



## Determination of aflatoxin M1 levels by elisa method in örgü (Knitted) cheese produced by traditional methods in Diyarbakır, Turkey

*Diyarbakır'da geleneksel yöntemlerle üretilen örgü peynirlerinde aflatoksin M1 düzeylerinin elisa metodu ile belirlenmesi*

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### MAKALE BİLGİSİ

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### ABSTRACT

In this study, the contamination levels of Aflatoxin M1 were determined on 184 Diyarbakır Örgü Cheese (DÖC) samples between May and December 2013 were taken from randomly selected markets of Diyarbakır centrum by ELISA method. Since there is only one study about the presence of AFM1 in DÖC, the results were mostly compared with other types of cheese. On the other hand, results of the study were compared with the European Commission's AFM1 level. The average AFM1 values of the samples were determined as 167.58 ng/kg. The minimum and maximum AFM1 values were detected as 50 and 800 ng/kg, respectively. The number of samples exceeding the limit specified for AFM1 in the European Commission are 32 (17.39%) samples. AFM1 contamination levels of 53 (28.80%), 73 (39.67%) and in 26 (14.13%) samples were determined as 51-250 ng/kg, 51-150 ng/kg and 0-50 ng/kg, respectively. The results of the analysis show that the AFM1 levels of the DÖC offered for sale at different points in Diyarbakır have a potential risk for consumers.

**Keywords:** Diyarbakır Örgü Cheese, knitted, ELISA, AFM1.

### ÖZET

Bu çalışmada, 2013 yılı Mayıs ve Aralık ayları arasında Diyarbakır merkez pazarlarından rastgele seçilmiş olan 184 Diyarbakır örgü peyniri örneklerinin aflatoksin M1 kontaminasyon seviyeleri Elisa yöntemi ile araştırılmıştır. Diyarbakır Örgü Peyniri'nde AFM1 varlığı ile ilgili tek bir çalışma olduğundan, sonuçlar çoğunlukla diğer peynir türleriyle karşılaştırıldı. Öte yandan, çalışmanın sonuçları Avrupa Komisyonu'nun AFM1 düzeyi ile karşılaştırıldı. Örneklerin ortalama AFM1 değerleri 167.58 ng / kg olarak belirlenmiştir. Minimum ve maksimum AFM1 değerleri sırasıyla 50 ve 800 ng / kg olarak tespit edildi. Avrupa Komisyonunda AFM1 için belirlenen sınırı aşan örnek sayısı 32 (% 17.39)' dir. AFM1 kontaminasyon seviyeleri; 53 (% 28.80), 73 (% 39.67) ve 26 (% 14.13) numunede sırasıyla 51-250 ng / kg, 51-150 ng / kg ve 0-50 ng / kg olarak belirlenmiştir. Analiz sonuçları, Diyarbakır'da farklı noktalarda satışa sunulan Diyarbakır Örgü Peynirinin AFM1 seviyelerinin tüketiciler için potansiyel bir risk taşıdığını göstermektedir.

**Anahtar Kelimeler:** Diyarbakır Örgü Peyniri, örgü, ELISA, AFM1.

## 1. INTRODUCTION

Presence of aflatoxins is one of the major food safety concerns in tropical and subtropical countries [1]. *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* species produce mycotoxins called zearalenone, ochratoxins, verotoxins, patulin, trichothecene, fumonisin and aflatoxins in animal feed and food under suitable climatic conditions. Aflatoxins among mycotoxins are toxic, mutagenic, teratogenic, immunosuppressive compounds for human and animals [2-7]. They cause especially on skin diseases and liver disorders [6].

Aflatoxins can be produced by *Aspergillus flavus*, *Aspergillus parasiticus* and rarely by *Aspergillus nomius*. *Aspergillus flavus* produces only B aflatoxins, while the other two species produce both B and G aflatoxins. Aflatoxin M1 (AFM1) and M2 (AFM2) are the hydroxylate metabolites of Aflatoxin B1 (AFB1) and B2 (AFB2) (22; 31). The mammals which digest AFB1-contaminating diet convert AFB1 to AFM1 known as "milk toxin" [7].

