution of mycotoxin in commodities,and analytical methodology. Economical and political factors such as commercial interests and sufficiency of food supply have their impact as well [6]. The Codex Alimentarious Commission (CAC) [7] has set a limit of 50 ng/kg for AFM_1 in milk products while the Turkish Food Codex [8] prescribed the maximum level for AFM_1 in milk products 10-fold higher (500 ng/l) than the current level in the CAC.

Dairy products have been produced and consumed widely in Turkey. Owing to the common presence and harmful effects of aflatoxin contamination, there is a need for detection and measurement of AFM_1 in dairy products. Ascribed to scientific literature, very few studies [5,9-12] have been published on the presence and level of AFM_1 in milk and dairy products in Turkey. The present study has been designed to investigate the presence of AFM_1 in butter and to compare the results with the maximum AFM_1 tolerance limits which are accepted by the CAC and TFC.

MATERIAL and METHODS

Samples

A total of 80 butter samples were obtained randomly from supermarkets between September 2007 and September 2009 in Erzurum city. The samples were transported to the laboratory in an insulated container at about 4°C and analyzed upon arrival.

Analysis of AFM_1 by ELISA

The quantitative analysis of AFM_1 in the butter samples was performed by competitive enzyme immunoassay using RIDASCREEN Aflatoxin M_1 30/15 (Art. No: R1111, R-Biopharm, Darmstadt, Germany) [13] test kit. Immunoaffinity column (Rida Aflatoxin Column Art. No: R5001/5002) [14] were used to run ELISA analyses.

Aflatoxin Column

Sample preparation procedures were performed according to the instructions of the test kit (Rida Aflatoxin Column Art. No: R5001/5002) [14] manual. 25 ml of methanol (70%) was added to 5 g of butter. Afterwards, the solution was extracted by mixing gently for 10 min at room temperature. The extract was filtered through a paper filter and 15 ml of distilled water were added to 5 ml of filtered solution. 0.25 ml Tween 20 were added and stirred for 2 min, followed by entire amount of the sample solution (20 ml) passing over the column. Clean up procedure was performed according to the kit's manual. Eluate containing toxin was diluted 1:9 with the sample dilution buffer (supplied with the test kit) and used 100 µl per well in the assay.

Test Procedure of AFM_1

One-hundred microliter of standard solutions and prepared samples were added into separate microtitre wells and incubated for 60 min at room temperature (22-25°C) in the dark. The liquid was then poured out and the wells were washed with washing buffer (250 µl) twice. In the next stage, 100 µl of the diluted enzyme conjugate was added to the wells, mixed gently by shaking the plate manually and incubated for 15 min at room temperature in the dark. Again, the wells were washed twice with washing buffer. Afterwards, 100 µl of substrate/chromogen was added, mixed gently and incubated in the dark at room temperature for 15 min. Finally, 100 µl of the stop reagent (1 N H_2SO_4) was added into the wells and the absorbance was measured at k=450 nm in ELISA plate reader (ELx800, Bio-Tek Instruments, USA) within 10 min.

Evaluation

The samples were evaluated according to the RIDAVIN computer program prepared by R-Biopharm. According to the instructions for use of the RIDASCREEN kit; the lower detection limit was 5 ng/kg.

RESULTS

In this study, a total of 80 butter samples were analysed for AFM_1 with the competitive ELISA. The presence and the distribution of AFM_1 concentration in various ranges in butter samples are presented in *Table 1.* As shown in *Table 1,* AFM_1 was detected in 66 of 80 of the butter samples above the detectable level of 5 ng/kg. In total 16.3% of AFM_1-contaminated samples exceeded the CAC regulation (50 ng/kg). However, none of the contaminated butter sample exceeded the limit (500 ng/kg) reported by TFC for AFM_1. Quantity

(concentration) of AFM1 in butter samples ranged from 10 to 121 ng/kg while the mean value was 30.4 ng/kg (*Table 1*).

