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RESEARCH ARTICLE

To Determine the Occurence of Aflatoxin M1 (AFM1) in Samples of Cyprus Traditional Cheese (Halloumi): A Cross-Sectional Study

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Summary

This Cross-Sectional present study was conducted to determine the occurence of aflatoxin M1 (AFM1) in samples of Turkish Republic of North Cyprus (TRNC) traditional cheese (halloumi). In the current study a total of 128 halloumi cheese samples including 36 industrial made and 92 home made were selected by using cluster sampling method and analyzed for AFM1 with the competitive ELISA. The percent of AFM1 contamination in halloumi cheese was found to be low, since 28.8% (0-16.66 ng/kg) of the samples were positive in industrial made and 21.7% (0-4.63 ng/kg) in home made. The mean, standard error of mean (SEM), 95% Confidence Interval (95% CI) values of aflatoxin M1 in halloumi cheese with industrial and home made samples were 0.84±0.24, (95% CI :0.35-1.35) and 1.28±0.32, (95% CI: 0.63-1.93) respectively. Both means were not significantly difference (P=0.422) and found very low from the limits of European Commission (EC) (250 ng/kg) and Turkish Food Codex (TFC) (500 ng/kg) (P<0.001). In order to prevent from introduction of aflatoxin M1 into cheese industry cycle, hygienic conditions, appropriate storage and control of livestock feed at all stages of planting and requires system that makes aflatoxin control are necessary.

Keywords: *Halloumi cheese, Aflatoxin M1, Cheese, Enzyme-linked immunosorbent assay, ELISA*

Kıbrıs Geleneksel Peynir (Hellim) Örneklerinde Aflatoksin M1 (AFM1) Oluşumunun Belirlenmesi: Kesitsel Bir Çalışma

Özet

Bu kesitsel çalışma ile Kıbrıs geleneksel peynir (Hellim) örneklerinde aflatoksin M1 (AFM1) oluşumunu belirlemek için yapılmıştır. Çalışmada küme örnekleme yöntemiyle 36 endüstriyel yapımı ve 92 ev yapımı olmak üzere toplam 128 Hellim peyniri örneğe seçildi. Peynir örneklerindeki AFM1 varlığı ELISA yöntemi ile analiz edilerek belirlendi. Endüstri yapımı Hellim peynirinde AFM1 pozitif bulunma oranı %28.8 (0.00-16.66 ng/kg), ev yapımı Hellim peynirinde ise bu oran %21.7 (0.00-4.63 ng/kg) şeklinde düşük bir oran olduğu belirlendi. Endüstri ve ev yapımı Hellim peynirindeki AFM1 değerinin ortalama, standart hata (SEM) ve %95 güven aralığında sırasıyla 0.84±0.24, (%95 CI: 0.35-1.35) ve 1.28±0.32, (%95 CI: 0.63-1.93) şeklinde bulundu. Her iki ortalama arasındaki farklılık önemli bulunmadı (P=0.422) ve European Commission (EC) (250 ng/kg) and Turkish Food Codex (TFC) (500 ng/kg) değerlerinden önemli derecede düşük olduğu bulundu (p<0.001). Aflatoksin M1 'in peynirin endüstri döngüsündeki girişini önlemek için, hijyenik koşullar, uygun depolama ve hayvan yemlerinin tüm aşamalarda kontrolünün sağlanması ve aflatoxin düzeyini denetleyen bir sistemin olması gerektiği bilinmelidir.

Anahtar sözcükler: *Hellim peyniri, Aflatoxin M1, Peynir, Enzyme-linked immunosorbent assay, ELISA*

INTRODUCTION

Halloumi is a firm pickled cheese with its origins in TRNC where it is made from sheep or goat milk or a mixture of both. It can also be made from cow milk. Starter is not used. The cheese may be eaten fresh or after storage in a cool store. If it is stored at below 12°C it will keep for several months. After salting the cheese pieces may also be stored in plastic bags without brining; if stored at about 10°C the cheese has a shelf-life of two to three months.

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About one kilogram of cheese will be obtained from nine liters of milk. The production and energy-nutritional values (100 g) of Halloumi cheese are presented as follows; the production of hallumi cheese was Milk, Coagulation, Processing of the Curd, Taking the Nor Cheese, Cooking, Salting and Folding, Packaging. Energy and nutritional values (100 g) of halloumi cheese were Energy: 352.6 kcal, Protein: 26 g, Calcium: 700 mg, Phosphorus: 590 mg, Carbohydrates: 1.4 g, Fat: 27 g [1].

