

## Determination of Aflatoxin M<sub>1</sub> Levels in Some Cheese Types Consumed in Erzurum - Turkey

Meryem AYDEMİR ATASEVER \* Gülşah ADIGÜZEL \*   
Mustafa ATASEVER \* Korhan ÖZTURAN \*\*

\* Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Ataturk University, TR-25240 Erzurum - TURKEY

\*\* Nineth Army Cops "A" Type Food Control Detachment Commandery, TR-25200 Erzurum - TURKEY

**Makale Kodu (Article Code): KVFD-2009-1639**

### Summary

In this study the total 304 cheese samples (85 white cheese, 75 kashar cheese, 62 civil cheese, 82 cream cheese) were examined in terms of AFM<sub>1</sub>. The AFM<sub>1</sub> content and concentrations of the samples were researched by competitive ELISA method. Determinable limit was 50 ng/kg and it was determined that white cheese samples included 82.4% AFM<sub>1</sub>, kashar cheese samples 80%, civil cheese samples 19.4% and cream cheese samples 84.2%. According to European Commission limit (250 ng/kg), the sample incidence exceeding the acceptable limits were 27.1%, 34.7%, 17.1% in white cheese, kashar cheese and cream cheese samples, respectively. The sample ratio exceeding the limits regulated by Turkish Food Codex (500 ng/kg) was determined in white cheese, kashar cheese and cream cheese samples as 16.5% (14/85), 14.7% (11/75) and 6.1% (5/82) respectively, any sample exceeding these limits was not met in civil cheese samples. As understood from these results, high AFM<sub>1</sub> level determined in some cheese types is an important problem threatening the public health in Turkey.

**Keywords:** AFM<sub>1</sub>, Cheese, ELISA

## Türkiye (Erzurum)'de Tüketilen Bazı Peynir Çeşitlerinde Aflatoxin M<sub>1</sub> Seviyesinin Belirlenmesi

### Özet

Bu çalışmada toplam 304 peynir örneği (85 beyaz peynir, 75 kaşar peynir, 62 civil peynir, 82 krem peynir) AFM<sub>1</sub> yönünden incelendi. Örneklerin AFM<sub>1</sub> içeriği ve konsantrasyonu kompetitiv ELISA metoduyla araştırıldı. AFM<sub>1</sub>'in belirlenebilir limiti 50 ng/kg olup, beyaz peynir örneklerinin %82.4, kaşar peynir örneklerinin %80, civil peynir örneklerinin %19.4 ve krem peynir örneklerinin ise %84.2'ünde bu maddeye rastlandı. Avrupa Komisyonu'na göre AFM<sub>1</sub> yönünden yasal limitleri (250 ng/kg) aşan numune oranı beyaz peynir, kaşar peynir ve krem peynir örneklerinde sırasıyla %27.1, %34.7, %17.1 olarak belirlendi. Türk Gıda Kodeksi'ne göre yasal limitleri (500 ng/kg) aşan numune oranı beyaz peynir, kaşar peynir ve krem peynir örneklerinde sırasıyla %16.5 (14/85), %14.7 (11/75) ve %6.1 (5/82) olarak belirlenirken civil peynir numunelerinde söz konusu limitleri aşan numuneye rastlanmadı. Bu sonuçlardan anlaşılacağı gibi yüksek AFM<sub>1</sub> düzeyi Türkiye'de halk sağlığını tehdit eden önemli bir problemdir.