Table 1. *Presence and distribution of AFM1 in butter samples*
Tablo 1. *Tereyağ örneklerinin AFM1 içeriği ve dağılımı*

Kind of Samples	Samples Tested (n)	Proportion of Positive Samples n (%)	Distribution of Samples [a] n (%)					Proportion of Samples Exceeding the CAC Legal Limit >50 ng/kg	Proportion of Samples Exceeding the TFC >500 ng/kg	Quantity of AFM1 (ng/kg)		
			<5*	5-25	26-50	51-100	>100			x±Sx	Min.	Max.
Butter	80	(82.5)	(17.5)	(28.8)	(37.5)	(15)	(1.3)	(16.3)	ND	30.4±23.9	10	121

** distribution of negative samples, **a:** ng/kg, **CAC:** Codex Alimentarious Commission, **TFC:** Turkish Food Codex, **( ):** indicates percent, **ND:** Not Detected, **x±Sx:** mean±standart deviation*
** negatif örneklerin dağılımı, **a:** ng/kg, **CAC:** Kodeks Alimentarius Komisyonu, **TFC:** Türk Gıda Kodeksi, **( ):** yüzde ifadesi, **ND:** Saptanmadı, **x±Sx:** ortalama±standat sapma*

Table 2. *Aflatoxin M1 contents of butter reported in previous studies*
Tablo 2. *Önceki çalışmalarda tereyağında bildirilen AFM1 içerikleri*

Sample	Country	No. of Samples Positive	Range of Samples Positive (ng/kg)	Exceed Legal Limit *	References
Butter	Turkey	92/92 (100)	10-7000	26/92 (28.3)	Tekinsen and Ucar [18]
	Turkey	5/5 (100)	13.5-16.6	0/5 (0)	Bakirci [19]
	Turkey	52/61(81)	ND	10/61 (16.4)	Aycicek et al. [15]
	Turkey	25/27 (92.6)	1-100	10/27 (3.7)	Aycicek et al. [16]

** CAC limits in butter is 50 ng/kg, **ND:** Not Detected, **( ):** indicates percent*
** Kodeks Alimentarius Komisyonu tereyağı limiti 50 ng/kg, **ND:** Saptanmadı, **( ):** yüzde ifadesi*

DISCUSSION

Dairy products play a significant role in human diet since they are rich sources of bioavailable calcium and proteins. However, many of the previous studies indicate the presence of AFM1 at high concentrations in dairy products [5,9-12].

A few studies in Turkey have addressed the issue of AFM1 contamination of butter. AFM1 levels determined in the butter consumed in Turkey by previous reports are indicated in *Table 2*.

As shown in *Table 1*, AFM1 was detected in 82.5% of the butter samples. These results are in parallel with the findings of Aycicek et al.[15,16]. In present study the contamination level of AFM1(as incidence) in butter samples was found to be low as compared to the results of earlier studies in the same area [17,18]. In a study by Tekinsen and Ucar [18], the number of AFM1 positive butter samples as well as the maximum AFM1 level is higher than the values reported in our study. These differences might be due to the differences in the AFM1 levels in the milk from which the butter is produced [19] and in the processing method of milk or due to the differences in the methods of analysis [20]. Moreover, differences in the hygiene and storage conditions at the dairies and sales department are other factors on the variations of the results [18,20,21]. In addition, the AFM1 level in the milk was signigicantly affected by the geographical region and the country [20]. The lower incidence of AFM1 found in butter samples may due to the number of samples analysed than other researches done in Turkey.

In this study, AFM1 concentration in contaminated butter samples exceeding CAC legal limit was lower than the results reported by Tekinsen and Ucar [18] and were higher than the results reported by Bakirci [17] and Aycicek et al.[16]. Similarly, results reported by Aycicek et al.[15] indicated that 10/61 (16.4%) samples had levels higher than CAC regulation.

The TFC has updated the maximum allowable AFM1 limit as 500 ng/kg for the foodstuff that has a potential to contain aflatoxin. This limit have been 50 ng/kg in the previous years. In the previous studies, the number of samples exceeding the Turkish legal limits has been high owing to that reason. The fact that no samples exceeding the legal limits regulated by the TFC encountered in our study is partially due to that reason. The recent limits put forward by the latest regulations are thought to be considerably high (10 times higher than that of the CAC limits) and a re-consideration of this arrangement is

thought to be required.

Shortly, the results of this study show that there is a risk from butter produced from milk obtained from animals fed with contaminated animals feed with aflatoxins, since all the age groups including infants and children consume milk and dairy products daily. The AFM_1 level of butter samples is closely related to the aflatoxins concentration in milk used for butter production and other related factors. Therefore the prevention of aflatoxin formation in feeds is very important. Avoiding contamination appears to be the only practical way to ensure the safety of milk and milk products for human consumption. For this reason, it is considered that food substances should be produced from healthy raw material and kept in convenient conditions to prevent aflatoxin formation.

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