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RESEARCH ARTICLE

Occurrence of Aflatoxin M_1 in UHT Milk in Erzurum-Turkey

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Summary

In this study, 150 UHT milk samples were analyzed for aflatoxin M_1. They were obtained from supermarkets in Erzurum city. The occurrence and concentration range of AFM_1 in the samples were investigated by ELISA method. Fifty-nine percent of the UHT milk samples contained AFM_1. AFM_1 levels in 16 (10.7%) UHT milk samples exceeded the maximum tolerable limit of the European Community and the Turkish Food Codex. It was concluded that high AFM1 level is an important problem threatening the public health in Turkey.

Keywords: *Aflatoxin M1, UHT milk, ELISA*

Türkiye (Erzurum)'de UHT Sütlerde Aflatoxin M_1 Oluşumu

Özet

Bu çalışmada toplam 150 UHT süt örneği AFM_1 yönünden incelendi. Örnekler Erzurum şehir merkezindeki marketlerden temin edildi. Örneklerin AFM_1 içeriği ve konsantrasyonu kompetitiv ELISA metoduyla araştırıldı. UHT süt örneklerinin %59'unun AFM_1 içerdiği belirlendi. 16 numunenin (10.7%) AFM_1 yönünden Türk Gıda Kodeksi ve Avrupa Birliği tarafından düzenlenen yasal limitleri aştığı belirlendi. Sonuç olarak yüksek AFM_1 düzeyi Türkiye'de halk sağlığını tehdit eden önemli bir problemdir.

Anahtar sözcükler: *Aflatoksin M1, UHT süt, ELISA*

INTRODUCTION

Aflatoxin is common contaminant of foods. This toxins is produced by fungal action during production, harvest, storage, and food processing. The toxin is produced as secondary metabolites by *Aspergillus flavus* and *A. 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. Aflatoxins M_1 and M_1 are the hydroxylated metabolites of aflatoxin B_1 and B_1. International Agency for Research and Cancer (IARC) [3] of WHO included AFB1 as primary and AFM_1 as secondary groups of carcinogenic compounds [4,5].

The residues of AFM_1 remain stable when milk is processed by heat or is fermented. There is no evidence that cold storage, freezing, heat-treating, fermenting, concentrating or drying of the contaminated milk changes the level of AFM_1 [6-8]. AFM_1 is mainly soluble in the aqueous phase of milk or adsorbed to casein particles; data of several studies show that a small proportion of AFM1 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 [9].

Milk is a major nutrient for infants, children, convalescents and old people. However, milk and milk products are the most potent source of aflatoxin among foods. To protect consumers several countries have established legislation to regulate the levels of AFB_1 in

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feeds and AFM_1 in milk and dairy products [10-12].

The European Community (EC) and the Turkish Food Codex (TFC) legal limit for AFM_1 in milk is 50 ng/kg [13,14]. Despite lots of studies on AFM_1 in cheese varieties [11,15-18] only a few are concerned with UHT milk. The aim of this study was to investigate the occurrence of AFM_1 in UHT milk and to compare the results with the maximum AFM_1 tolerance limits which are accepted by the EC and TFC.

MATERIAL and METHODS

Samples: A total of 150 samples of UHT milk (whole milk) samples were obtained randomly from supermarkets between September 2006 and September 2007 in Erzurum city. The samples were transported to the laboratory in an insulated container at about 4°C and analyzed upon arrival. All samples were analyzed before their expiry date.

Methods: AFM_1 concentrations of the samples were analyzed by competitive ELISA (RIDASCREEN Aflatoxin M_1, R-Biopharm). The samples were evaluated according to the RIDAVIN computer program prepared by R-Biopharm [19]. According to the instructions for use of the RIDASCREEN kit; the lower detection limit was 5 ng/kg.

Calculation of extrapolated values of AFB_1 concentration in cattle feeding stuffs based on AFM_1 in UHT milk samples

It has been suggested that only 1.6% of ingested AFB_1 is converted to AFM_1 by the dairy cattle [10,20-22].