**Anahtar sözcükler:** AFM<sub>1</sub>, Peynir, ELISA

### INTRODUCTION

Aflatoxins are toxic metabolites produced by fungi, eg, *Aspergillus flavus*<sup>1</sup>, *A. parasiticus* and *A. nomius*. *A. flavus* produces only B aflatoxin, while the other two species produce both B and G aflatoxins. Aflatoxins

contaminate the feeds, especially cereals and oilseeds both in the pre-harvest and postharvest seasons. They present toxigenic, carcinogenic, teratogenic and mutagenic potential<sup>2,3</sup>. Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and aflatoxin



**İletişim (Correspondence)**



+90 442 2315533



gulsah@atauni.edu.tr

M<sub>1</sub> (AFM<sub>1</sub>) are classified as human carcinogen in group 1 and 2B, respectively <sup>4</sup>. There is a linear relationship between the amount of AFM<sub>1</sub> in milk and AFB<sub>1</sub> in feed consumed by animals. It has been reported that 0.3-6.2% of AFB<sub>1</sub> in animal feed is transformed to AFM<sub>1</sub> and excreted in milk <sup>3</sup>. AFM<sub>1</sub> is comparatively stable during pasteurization, sterilization, preparation, and storage of various dairy products <sup>5-9</sup>. Most of the developed countries have regulated the maximum permissible levels of AFM<sub>1</sub> in milk and milk products, which vary from country to country <sup>10</sup>. The European Commission (EC) has approved a maximum admissible level of 250 ng/kg for AFM<sub>1</sub> in cheese <sup>11</sup>. However, the Turkish Food Codex (TFC), has accepted 500 ng/kg as the action level for AFM<sub>1</sub> <sup>12</sup>.

Although there is some information about the occurrence of AFM<sub>1</sub> in white, kashar and cream cheese there is not enough information about the occurrence of AFM<sub>1</sub> in civil cheese in Turkey. For this purpose, this study was designed to determine the presence and levels of AFM<sub>1</sub> in white, kashar, civil and cream cheese that especially sold and consumed in Erzurum province of Turkey, and to compare the results with the legal regulations for AFM<sub>1</sub> legislated by EC and TFC.

## MATERIAL and METHODS

### Samples

In this study the total 304 cheese samples (85 white cheese, 75 kashar cheese, 62 civil cheese, 82 cream cheese) put up for sale in various places in Erzurum between the dates September 2006 - September 2007 were examined in terms of AFM<sub>1</sub>.

### Methods

AFM<sub>1</sub> concentrations of the samples were analyzed by competitive ELISA (RIDASCREEN Aflatoxin M<sub>1</sub>, R-Biopharm) as procedure described by R-Biopharm GmbH <sup>13</sup>. 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 50 ng/kg. The statistical analysis was performed by SPSS Statistical Program.

## RESULTS

In this study a total of 304 cheese samples including 85 white cheese, 75 kashar cheese, 62 civil cheese and 82 cream cheese were analysed for AFM<sub>1</sub> with the competitive ELISA.

The occurrence and the distribution of AFM<sub>1</sub>

concentration in various ranges in cheese samples are presented in [Table 1](#).

Aflatoxin M<sub>1</sub> was found above measurable level (50 ng/kg) in 82.4% (70/85), 80% (75/65), 19.4% (12/62) and 84.2% (69/82) of white cheese, kashar cheese, civil cheese and cream cheese samples, respectively. Another expression of the results, it was found that 17.7% (15/85) of white cheese, 20% (15/75) of kashar cheese, 80.7% (50/62) of civil cheese and 15.9% (13/82) of cream cheese have no AFM<sub>1</sub> in detectable level by ELISA. According to the European Commission limit (250 ng/kg), 23 (27.1%), 26 (34.7) and 14 (17.1) contaminated white, kashar and cream cheese samples had AFM<sub>1</sub> in concentrations in excess of the maximum tolerance limit, respectively. The sample ratio exceeding the limits regulated by Turkish Food Codex (500 ng/kg) was determined in white cheese, kashar cheese and cream cheese samples as 16.5% (14/85), 14.7% (11/75) and 6.1% (5/82) respectively, any sample exceeding these limits was not met in civil cheese samples. AFM<sub>1</sub> content of positive cheese samples were determined as minimum 51 ng/kg, maximum 860 ng/kg and mean 263.4±198.1 ng/kg.

In other studies made on various cheeses, presence and level of AFM<sub>1</sub> were showed in [Table 2](#).

