A Research on the Determination of Aflatoxin M1 Levels in Milk and Dairy Products for Sale in Diyarbakır by ELISA

Ömer Faruk YEŞİL1, Abdulkerim HATİPOĞLU 2*, Abdunnasır YILDIZ 3, Aydın VURAL 4, Mehmet Emin ERKAN 4

1 Food Technology Programme, Department of Food Processing, Diyarbakır Agricultural Vocational High School, Dicle University, 21280, Diyarbakır, Turkey
2 Department of Nutrition and Dietetics, Health College, Mardin Artuklu University 47200, Mardin, Turkey
3 Department of Biology, Faculty of Science, Dicle University, 21280, Diyarbakır, Turkey
4 Department of Food and Nutrition Hygiene, Faculty of Veterinary Medicine, Dicle University, 21280, Diyarbakır, Turkey

Geliş / Received: 18/12/2018, Kabul / Accepted: 08/03/2019

Abstract
In this study, the contamination levels of Aflatoxin M1 of 248 sterilized milk, Beyaz, Cecil, Cokelek, Cream, Lor, Van Otlu (herbed), Van Otlu Lavash Cheese and butter samples randomly obtained from selected market and supermarkets from Diyarbakır province center were determined by ELISA method. The average AFM1 contamination value of the cheese samples investigated was 138.65 ng/kg; while the minimum and maximum values were 50 and 595.31 ng/kg, respectively. On the other hand, the average AFM1 value of 13 sterilized milk samples was 52.59 ng/kg; minimum and maximum values were determined as 25.97 and 80.0 ng/kg, respectively. In addition, the average AFM1 value of 18 butter samples was 97.54 ng/kg; the minimum and maximum values were calculated as 50 and 308.13 ng/kg. In the study, the level of AFM1 contamination in the 26 samples of cheese samples were exceeded the limits set by the European Commission for AFM1. In terms of Turkish Food Codex, only 2 cheese samples exceeded the AFM1 tolerance limit. According to the study, the level of AFM1 contamination in the 7 milk samples and 15 butter samples exceeded both the European Commission and Turkish Food Codex AFM1 tolerance limit.

Keywords: ELISA, AFM1, cheese, butter, milk

Diyarbakır’da Tüketime Sunulan Süt ve Ürünlerinde Aflatoksin M1 Düzeylerinin ELISA ile Belirlenmesi Üzerine Bir Araştırma

Öz
Bu çalışmada, Diyarbakır il merkezinde bulunan toplu ve perkakende satış noktalarında tüketime arz edilen 248 adet sterilize süt, Beyaz, Cecil, Cokelek, Cream, Lor, Van Otlu (herbed), Van Otlu Lavash Peynir ve tereyağı örneğinin Aflatoksin M1 (AFM1) bulaşma düzeyi ELISA yöntemi kullanılarak tespit edilmiştir. Araştırma boyunca toplam 26 peynir örnekleri ortalama AFM1 değeri 138.65 ng/kg; minimum ve maksimum değerleri ise 50 ve 595.31 ng/kg olarak ortaya konmuştur. Diğer taraftan 13 adet sterilize süt örneğinin ortalama AFM1 değeri 52.59 ng/kg; minimum ve maksimum değerleri ise 25.97 ve 80.0 ng/kg olarak tespit edilmiştir. Ayrıca 18 adet tereyağı örneğinin ortalama AFM1 değeri 97.54 ng/kg; minimum ve maksimum değerleri ise 50 ve 308.13 ng/kg olarak hesaplanmıştır. Araştırımda toplam 26 peynir örneklerinde AFM1 kontaminasyon düzeyi Avrupa Komisyonu’nda AFM1 için belirlenen limiti aşmıştır. Türk Gıda Kodeksi açısından ise sadece 2 peynir örneklerinin AFM1 kontaminasyonu tolerans limitini aşmıştır. Çalışma sonucuna göre, 7 süt örnek ile 15 tereyağı örneğinde tespit edilen AFM1 düzeyi hem Avrupa Komisyonu hem de Türk Gıda Kodeksi AFM1 tolerans limitini aşmıştır.