Hence, the values of AFB_1 contamination in feeding stuffs were back calculated by using the formula:

$$AFB_1\ (\mu g/kg) = \frac{AFM_1\ (ng/kg) \times 100}{1.6 \times 1000}$$

RESULTS

In this study a total of 150 UHT milk (whole milk) samples were analyzed for AFM_1 with the competitive ELISA. The occurrence and the distribution of AFM_1 concentration in various ranges in UHT milk samples are presented in *Table 1*.

As shown in *Table 1*, AFM_1 was found above the detectable level in 59% (89/150) in UHT milk samples. AFM_1 levels in 16/150 (10.7%) UHT milk samples were found to be higher than the maximum acceptable limits (milk; 50 ng/kg) of the EC [13] and TFC [14]. AFM_1 content of positive samples were determined in UHT milk samples as minimum 5 ng/kg, maximum 185 ng/kg, and mean 36±38 ng/kg.

Earlier studies have shown that contamination of AFM_1 in milk and dairy products is a result of exposure of AFB_1 to dairy cattle through feedstuffs [23]. Further, investigators have suggested that on an average 1.6% of AFB_1 fed to the lactating cattle is excreted in milk as AFM_1 [20,21]. Using this factor, the content of AFB_1 in the dairy cattle feeding stuffs was extrapolated from AFM_1 contamination in the UHT milk samples *(Table 2)*.

Table 1. *Occurrence and distribution of AFM_1 in UHT milk samples*
Tablo 1. *UHT süt örneklerinin AFM_1 içeriği ve dağılımı*

Kind of Samples	Samples Tested (n)	Proportion of Positive Samples n (%)	Distribution of Samples [a] n (%)					Proportion of Samples Exceeding the EC and TFC Legal Limit >50 ng/kg	Quantity of AFM_1 (ng/kg)		
			<5*	5-25	26-50	51-100	>100		x±Sx	Min.	Max.
UHT Milk	150	89/150 (59)	61/150 (40.0)	42/150 (28)	31/150 (20.7)	10/150 (6.7)	6/150 (4)	16/150 (10.7)	36±38	5	185

** distribution of negative samples, **a:** ng/kg, **EC:** European Community, **TFC:** Turkish Food Codex, **( ):** indicates percent,*
x±Sx: *mean±standart deviation*
** negatif örneklerin dağılımı, **a:** ng/kg, **EC:** Avrupa Birliği, **TFC:** Türk Gıda Kodeksi, **( ):** yüzde ifadesi, **x±Sx:** ortalama±standat sapma*

Table 2. *Extrapolated AFB_1 concentration in cattle feedstuffs based on AFM_1 contamination in UHT milk samples*
Tablo 2. *UHT süt örneklerin AFM_1 kontaminasyonuna dayanılarak tahmin edilen AFB_1 konsantrasyonları*

Kind of Samples	Samples Tested (n)	Level of Positive Samples n (%)	Range (µ/kg)	Exceeding EC and TFC (5 µg/kg)	Positive Samples (µ/kg)		
					x±Sx	Min.	Max.
UHT Milk	150	89/150 (59)	0-11.6	6/150 (4)	2.3±2.4	0.3	11.6

x±Sx: *mean±standart deviation, **EC:** European Community, **TFC:** Turkish Food Codex, **( ):** indicates percent*
x±Sx: *ortalama±standat sapma, **EC:** Avrupa Birliği, **TFC:** Türk Gıda Kodeksi, **( ):** yüzde ifadesi*

It can be seen from the results that the contamination of feed with AFB_1 in cattle feed may range of 0-11.6, with an average of 2.3±2.4 µg/kg. Moreover, 4% (6/150) of the samples exceeded the limits recommended by EC and TFC regulations.

In some studies made on UHT milk, presence and level of AFM_1 were showed in *Table 3*.