## DISCUSSION

Milk and dairy product have an important place in a healthy human diet since they are good sources calcium and proteins. The production and consumption of cheese is widespread in Turkey. AFM<sub>1</sub> has affinity to casein of milk. Therefore AFM<sub>1</sub> concentration is higher than in corresponding milk <sup>14</sup>. For these reasons, cheese could be the most potent source of aflatoxin among dairy products. In some studies made on cheese, AFM<sub>1</sub> was determined in high or low levels in some of them but it was not able to be determined in some cheese samples <sup>15-28</sup> ([Table 2](#)).

In our study AFM<sub>1</sub> was determined in 216 of the total 304 cheese samples. AFM<sub>1</sub> incidence determined as 71.1% (216/304) in cheese samples is high. It is known that this situation can be sourced from the feeds of animals from which milk is got become contaminated with aflatoxin or *Aspergillus spp.* This information was verified by Bakirci <sup>14</sup> who determined high amounts of AFM<sub>1</sub> in raw milk samples in spring months. While the findings got in our study are in parallel of the studies <sup>15-24</sup> stating that they determined AFM<sub>1</sub> in cheese samples in various levels, they are contradicts with the data <sup>25-28</sup> stating that this substance was not met in determinable

**Table 1.** Distribution of aflatoxin M1 contents in various range in white cheese, kashar cheese, civil cheese and cream cheese samples  
**Tablo 1.** Beyaz peynir, kaşar peynir, civil peynir ve krem peynir örneklerinin aflatoxin M1 içeriğinin dağılımı

Kind of Cheese	Samples Tested (n)	Proportion of Positive Samples n (%)	Distribution of Samples n (%)					Proportion of Samples Exceeding the European Commission >250 ng/kg	Proportion of Samples Exceeding the Legal Limit >500 ng/kg	Quantity of AFM1 (ng/kg)				
			<50	50-150	151-250	251-450	451-650			651-800	>800	x±Sx	Min. Max.	
White	85	70 (82.4)	15 (17.7)	16 (18.8)	31 (36.5)	5 (5.9)	10 (11.8)	7 (8.2)	1 (1.2)	23 (27.1)	14 (16.5)	297.6±216.1	58	860
Kashar	75	65 (80)	15 (20)	12 (16)	22 (29.3)	14 (18.7)	5 (6.7)	5 (6.7)	2 (2.7)	26 (34.7)	11 (14.7)	309.4±206.7	55	850
Civil Cheese	62	12 (19.4)	50 (80.7)	12 (19.4)	0	0	0	0	0	0	0	66.8±22.6	51	116
Cream Cheese	82	69 (84.2)	13 (15.9)	25 (30.5)	30 (36.6)	9 (11)	2 (2.4)	1 (1.2)	2 (2.4)	14 (17.1)	5 (6.1)	222.9±158.5	52	860
Total	304	216 (71.1)	93 (30.6)	65 (21.4)	83 (27.3)	28 (9.2)	17 (5.6)	13 (4.3)	5 (1.6)	63 (20.7)	30 (9.9)	263.4±198.1	51	860

**x±Sx:** mean±standard deviation, **<50:** distribution of negative samples, **a:** ng/kg  
**x±Sx:** ortalama±standart sapma, **<50:** negatif örneklerin dağılımı, **a:** ng/kg