Anahtar Kelimeler: ELISA, AFM1, peynir, tereyağı, süt

1. Introduction
Presence of aflatoxins (AFs) is one of the major food safety concerns in tropical and subtropical countries (Skrbic et al., 2015). The AFs among mycotoxins detected in dairy products are toxic, teratogenic, mutagenic, immunosuppressive compounds for human
A Research on the Determination of Aflatoxin M1 Levels in Milk and Dairy Products for Sale in Diyarbakır by ELISA

and animals (Fallah et al., 2009; Kamkar, 2008; Anfossi et al., 2012; Mohajeri et al., 2013). They cause especially on skin diseases and liver disorders (Rodas et al., 2018).

Afs are secondary metabolites produced by fungi, mainly Aspergillus flavus, Aspergillus parasiticus and rarely by Aspergillus nomius under suitable environmental conditions in animal feed and food. There are at least 20 different types of naturally-occurring AFs and the most recognized are B1, B2, G1 and G2 (Prodanov-Radulović et al., 2017). A. parasiticus and A. nomius produce both B and G Afs, while A. flavus only produces B Afs. Aflatoxin M1 (AFM1) and M2 (AFM2) are the hydroxylate metabolites of Aflatoxin B1 (AFB1) and B2 (AFB2), respectively. There are two main ways of aflatoxin contamination in the milk and dairy products. Firstly, when the animals in lactation period consume feeds contaminated with aflatoxin, aflatoxin B1 and B2 transform into AFM1/milk toxin and M2 by hepatic cytochrome p450 after metabolized in the animal body. These metabolized toxins pass to the milk produced from the animal and the contamination occurs. Secondly, the contamination occurs when the molds synthesizing aflatoxin pass to the milk and produce aflatoxin during transport, process and storage phases after milking (Torkar and Venguust, 2008; Fallah, 2010; Anfossi et al., 2012; Durakovic, 2012; Li et al., 2017, Berna et al., 2018, Hassan et al., 2018, Madali et al., 2018).

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, and yet a smaller proportion to butter. The remainder of AFM1 in milk, however, remains in skim milk and buttermilk (Atasever et al., 2010b). 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 (Aksoy et al., 2016).

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 (Langat et al., 2016). 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 (Anonymous, 2001). Therefore, many countries have set legal regulations to control aflatoxin M1 level in milk and dairy products.

The present study, AFM1 contamination levels of White, Cecil, Cokelek, Cream, Lor, Van Otlu, Van Otlu Lavash Cheese, butter and sterilized/UHT milk samples (248 samples) sold in Diyarbakır were determined. The results were compared with the European Commission’s (Anonymous, 2010) and Turkish Food Codex’s AFM1 levels (Anonymous, 2008).

2. Material and Methods

2.1. Collecting of Samples

In our study, A total of 248 samples of Beyaz, Cecil, Cokelek, Cream, Lor, Van Otlu (herbed), Van Otlu Lavash Cheese, butter and sterilized/UHT milk samples (248 samples) sold in Diyarbakır were determined. The results were compared with the European Commission’s (Anonymous, 2010) and Turkish Food Codex’s AFM1 levels (Anonymous, 2008).
A Research on the Determination of Aflatoxin M1 Levels in Milk and Dairy Products for Sale in Diyarbakır by ELISA

preserving cold chain. The samples (except sterilized milk) were stored at -18 °C in the laboratory until they were analyzed by ELISA method.

2.2. Preparation Of Milk Samples

Milk samples were centrifuged at 3500 rpm for 10 minutes at 10 °C. After centrifugation, the remaining oil layer top of the tube was removed using Pasteur pipette. 100 μl of the supernatant was used for direct analysis. Dilution factor was accepted as "1" for milk samples.

2.3. Preparation of Cheese Samples

40 ml of dichloromethane was added to 2 g of cheese, 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 (Whatman filter No:1, 125 mm), 10 ml of the extract was taken into the test tube and evaporated in a hot water bath at 60 °C. Then, it was evaporated at 60 °C by the weak nitrogen gas until the oil layer remained at the bottom of the tube. The residue, with the addition of 0.5 mL of methanol, 0.5 mL of Phosphate Buffered Saline (PBS) buffer and 1 mL of heptane, was centrifuged at 2700 rpm for 15 min (15 °C). After removing the supernatant heptane layer with a pasteur pipette, 100 μl of the bottom methanolic liquid phase and added 400 μl Buffer 1. 100 μl of this mixture was centrifuged (for 10 minutes at 4000 rpm and 10 °C). 50 μl of the bottom liquid phase with buffer1 were diluted 1:17 in a new tube. 100 μl of this sample was used for analysis. Dilution factor was accepted as "10" for butter samples.