Table 3. *AFM1 contents of UHT milk reported in previous studies*
Tablo 3. *Önceki çalışmalarda UHT sütlerde bildirilen AFM1 içerikleri*

Sample	Country	No. of Samples Positive	Range of Samples Positive (ng/kg)	Exceed Legal Limit *	References
UHT Milk	Turkey	75/129 (58.1)	10-543.6	61/129 (47)	Unusan [22]
	Portugal	60/70 (85.7)	5-61	20/70 (2.9)	Martin and Martin [27]
	Turkey	14/24 (58.3)	10-50.5	1/24(4.2)	Gurbay et al.[24]
	Turkey	67/100 (67)	10-630	31/100 (31)	Tekinsen and Eken [25]

** Turkish Food Codex limits in UHT milk is 50 ng/kg,* ***( ):*** *indicates percent*
** Türk Gıda Kodeksi UHT süt limiti 50 ng/kg,* ***( ):*** *yüzde ifadesi*

DISCUSSION

In our study, AFM_1 was determined in 59% of the UHT milk samples. These results are in parallel with the findings of some previous reports [22,24,25] which pointed out the presence of AFM_1 in all or most of the UHT milk samples in Turkey *(Table 3)*.

These reports and the present findings suggest that the milk which is processed in to dairy products may contain a high concentration of AFM_1 and/or be contaminated with *Aspergillus spp.* The results confirm the findings of Bakirci [26] who reported high concentrations of the AFM_1 in raw milk during spring. The AFM_1 levels (as incidence) also were lover than the reported results by Martin and Martin [27], Oliveira and Ferraz [28]. In addition, the AFM_1 level in the milk was significantly affected by the geographical region and the country [16,29]. Moreover, differences in the hygiene and storage conditions at the dairies and retail points are other key factors on the variations of the results [30,31].

Some of the AFM_1 amounts in the UHT milk samples were at the risk level for human health because the AFM_1 in 16 UHT milk samples (10.7%) exceeded the EC and TFC legal limits of 50 ng/kg. In this study AFM_1 incidence of exceeding legal limit in UHT milk samples was lower than the reported results by Unusan [22], Tekinsen and Eken [25], Oliveira and Ferraz [28] and were higher than the results reported by Gurbay et al.[24], Martin and Martin [27]. Very high AFM_1 levels (51 - >100 ng/kg) in 10.7% of the UHT milk samples are of great importance. Also it should be kept in mind that, total daily aflatoxin intake from other foods could be an important risk factor for people as well. The weighted mean concentration of AFM_1 in milk is 0.023 µg/kg in the European-type diet, 0.022 µg/kg in the Latin American diet, 0.36 µg/kg in the Far Eastern diet, 0.005 µg/kg in the Middle Eastern diet and 0.002 µg/kg in the African diet. These mean concentrations are based on a large number of milk samples analyzed. The intake of AFM_1 from milk is calculated to be 6.8 ng/person per day for the European diet, 3.5 ng/person per day for the Latin American diet, 12 ng/person per day for the Far Eastern diet, 0.7 ng/person per day for the Middle Eastern diet and 0.1 ng/person per day for the African diet [2,22].

The content of AFB_1 in the dairy cattle feeding stuffs was extrapolated from AFM_1 contamination in the UHT milk samples *(Table 2)*. In a previous study, contamination of AFB_1 in Turkey cattle feedstuffs was found to be in the range of 0-33.98 µg/kg [22]. At the present study, AFB_1 incidence of exceeding legal limit in UHT milk samples was lower than the reported results by Unusan [22].

In conclusion, AFM_1 is common contaminant UHT milk can be considered to be a main concern for public health. So the public health authorities should take necessary measures and the producers should be informed. Moreover the prevention of aflatoxin formation in feeds is very important. Because the consumption of contaminated feeds by dairy animals causes AFM_1 formation in milk. So the easiest and shortest way of reducing AFM_1 amount forming in milk to minimum focuses on the prevention of AFB_1 formation in feeds. For this, it is necessary to control well the feeds given to dairy animals and to reduce AFB_1 amount permitted to take place in feeds to lower levels. In addition, it is considered that food substances should be produced and kept in convenient conditions to prevent aflatoxin formation.

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