**Table 2.** Presence and level of AFM<sub>1</sub> in various cheeses

**Tablo 2.** Çeşitli peynirlerde AFM<sub>1</sub> varlığı ve seviyeleri

Country	Cheese Variety	No. of Samples Positive (%)	Range (ng/kg)	Exceed Legal Limit (%)	References	Country	Cheese Variety	No. of Samples Positive (%)	Range (ng/kg)	Exceed Legal Limit (%)	References
Turkey	White Cheese	12/25 (48)	51-510	NP	Dagloglu et al. <sup>15</sup>	Iran	Feta	66/80 (82.5)	350-520	48/80 (60.6)	Kamkar <sup>24</sup>
Turkey	White Cheese	51/57 (89.47)	40-810	7/57 (12.28)	Oruc & Sonal <sup>16</sup>	Turkey	White	0/30 (0.0)	ND	NP	Demirer <sup>25</sup>
Turkey	White Cheese	82/100 (82)	51-800	27/100 (27)	Sarimehmetoglu et al. <sup>17</sup>	Turkey	Kashar	0/12 (0.0)	ND	NP	
Turkey	Kashar	85/100 (85)	51-800	34/100 (34)		Turkey	Tulum	0/26 (0.0)	ND	NP	
Turkey	Tulum	81/100 (81)	51-800	24/100 (24)		Turkey	Konya Küfü Tulum	0/10 (0.0)	ND	NP	
Turkey	Cream	79/100 (79)	51-650	25/100 (25)	Seyrek <sup>18</sup>	Turkey	Konya Küfü Tulum	0/10 (0.0)	ND	NP	Demirer <sup>33</sup>
Turkey	White Cheese	101/110 (91.8)	10-2000	17/110 (15.5)	Tabata et al. <sup>19</sup>	Turkey	Diyarbakır Otlı	0/10 (0.0)	ND	NP	
Japan	Cheese	44/303 (14.50)	200-1200	NP	Trucksess and Page <sup>20</sup>	Turkey	Erzincan Küfü Tulum	0/71 (0.0)	ND	NP	
USA	Cheese	80/118 (6.80)	100-1000	NP	Tekinsin & Tekinsin <sup>21</sup>	Turkey	Tulum	0/4 (0.0)	ND	NP	Coksoyler <sup>26</sup>
Turkey	Van Otlı	52/60 (86.7)	160-7260	12/60 (20)	Yaroglu et al. <sup>22</sup>	Turkey	Çökelek	0/9 (0.0)	ND	NP	Kivanc <sup>27</sup>
Turkey	White Brine	31/50 (62.0)	10-5200	30/50 (60)		Turkey	White	0/25 (0.0)	ND	NP	
Turkey	White	10/200 (5.0)	100-600	2/200 (1)		Turkey	Van otlı	0/25 (0.0)	ND	NP	
Turkey	Kashar	24/200 (12.0)	120-800	2/200 (1)		Turkey	White	0/50 (0.0)	ND	NP	Kardes <sup>28</sup>
Turkey	Cream	16/200 (8.0)	100-700	2/200 (1)		Turkey	Kashar	0/50 (0.0)	ND	NP	
Turkey	White Brine	159/193 (82.4)	52- 860	51/193 (26.4)	Ardic et al. <sup>23</sup>						

**NP:** Not presented, **ND:** Not detected

**NP:** Bildirilmemiş, **ND:** Belirlenmemiş

limits in cheese. It was stated that these variations between the data could be sourced from the difference of AFM<sub>1</sub> level in milk used in the production due to seasonal changes<sup>14,17,21,29,30</sup> and from different operation and analysis methods<sup>30-32</sup>.

That AFM<sub>1</sub> determined in civil cheese in relatively low levels (mean; 66.8±22.6 ng/kg) is in accord with the studies in which AFM<sub>1</sub> can not be determined in some civil cheese types such as Konya moldy tulum cheese (its original name is Konya Küflü Tulum) and Erzincan moldy tulum cheese (its original name is Erzincan Küflü Tulum)<sup>25,26,33</sup>. In this study the samples ratio (20.7%) exceeding the EC legal limits is similar to the findings of researchers<sup>17,18,21,23,-25</sup>. The AFM<sub>1</sub> levels exceeding the TFC legal limits (500 ng/kg), also were lower than the reported results by Sarimehmetoglu et al.<sup>17</sup>, Tekinsen and Tekinsen<sup>21</sup>, Ardic et al.<sup>23</sup>, Kamkar<sup>24</sup>, and were similar to the results reported by Oruç and Sonal<sup>16</sup> and Seyrek<sup>18</sup>. This similarity can be sourced from the highness of AFM<sub>1</sub> level regulated by TFC. It was concluded that AFM<sub>1</sub> incidence determined in white, kashar and cream cheese were quite higher than legal limits, so it could form an important risk for health. But there isn't a sample in civil cheeses exceeding legal limits, it might be said that civil cheese can carry less risk in terms of AFM<sub>1</sub> content.