2.5. 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.

2.6. Determination of Aflatoxin M1 by ELISA

The presence of aflatoxin M1 was determined by 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).

2.7. 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 AFM1 values of the cheese samples are presented in Table 1. According to the
results, AFM1 was detected all of the samples analyzed. The average AFM1 value of cheese samples was 133.51 ng/kg; the minimum and maximum values were determined as 50 ng/kg and 595.31 ng/kg, respectively. 11.98% of the samples (26 samples of cheese) were found to exceed the limit for AFM1 (250 ng/kg) of the European Commission (EC) (Anonymous, 2010). On the other hand, 0.92% of the samples (2 samples of cheese) were found to exceed the tolerance limit for AFM1 (500 ng/kg) of the Turkish Food Codex (TFC)(Anonymous, 2008).

Some of the researchers found that the maximum AFM1 contamination in cheeses were between 17 and 19 ng/kg (Gürses et al., 2004; Öztürk et al., 2014); while the others reported that were between 800 and 2000 ng/kg (Günsen and Büyükyörük, 2003; Alkan and Günülalan, 2006; Atasever et al., 2010a; Filazi et al., 2010; Hatipoğlu et al., 2016).

A number of researchers reported that AFM1 contamination rates did not exceed the tolerance limits (EC and TFC) in their studies on the Kashar, Hellim, Beyaz, Urfa Beyaz, Civil, Tulum and Lor cheese samples (Gürses et al., 2004; Kök, 2006; Atasever et al., 2010a; Dinçel et al., 2012; Öztürk et al., 2014). On the other hand, different investigators determined that AFM1 level in

Table 1. Aflatoxin M1 levels of the cheese samples.

| Samples          | The Number of Samples | Concentration (ng/kg) |
|------------------|-----------------------|-----------------------|
|                  | Total | 0-50 ng/kg | 51-150 ng/kg | 151-250 ng/kg | >250<sup>a</sup> (ng/kg) | >500<sup>b</sup> (ng/kg) | Mean | Minimum - Maximum |
| Beyaz Cheese     | 131 (60.37%) | 27 | 63 | 26 | 14 | 1 | 132.94 | 50-595.31 |
| Cecil Cheese     | 7 (3.2%) | 0 | 1 | 2 | 4 | 0 | 249.84 | 70.06-406.76 |
| Cokelk            | 6 (2.76%) | 2 | 3 | 1 | 0 | 0 | 85 | 50-176.2 |
| Cream Cheese     | 5 (2.30%) | 0 | 4 | 1 | 0 | 0 | 136.96 | 99.46-181.14 |
| Lor Cheese       | 12 (5.53%) | 2 | 3 | 6 | 1 | 0 | 159.01 | 50-393.82 |
| Van Otlu Cheese  | 43 (19.82%) | 16 | 11 | 10 | 5 | 1 | 140.47 | 50-518.78 |
| Van Otlu Lavash Cheese | 13 (5.99%) | 8 | 4 | 1 | 0 | 0 | 50-160.46 |
| Total            | 217 (100%) | 55 (25.35%) | 89 (41.01%) | 47 (21.66%) | 24 (11.06%) | 2 (0.92%) | 138.65 | 50-595.31 |

<sup>a</sup>: European Commission limit (250 ng/kg), <sup>b</sup>: Turkish Food Codex limit: (500 ng/kg).
A Research on the Determination of Aflatoxin M1 Levels in Milk and Dairy Products for Sale in Diyarbakır by ELISA

some Orgu, Kashar, Beyaz and Cream cheese samples exceeded the tolerance limit (Anonymous, 2010). The AFM1 contamination rates of the mentioned cheeses were reported as 14% (13), 17% (32); 4% (1), 17% (1), 30% (6), 33% (16), 44% (20), 35% (26), 33% (28); 2% (1), 20% (4), 10% (5), 21% (16), 27% (23), 28% (33); 17% (14), 19% (18), respectively (Günsen and Büyükyörük, 2003; Gürses et al., 2004; Çetin et al., 2005; Alkan and Gönülalan, 2006; Kök, 2006; Kireççi et al., 2007; Erkan et al., 2009; Fallah et al., 2009; Atasever et al., 2010a; Dinçoğlu et al., 2012; Filazi et al., 2010; Kögüstün, 2013; Hatipoğlu et al., 2016).