AFM1 is mainly soluble in the aqueous phase of milk or adsorbed to casein particles; a small ratio of AFM1 in milk is carried-over to cream. The remainder of AFM1 in milk, however, remains in skim milk and buttermilk (8). In other words, the stability of AFM1 determines its persistence in foodstuffs such as butter, yogurt, cheese, cream and ice cream. However, this toxin is not inactivated by the thermal processing (pasteurization and ultra-high-temperature (UHT) treatment) used in the dairy industry [9].

Human exposure to AFM1 is due to the consumption of contaminated milk and dairy products of which daily intake could be highly variable in the world. Infants represent the most susceptible exposed population due to their high consumption of dairy products either as animal's milk and related by-products in their diet or from breast milk where the mycotoxin can be excreted (10). The Joint FAO/WHO Expert Committee on Food Additives reported that the intake of AFM1 from milk was 6.8 ng/person/day for the European diet, 0.7 ng/person/day for the Middle Eastern diet, 12 ng/person/day for the Far Eastern diet, 3.5 ng/person/day for the Latin American diet and 0.1 ng/person/day for the African diet [11]. Therefore, many countries have set legal regulations to control aflatoxin M1 level in milk and dairy products.

In this study, AFM1 contamination levels of DÖC were determined. DÖC, which is a traditional Anatolian cheese, is produced mostly in the Karacadağ Basin situated in the South of the province of Diyarbakır. Due to the small arable areas of the basin and the presence of large meadow-pasture areas,

animal husbandry, especially sheep farming, is carried out in general [12]. DÖC is widely consumed in Diyarbakır and surrounding provinces, and is sold just above the cost in markets in cities such as Istanbul, Ankara and İzmir. The production process of DÖC is similar to that of pasta-filata cheeses such as Mozzarella, Caciocavallo and Povola dei Nebrodi. Additionally, it has been received Protected Denomination of Origin certificate (certificate of geographical origin) since 2010.

The results of the study were compared with the European Commission's AFM1 level [13].

## 2. MATERIAL AND METHODS

### 2.1. Collecting of samples

In this study, 184 traditional DÖC samples (each sample about 250 g) between May and December 2013 were taken to sterile sampling bags from randomly selected markets of Diyarbakır centrum. By the sampling procedure, attention was paid to collect different brands and lots. The samples were delivered to the laboratory by preserving the cold chain.

### 2.2. Preparation of the samples

It was conducted according to the method reported by Ardic et al. (2009) [14]. 40 mL of dichloromethane was added to 2 g of DÖC, which had been thoroughly shredded and crushed into a stomacher bag, and homogenized on a stomacher for 2 minutes. After the obtained suspension was filtered, 10 mL of the extract was taken and evaporated at 60 °C. The residue, with the addition of 0.5 mL of methanol, 0.5 mL of PBS buffer and 1 mL of heptane, was centrifuged at 2700 rpm for 15 min (15 °C). One-hundred microliters of this aliquot brought up to a 10% methanol content by addition of 400 µL Ridascreen buffer 1 and 100 µL was used per well in the test.

### 2.3. Aflatoxin M1 test procedure

100 µL of the prepared sample and the standards were incubated for 60 minutes. Plates were washed 2 times in the automatic washer, then 100 µL of enzyme conjugate was added and incubated for 60 minutes in room temperature. After 3 washes in the automatic washer, 100 µL of substrate/chromogen was added to each well, thoroughly mixed, and incubated for 30 minutes in the dark at room temperature. After adding 100 µL stock solution to each well, it was thoroughly mixed and read at 450 nm with a packet program. Dilution factor was accepted as "10" for cheese samples.

### 2.4. Determination of the presence of Aflatoxin M1 by ELISA method

The presence of aflatoxin M1 was determined by the ELISA method using Ridascreen® Aflatoxin M1

Art. No. ELX 50 Automatic Microplate Washer and ELX 800 Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA) devices with the ELISA test kit R1101 (RBIopharm AG, Darmstadt, Germany). The results were evaluated in the RIDAWIN Package Program (RIDA®SOFT Win, RBIopharm AG, Darmstadt, Germany).

### 2.5. Statistical analysis

The results were analyzed by SPSS statistical program for Windows developed by IBM (IBM® SPSS© Statistics Version 21). Results were expressed as mean and also as minimum and maximum concentration of AFM1.