In conclusion, AFM<sub>1</sub> determined in high levels in three cheese types in the present study and the other researchs made in Turkey show that this subject is still an important public health problem in Turkey. So the public health authorities should train the farmers, dairy companies and dairy product consumers on the potential health results of aflatoxins. Moreover the prevention of aflatoxin formation in feeds is very important. Because the consumption of contaminated feeds by dairy animals causes AFM<sub>1</sub> formation in milk. So the easiest and shortest way to deal with this problem is reducing the AFB<sub>1</sub> concentration in animal feed by improved processing and storage practices. For this, it is necessary to control well the feeds given to dairy animals and to reduce AFB<sub>1</sub> 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.

## REFERENCES

- Doğan A, Bayezit M:** Kars yöresinde yemlerde aflatoksin B<sub>1</sub> düzeylerinin ELISA yöntemi ile belirlenmesi. *Kafkas Univ Vet Fak Derg*, 5 (1): 63-70, 1999.
- Sweeney MJ, Dobson ADW:** Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species. *Int J Food Microbiol*, 43, 141-158, 1998.
- Creppy EE:** Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol Lett*, 127, 19-28, 2002.
- IARC (International Agency for Research on Cancer):** IARC monographs on the evaluation of carcinogenic risks to humans, some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Scientific Publication, No. 56, IARC, Lyon, 1993.
- Stubblefield RD, Shannon GM:** Aflatoxin M<sub>1</sub>: Analysis in dairy products and distribution in dairy foods made from artificially contaminated milk. *J Assoc Off Anal Chem*, 57, 847-851, 1974.
- Barbieri G, Bergamini C, Ori E, Reska P:** Aflatoxin M<sub>1</sub> in parmesan cheese: HPLC determination. *J Food Sci*, 59, 1313-1331, 1994.
- Blanco JL, Dominguez L, Gomez-Lucia E, Garayzabal JFF, Garcia JA, Suarez G:** Presence of aflatoxin M<sub>1</sub> in commercial ultra-high-temperature-treated milk. *App Environ Microb*, 54, 1622-1623, 1988.
- Stoloff L:** Aflatoxin M<sub>1</sub> in perspective. *J Food Protect*, 43, 226-230, 1989.
- Galvano F, Galofaro V, Galvano G:** Occurrence and stability of aflatoxin M<sub>1</sub> in milk and milk products. *J Food Protect*, 59, 1079-1090, 1996.
- Kaniou-Grigoriadou I, Eleftheriadou A, Mouratidou T, Katikou P:** Determination of aflatoxin M<sub>1</sub> in ewe's milk samples and the produced curd and Feta cheese. *Food Control*, 16, 257-261, 2005.
- European Commission (EC):** No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off J Eur Union*, 364, 5-24, 2006.
- Turkish Food Codex (TFC):** Gıda Maddelerinde Belirli bulaşanların maksimum seviyelerinin belirlenmesi hakkında teblig. *Resmi Gazete*, 17 Mayıs 2008. Sayı: 26879 Basbakanlık Basimevi. Ankara, Turkey, 2008.
- R-Biopharm GmbH:** Enzyme immunoassay for the quantitative analysis of aflatoxins. Ridascreen Aflatoxin M<sub>1</sub> Art. No: R-1101. Darmstadt, Germany, 1999.
- Bakirci I:** A study on the occurrence of aflatoxin M<sub>1</sub> in milk and milk products produced in Van province of Turkey. *Food Control*, 12, 47-51, 2001.
- Dagoglu G, Keles O, Yıldırım M:** Peynirlerde aflatoksin düzeylerinin ELISA ile araştırılması. *Istanbul Univ Vet Fak Derg*, 21, 313-317, 1995.
- Oruç HH, Sonal S:** Determination of aflatoxin M<sub>1</sub> levels in cheese and milk consumed in Bursa, Turkey. *Vet Hum Toxicol*, 43, 292-293, 2001.
- Sarimehmetoglu B, Kuplulu O, Celik TH:** Detection of aflatoxin M<sub>1</sub> in cheese samples by ELISA. *Food Control*, 15, 45-49, 2004.
- Seyrek K:** Türk Silahlı Kuvvetleri'ne bağlı birliklerde tüketilen beyaz peynirlerdeki aflatoxin M<sub>1</sub> seviyesinin ELISA metodu ile saptanması. *Vet Hek Der Derg*, 72, 55-58, 2001.
- Tabata S, Kamimura H, Tamura Y, Yasuda K, Ushuyama H, Hasimoto H, Nishijima, M, Nishima T:** Investigation of aflatoxins contamination in food and foodstuffs. *J Food Hyg Soc Jpn*, 28, 395-401, 1987.
- Truckness MV, Page SV:** Examination of imported cheese for aflatoxin M<sub>1</sub>. *J Food Protect*, 49, 632-633, 1986.
- Tekinsen KK, Tekinsen OC:** Aflatoxin M<sub>1</sub> in white pickle