In this study, the total AFM1 rate of cheese samples (11.98%), which was exceeding the European Commission tolerance limit, was determined higher than the values reported by Çetin et al. (2005) in Kashar cheese (4%), Alkan and Gönülalan (2006) in Beyaz cheese (2%) and Filazi et al. (2010) in Beyaz cheese (10%). Conversely, the rate was determined lower than the values reported by Erkan et al. (2009) and Hatipoğlu et al. (2016) in Orgu cheese (14%, 17%); Günsen and Büyükyörük (2003), Kök (2006), Kireççi et al. (2007), Atasever et al. (2010a), Dinçoğlu et al. (2012) and Kögüstün (2013) in Kashar cheese (33%, 17%, 30%, 35%, 33%, 44%); Kireççi et al. (2007), Fallah et al. (2009), Atasever et al. (2010a) and Dinçoğlu et al. (2012) in Beyaz cheese (20%, 28%, 27%, 21%); Fallah et al. (2009) and Atasever et al. (2010a) in Cream cheese (19%, 17%).

Table 2. Aflatoxin M1 levels of sterilized/UHT milk and butter samples

| Samples      | The Number of Samples | Concentration (ng/kg) |
|--------------|-----------------------|-----------------------|
|              | Total | 0-50 ng/kg | 51-150<sup>a</sup> ng/kg | 151-250 ng/kg | >250 (ng/kg) | Mean | Minimum-maximum |
| Sterilized/UHT milk | 13 (100%) | 6 (46.16%) | 7 (53.84%) | 0 | 0 | 52.59 | 25.97-80 |
| Butter       | 18(100%) | 3 (16.66 %) | 12 (66.67 %) | 2(11.11 %) | 1 (5.56%) | 97.54 | 50-308.13 |

<sup>a</sup>: European Commission and Turkish Food Codex limit for milk and butter:50 ng/kg

On the other hand, many investigators reported that almost all of the samples analyzed in their researches related to raw, pasteurized and sterilized milk exceeded the permitted limits (Anonymous, 2008; Anonymous, 2010; Bakırcı, 2001; Akdemir and Altuntaş, 2004; Çelik et al., 2005; Kök, 2006; Ünüsan, 2006; Özdemir, 2007; Kamkar, 2008; Elzupir and Elhussein, 2010; Fallah, 2010; Hazer, 2011; İşleyici et al., 2012; Yurt and Uluçay, 2016; Karadal et al., 2018). Some of the researchers determined that the maximum AFM1 contamination in milk were between 37 and 94 ng/kg (Durakovic et al., 2012; İşleyici et al., 2012; Karadal et al., 2018), whereas the other part of the researchers determined were between 352 and 6900 ng/kg (Akdemir and Altuntaş, 2004; Ünüsan, 2006; Fallah, 2010; Elzupir and Elhussein, 2010; Yurt and Uluçay, 2016; Xiong et al. 2018). According to the results of our study (Table 2), it was revealed that 7
out of 13 sterilized milk samples (53.84%) exceeded the acceptance limits (Anonymous, 2008; Anonymous, 2010). The AFM1 contamination rates of the milk samples were determined higher than raw milks’ values reported by Bakırcı (2001), Akdemir and Altıntaş (2004), Özdemir (2007), Hazer (2011), Rama et al. (2015), Mashak et al. (2016), Karadal et al. (2018), Quevedo-Garza (2018), Xiong et al. (2018) and Türkoğlu and Keyvan (2019). Likewise, the rates were determined higher than those in pasteurized and sterilized milks (Ünüşan, 2006; Fallah, 2010; İşleyici et al., 2012). On the other hand, the AFM1 contamination rates of the milk samples were lower than the values reported by Çelik et al. (2005), Kök (2006), Kamkar (2008), Elzupir and Elhussein (2010) and Yurt and Uluçay (2016) as 64% (48), 62% (8), 77% (40), 83% (35) and 80% (20), respectively. According to the results of a study with 520 milk samples in Pakistan, the lowest AFM1 levels in milk were observed in summer and the highest AFM1 level in winter months. The same researchers reported that AFM1 levels in the morning milk were higher than evening milk (İsmail et al., 2016).