### 3. RESULTS AND DISCUSSION

The cheese samples AFM1 values are presented in Table 1 and Table 2. According to the results, AFM1 was detected in 158 samples of 184 (85.87%). The average AFM1 value was 164.73 ng/kg; the minimum and maximum values were determined as 51.73 ng/kg and 800 ng/kg. 17.39% of the samples (32 samples), were found to exceed the limit for AFM1 (250 ng/kg) of the European Commission (13). 28.80% of the samples (53 samples) AFM1 values were between 151-250 ng/kg. 39.67% of the samples (73 samples) were between 51-150 ng/kg and 14.13% of the samples (26 samples) were between 0-50 ng/kg.

**Table 1.** Different range distribution of Aflatoxin M<sub>1</sub> contents in DÖC samples.

Aflatoxin M <sub>1</sub> Level (ng/kg)	The Number of Samples (n=184)	AFM <sub>1</sub> Detected Sample
	<u>n</u>	%
ND <sup>a</sup> <50	26	14.13
51-150	73	39.67
151-250	53	28.80
250<	32	17.39

ND: Not detected (Aflatoxin M1 level less than 50 ng/kg).

**Table 2.** Aflatoxin M<sub>1</sub> levels in DÖC samples.

The Number of Samples			Concentration (ng/kg)			
Positive	Negative	Exceeding the Acceptance Limit <sup>b</sup>	Total Sample Mean ±SEM	Positive Sample Mean±SEM	Min.	Max.
158 <sup>a</sup> (%85.87)	26 <sup>a</sup> (%14.13)	32(%17.39)	164.73±18.2	183.61±24.3	51.73	800

<sup>a</sup>: The number of samples.

<sup>b</sup>: European Commission limit (250 ng/kg).

Since there is only one study (15) about the presence of AFM1 in DÖC, the results were mostly compared with other types of cheese.

Some researchers could not detect the presence of AFM1 in their studies on the Beyaz, Tulum, and Cheddar cheese samples (16-18). On the other hand, different researchers determined AFM1 contamination level was over 650 ng/kg as well as below 50 ng/kg in the Beyaz, Kashar, Tulum and Cream cheese (2; 19; 20). In this study, the AFM1 contamination rate (85.87%) in DÖC was higher than the results obtained in many studies about Beyaz, Cream, Kashar, Tulum, Civil, Lor, Halloumi and Feta cheese (2; 16; 19-26). On the other hand, was determined lower the values of Beyaz (100%) reported by (27) and the values of cream cheese (99%) reported by (28).

Many investigators reported that AFM1 contamination rates exceeding the European Commission tolerance limit (250 ng/kg) in DÖC, Beyaz, Kashar and Cream cheese samples were 14%; 10%, 12%, 20%, 21%, 27%, 28%; 30%, 33%, 34%; 10%, 17%, 19%, respectively (2; 15; 18; 21- 23; 29).

On the other hand, other researchers determined that AFM1 concentration was less than 250 ng/kg in cheese samples including the Kashar, Beyaz, Tulum, Civil and Lor cheese (25; 28; 30). In our study, the number of samples exceeding the maximum values (250 ng/kg) accepted by the European Commission (17.39%) is lower than the values reported by (2; 18; 29; 31); whereas higher than the values reported by (23).

As seen above, the contamination levels of cheese by AFM1 vary from one study to another. This variability can be explained by different factors: cheese-making procedures, conditions of cheese ripening, type of cheese studied, geographical region, analysis methods and degree of milk contamination according to seasonal changes (26; 32-36).

### 4. CONCLUSION

Analysis results show that AFM1 levels of DÖC are a potential risk for consumers. It is especially important to keep humidity and temperature levels (temperature <12 °C or > 42 °C, humidity <80%) under control in storage areas of animal feed (especially

silage), from where raw milk/cheese to prevent the risk (12; 37; 38). Feed storage has to be designed appropriately. On the other hand, can be suggested the establishment of sustainable modern milk production farms. Moreover, training of feed and cheese producers/employees should be required in terms of hygiene and sanitation rules during their production. As the last sentence, consumers' awareness about this issue should be enhanced.

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