and Van otlı (herb) cheeses consumed in southeastern Turkey. *Food Control*, 16, 565-568, 2005.

**22. Yaroglu T, Oruc HH, Tayar M:** Aflatoxin M<sub>1</sub> levels in cheese samples from some provinces of Turkey. *Food Control*, 16, 883-885, 2005.

**23. Ardıc M, Karakaya Y, Atasever M, Adiguzel, G:** Aflatoxin M<sub>1</sub> levels of Turkish white brined cheese. *Food Control*, 20, 196-199, 2009.

**24. Kamkar A:** A study on the occurrence of aflatoxin M<sub>1</sub> in raw milk produced in Sarab city of Iran. *Food Control*, 16, 593-599, 2005.

**25. Demirer MA:** Süt ve süt mamüllerinde aflatoxin M<sub>1</sub> ve B<sub>1</sub> aranması üzerine araştırmalar. *Ankara Univ Vet Fak Derg*, 36, 85-107, 1973.

**26. Coksoyler N:** Süt ve Süt Mamüllerinde Aflatoxin Oluşumu Üzerine Araştırmalar. *Diploma Sonrası Yükseköğretim İhtisas Tezi*, Ankara Univ Ziraat Fak, Ankara, 1977.

**27. Kivanc M:** Mold growth and presence of aflatoxin in some Turkish cheeses. *J Food Safety*, 10, 287-294, 1990.

**28. Kardes E:** Türk Silahlı Kuvvetleri'ne bağlı birliklere alınan peynirlerde aflatoxin B<sub>1</sub> ve M<sub>1</sub> varlığının ve seviyelerinin saptanması. *MS Thesis*, Ankara University, 2000.

**29. Amra HA:** Survey of aflatoxin M<sub>1</sub> in Egyptian raw milk by enzyme-linked immunosorbent assay. *Rev Med Vet*, 149, 695, 1998.

**30. Tekinsen KK, Ucar G:** Aflatoxin M<sub>1</sub> levels in butter and cream cheese consumed in Turkey. *Food Control*, 19, 27-30, 2008.

**31. Galvano F, Galofaro V, Ritieni A, Bognanno M, De Angelis, A, Galvano G:** Survey of the occurrence of aflatoxin M<sub>1</sub> in dairy products marketed in Italy: Second year of observation. *Food Addit Contam*, 18, 644-646, 2001.

**32. Wiseman DW, Marth EH:** Behavior of aflatoxin M<sub>1</sub> during manufacturing and storage of queso blanco and bakers cheese. *J Food Protect*, 46, 910-913, 1983.

**33. Demirer MA:** Bazı peynirlerimizden izole ettiğimiz küfler ve bunların aflatoxin yeteneklerinin araştırılması. *Ankara Univ Vet Fak Derg*, 21, 1-2, 1974.