The present study, the total AFM1 rate of butter samples (83.34%), which was exceeding the EC and TFC tolerance limits, was determined higher than the values reported by Atasever et al. (2010b) and Iqbal and Asi (2013) in the butter (16.3% and 22.97%). On the other hand, Aksoy et al. (2016) reported that they could not detect AFM1 in any of the 40 butter samples in their study by ELISA and HPLC. Additionally, Hassan et al. (2018) reported that the level of AFM1 were below 50 ng/kg in 18 butter samples. Some researchers claimed the storage conditions of products including humidity and temperature were also important for toxin production in butter (Iqbal and Asi, 2013).

As seen above, different studies have been carried out to reveal the level of AFM1 contamination in milk and milk products. The differences in the results of the research can be attributed to the level of milk contamination due to seasonal changes, the geographical region, the milk/butter/cheese type or production technique, the cheese maturity level, the analysis methods used, the diet of dairy animals (rations) and the lactation period (Blanco et al., 1988; Barrios et al., 1996; Galvano et al., 1996; Pitter, 1998; Sarımehmetoğlu et al., 2004; Kamkar, 2008; Fallah, 2010; Rahimi et al. 2010; Tavakoli et al., 2012; Malissiova et al. 2013, İsmail et al., 2016).

4. Conclusion
As a result, 53.84% of milk samples and 83.34% of butter samples sold in Diyarbakır exceeded the limit for AFM1 of the European Commission and Turkish Food Codex. Additionally, 11.98% of cheese samples sold in Diyarbakır exceeded the limit for AFM1 of the European Commission and 0.92% of cheese samples exceeded the tolerance limit for AFM1 of Turkish Food Codex. This situation poses a potential risk to public health and indicates the sporadic use of contaminated feedstuff at farms. For this reason, the following points should be noted: It is especially important to keep humidity and temperature levels under control in storage areas of animal feed (especially silage) from where raw milk/cheese/butter to prevent the risk. On the other hand, training of feed and dairy manufacturers/employees should be required about the health risks of aflatoxins and possible measures. Moreover, can be suggested the establishment of sustainable modern milk production farms. Finally, the competent authorities should take urgent precautions in these matters.

Acknowledgements
This study was financially supported by Scientific Research Project Coordinatorship of Dicle University (Project No: DUBAP-12-MYO-73).

5. References

Akdemir, C., Altıntaş, A., 2004. Investigation of aflatoxin-M1 presence and levels in milk processed in Ankara by HPLC. *The Journal of Ankara Univ. Vet. Fac.*, 51, 175-179.

Aksoy, A., Atmaca, E., Yazıcı, F., Güvenç, D., Gül, O., Dervişoğlu, M., 2016. Comparative Analysis of Aflatoxin M1 in Marketed Butter by ELISA and HPLC. *The Journal of Kafkas Univ.Vet.Fac.*, 22(4), 619-621.

Alkan, Y., Gönülalan, Z., 2006. A study on aflatoxin M1 moisture and acidity values in Beyaz cheeses offered for sale in Amasya. *Journal of Health Sciences*, 15 (2), 91-98.

Anfossi, L., Baggiani, C., Giovannoli, C., D’Arco, G., Passini, C., Giraudi, G., 2012. Occurrence of aflatoxin M1 in Italian cheese: results of a survey conducted in 2010 and correlation with manufacturing, production season, milking animals, and maturation of cheese. *Food Control*, 25, 125-130.

Anonymous, 2001. Fifty-Sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Safety Evaluation of Certain Mycotoxins in Food. Food Additive Series, World Health Organization, Geneva, 47.

Anonymous, 2008. Turkish Food Codex, Notification On Maximum Limits Of Contaminants (Notification No: 2008/26). Ankara: Prime Minister’s Printery. Official Newspaper, 17 May 2008, No: 26879.

Anonymous, 2010. European Commission (EC) Regulation: Commission Regulation No. 165/2010 of 26 February 2010 Amending Regulation (EC) No. 1881/2006. Setting maximum levels for certain contaminants in foodstuffs As Regards Aflatoxins. Official Journal European Union, L 50/8.

Atasever, M.A., Adıgüzel, G., Atasever, M., Özturan, K., 2010a. Determination of Aflatoxin M1 Levels in Some Cheese Types Consumed in Erzurum, Turkey. *The Journal of Kafkas Univ.Vet.Fac.*, 16(Suppl-A), 87-91.

Atasever, M.A., Atasever, M., Özturan, K., Urcar, S., 2010b. Determination of Aflatoxin M1 Level in Butter Samples Consumed in Erzurum, Turkey. *The Journal of Kafkas Univ.Vet.Fac.*, 16(Suppl-A), 159-162.

Bakırç, I, 2001. A study on the occurrence of aflatoxin M1 in milk and milk products produced in Van province of Turkey. *Food Control*, 12(1), 47-51.

Barrios, M.J., Gualda, M.J., Cabanas, J.M., Medina, L.M., Jordano, R., 1996. Occurrence of aflatoxin M1 in cheeses from the South of Spain. *Journal of Food Protection*, 59, 898-900.

Blanco, J.L., Dominguez, L., Gomez-Lucia, E., Garayzabal J.F.F., Goyache, J., Suarez, G., 1988. Behaviour of aflatoxin during manufacture, ripening and storage of Manchego type cheese. *Journal of Food Science*, 53, 1373-1376.

Çelik, T.H., , Sarımehtoğlu, B., Kuplulu, O., 2005. Aflatoxin M1 contamination in pasteurised milk, *Veterinarski Arhiv*, 75 (1), 57-65.

Çetin, T., Gürsoy, A., Deveci, O., Sezgin, E., 2005. Determination of aflatoxin M1
A Research on the Determination of Aflatoxin M1 Levels in Milk and Dairy Products for Sale in Diyarbakir by ELISA

contents of Kashar cheese presented for sale in Ankara market. Food Technology, 9, 93-95.

Dinçel, A., Demli, F., Durlu-Özkaya, F., Alatan, F., Uzun, R., Subaşı, S.A., 2012. Analysis of aflatoxin M1 in various cheese samples by HPLC. Journal of Turkish Hygiene and Experimental Biology, 69(2), 89-96.

Dinçoğlu, A.H., Gönülalan, Z., Kök, H., 2012. Determination of Aflatoxin M1 Levels in Cheese Sold in Izmit Region by Elisa. e-Journal of New World Sciences Academy, NWSA - Veterinary Sciences, 3B0019, 7(1), 1-6.

Durakovic, L., Mrkonjic-Fuka, M., Skelin, A., Durakovic, S., Redzepovic, S., 2012. Incidence of aflatoxin M1 in milk used for the production of Istrian cheese. The 47th Croatian and 7th International Symposium on Agriculture, Opatija, Crotia, 679-683.

Elzupir, A.O., Elhussein, A.M., 2010. Determination of aflatoxin M1 in dairy cattle milk in Khartoum State, Sudan. Food Control, 21, 945–946.

Erkan, M.E., Vural, A., Güran, H.Ş., 2009. Diyarbakır Örgü Peynirinde Aflatoksibn M1 ile Verotoksin 1 ve 2 Varlığının Araştırılması. The Journal of Dicle Univ. Vet. Fac., 1(1), 19-25.

Fallah, A.A., Jafari, T., Fallah, A., Rahnama, M., 2009. Determination of aflatoxin M1 levels in Iranian White and cream cheese. Food and Chemical Toxicology, 47, 1872-1875.

Fallah, A.A., 2010. Assessment of aflatoxin M1 contamination in pasteurized and UHT milk marketed in central part of Iran. Food and Chemical Toxicology, 48, 998-991.

Filazi, A., İnce, S., Temamoğulları, F., 2010. Survey of the occurrence of aflatoxin M1 in cheeses produced by dairy ewes' milk in Urfa city, Turkey. The Journal of Ankara Univ. Vet. Fac., 57, 197-199.

Galvano, F., Galofaro, V., Galvano, G., 1996. Occurrence and stability of aflatoxin M1 in milk and milk products: a world wide review. Journal of Food Protection, 10,1079- 1090.

Günsen, U., Büyükyörük, I., 2003. Determination of bacteriological quality and AFM1 levels of fresh Kashar cheese supplied from the market. Turk Journal Vet. Animal Science 2, 821-825.

Gürses, M., Erdoğan, A., Çetin, B., 2004. Occurrence of Aflatoxin M1 in Some Cheese Types Sold in Enzurum, Turkey. Turk J. Vet. Anim Sci., 28, 527-530.

Hassan, Z. U., Al-Thani, R., Atia, F.A., Almeer, S., Balmas, V., Migheli, Q., Jaoua, S., 2018. Evidence of low levels of aflatoxin M1 in milk and dairy products marketed in Qatar. Food Control, 92, 25-29.

Hatipoğlu, A., Yeşil, Ö.F., Vural, A., Erkan, M. E., Yıldız, A., 2016. Determination of Aflatoxin M1 Levels in Orgu Cheese Produced by Traditional Methods in Diyarbakır by ELISA. 23rd National Biology Congress, 5-9 September, Gaziantep, 103.

Hazer, A., 2011. The Investigations of Prevalence and Amount of Aflatoxin M1 in Raw Milks Obtained from Denizli and Aydın. Adnan Menderes University Institute of Health Sciences Department of Food Hygiene, M.Sc. Thesis, 57p.
Iqbal, S.Z., Asi, M.R., 2013. Assessment of aflatoxin M1 in milk and milk products from Punjab, Pakistan. *Food Control*, 30(1), 235-239.

Ismail, A., Riaz, M., Levin, R.E., Akhtar, S., Gong, Y.Y., Hameed, A., 2016. Seasonal prevalence level of aflatoxin M1 and its estimated daily intake in Pakistan. *Food Control*, 60, 461-465.

İşleyici, Ö., Morul, F., Sancak, Y.C., 2012. Investigation of aflatoxin M1 level in UHT sterilized cow milk presented in Van. *Yuzuncu Yıl University The Journal of Veterinary Faculty*, 23(2), 6569.

Karadal, F., Onmaz, E.N., Hızlısoy, H., Yıldırım, Y., Al, S., Gönulalan, Z., 2018. Aflatoxin M1 Levels In Raw Sheep, Goat And Cow Milks In Niğde Province. *Kocatepe Veterinary Journal*, 11(2), 119-125.

Kamkar, A., 2008. Detection of Aflatoxin M1 in UHT milk samples by ELISA. *J. Vet.Res.*, 63(2), 7-12.

Kireççi, E., Savaşçı, M., Ayyıldız, A., 2007. Determination of the presence of aflatoxin M1 in milk and cheese products consumed in Sarıkamış. *Turkish Journal of Infection*, 21(2), 93-96.

Kök, Z., 2006. Investigation of Aflatoxin Presence in Milk and Dairy Products Produced in Aydin and Its Environrs. *Adnan Menderes University Institute of Health Sciences*, M.Sc. Thesis, 48-49.

Kögüstün, F., 2013. Investigation of Aflatoxin M1 Presence in Fresh Kashar and Melting Cheese Samples Presented in Aydin and Izmir by ELISA. *Adnan Menderes Univ. Institute of Health Sciences Department of Food Hygiene and Technology*, M.Sc. Thesis, 45p.

Langat, G., Tetsuhiro, M., Gonoi, T., Matiru, V., Bii, C., 2016. Aflatoxin M1 Contamination of Milk and Its Products in Bomet County, Kenya. *Advances in Microbiology*, 6, 528-536.

Li, S., Min, L., Wang, P., Zhang, Y., Zheng, N., 2017. Jiaqi Wang Occurrence of aflatoxin M1 in pasteurized and UHT milks in China in 2014-2015. *Food Control*, 78, 94-99.

Madali, B., Güleç, A., Ayaz, A., 2018. A survey of aflatoxin M1 in different milk types in Turkey: risk assessment of children’s exposure. *Progress in Nutrition*, 20 (4), 659-664.

Malissiova, E., Tsakalof, A., Arvanitoyannis, I.S., Katsafliaka, A., Katsioulis, A., Tserkezou, P., Koureas, M., Govarise, A., Hadjichristodoulou, C., 2013. Monitoring Aflatoxin M1 levels in ewe's and goat's milk in Thessaly, Greece; potential risk factors under organic and conventional production schemes. *Food Contr.*, 34, 241-248.

Mashak, Z., Sohia, H.J., Heshmatib, A., Nejad, A.S.M., 2015. Assessment of AflatoxinM1 Contamination in UHT Flavored Milk Samples in Karaj, Iran. *Iranian Journal of Pharmaceutical Research*, 15 (3), 407-411.

Mohajeri, F.A., Ghalebib, S.R. Rezaeianc, M., Gheisarid, H.R., Azade, H.K., Zolfagarid, A., Fallah, A.A., 2013. Aflatoxin M1 contamination in white and Lighvan cheese marketed in Rafsanjan, Iran. *Food Control*, 33(2), 525-527.

Özdemir, M., 2007. Determination of aflatoxin M1 levels in goat milk consumed in Kilis province. *Ankara Univ The Journal of Veterinary Faculty*, 54, 99-103.
Öztürk, B., Çelik, F., Çelik, Y., Kabaran, S., Ziver, T., 2014. To Determine the Occurrence of Aflatoxin M1 (AFM1) in Samples of Cyprus Traditional Cheese (Halloumi): A Cross-Sectional Study. *Kafkas University The Journal of Veterinary Faculty*, 20 (5), 773-778.

Peña-Rodas, O., Martinez-Lopez, R., Hernandez-Rauda, R., 2018. Occurrence of Aflatoxin M1 in cow milk in El Salvador: Results from a two year Survey. *Toxicology Reports*, 5, 671–678.

Pitter, A., 1998. Natural Occurrence Of Mycotoxins in Foods and Feeds an Updated Review. *Revue de Medicine Veterinaire*, 149, 479-492.

Prodanov-Radulović, J., Živkov-Baloš, M., Jakšić, S., Grgić, Z., Stojanov, I., Bojkovski, J., Tassis, P., 2018. Aflatoxin M1 levels in sow milk. *Journal of the Hellenic Veterinary Medical Society*, 68(3), 341-346.

Quevedo-Garza, P.A., Amador-Espejo, G.G., Cantù-Martínez, P.C., Trujillo-Mesa, J.A., 2018. Aflatoxin M1 occurrence in fluid milk commercialized in Monterrey, Mexico. *Journal of Food Safety*, 38 (6), e12507. https://doi.org/10.1111/jfs.12507

Rahimi, E., Bonyadian, M., Rafei, M., Kazemeini, H.R., 2010. Occurrence of aflatoxin M1 in raw milk of five dairy species in Ahvaz, Iran. *Food Chem Toxicol.*, 48(1), 129-131.

Rama, A., Latifi, F., Bajraktari, D., Ramadani, N., 2015. Assessment of aflatoxin M1 levels in pasteurized and UHT milk consumed in Prishtina, Kosovo. *Food Control*, 57, 351-354.

Sarmimehetoğlu, B., Kuplulu, O., Çelik, T.H., 2004. Detection of aflatoxin M1 in cheese samples by ELISA. *Food Control*. 15, 45-49.

Skribic, B., Antic, I., Zivancev, J., 2015. Presence of aflatoxin M1 in white and hard cheese samples from Serbia. *Food Control*, 50, 111-117.

Tavakoli, H.R., Riazipour, M., Kamkar, A., Shaldehi, H.R., Nejad, A.S.M., 2012. Occurrence of aflatoxin M-1 in Beyaz cheese samples from Tehran, Iran. *Food Control*, 23, 293-295.

Torkar, K.G., Vengust, A., 2008. The presence of yeasts, moulds and aflatoxin M1 in raw milk and cheese in Slovenia. *Food Control*, 19(6), 570–577.

Türkoğlu, Ç., Keyvan, E., 2019. Determination of Aflatoxin M1 and Ochratoxin A in Raw, Pasteurized and UHT Milk in Turkey. *Acta Scientiae Veterinariae*, 47, 1626. DOI: 10.22456/1679-9216.79176.89667

Ünüşan, N., 2006. Occurrence of aflatoxin M1 in UHT milk in Turkey. *Food and Chemical Toxicology*, 44, 1897-1900.

Xiong, J., Xiong, L., Zhou, H., Liu, Y., Wu, L., 2018. Occurrence of aflatoxin B1 in dairy cow feedstuff and aflatoxin M1 in UHT and pasteurized milk in central China. *Food Control*, 92, 386-390.

Yurt, B., Uluçay, B., 2016. Determination of Some Chemical Properties and Aflatoxin M1 of Raw Cow Milk Produced on Igdır and Region. *International Conference on Natural Science and Engineering (ICNASE’16)*, March 19-20, Kilis, p3624-3635.