Aflatoxins are carcinogenic compounds produced predominantly by certain strains of the Aspergillus genus. They are both acutely and chronically toxic, mutagenic, teratogenic and carcinogenic compounds for animal and human Contamination of milk and dairy products to aflatoxin M_1 is a risk for human health. Afloatoxin M_1 (AFM_1) is relatively stable during milk pasteurization and storage as well as during the preparation of various dairy products. Aflatoxin M_1 is the principle hydroxylated metabolite of aflatoxin B_1 which is transformed at the hepatic level by means of cytochrome p450 enzymes and excreted into the milk in the mammary glands of both human and lactating animal after ingestion by the animal of pellets and forage contaminated with aflatoxin B_1 [2-5].

Of the 550.000-600.000 new hepatocellular carcinoma (HCC) cases worldwide each year, about 25.200-155.000 may be attributable to aflatoxin exposure. Most cases occur in sub-Saharan Africa, Southeast Asia, and China where populations suffer from both high HBV prevalence and largely uncontrolled aflatoxin exposure in food. Liver cancer, is the third leading cause of cancer deaths worldwide, with prevalence 16-32 times higher in developing countries than in developed countries. Aflatoxin may play a causative role in 4.6-28.2% of all global HCC cases [6-8].

When cheese-making is carried out using AFM_1 contaminated milk, this toxin is likely to have become enriched in the final curd compared to that found in milk. This could be explained by both the capacity of AFM_1 to somehow bind caseins and increased dry matter content. The affinity of AFM_1 has been tested not only with these proteins, but also with other different ones present in whey as a larger amount of this toxin has been demonstrated to be present in the retentive where the protein-rich fraction appears. Therefore it is necessary to note whether AFM_1 is present in final products like cheese because its concentration in them has been reported to be around 2.1-4.5 times higher than in the original milk used, depending on the cheese type [9,10].

The measured AFM_1 concentration was correlated to four factors which were presumed to influence the contamination level: manufacturing, production season, milking animal, and maturation. Statistical analyses demonstrated that milking animals and manufacturing affect AFM_1 concentrations, as cheeses obtained from cows' milk and from artisanal production are more contaminated than cheeses produced with milk belonging to other animals and in industrial contexts [11].

Markets in developing countries generally do not reward reduced aflatoxins in crops because it is difficult to discern aflatoxin contamination or its risks. The presence of mold is a potential, but highly imperfect, indicator of aflatoxin contamination. Surveys in a few African countries show that farmer knowledge and awareness are far from perfect, as are storage and drying practices. While some moldy grain is diverted to uses that somewhat reduce direct human exposure (such as for brewing and animal feeds), quality differentiation based on either market rewards or public standards is still unusual in most developing countries [12].

Vacuum packaging is sometimes used to inhibit fungal growth on cheese, but some fungal species are able to grow under these condition. It has been isolated that several fungal species from vacuum packaged cheeses, the most commonly occurring being species of Cladosporium, Penicillium and Phoma [13,14].

The European Commission (EC) has approved a maximum admissible level of 250 ng/kg for AFM_1 in cheese [15]. However, the Turkish Food Codex (TFC), has accepted 500 ng/kg as the action level for AFM_1 [16].

Although there are some literature published about the occurrence of AFM_1 in various cheese like feta, Parmesan, Manchego, Kahramanmaraş, white, kashar, cream, civil and cheeses produced by dairy ewe's milk [17-24], there is not any information about the occurrence of AFM_1 in Halloumi is a firm pickled cheese with its origins in Cyprus.

For this purpose, the current study was designed to determine the presence and levels of AFM_1 in halloumi cheese that especially consumed fresh or after storage in a cool store in Cyprus province and to suggestion how could it protect from aflatoxin. Also the levels of AFM_1 found for halloumi cheese will compare the results with the legal regulations for AFM_1 legislated by EC and Turkish Food Codex (TFC).

MATERIAL and METHODS

Collection of Samples

A total of 128 samples of TRNC traditional cheese (halloumi) were collected during November and December 2013 from main districts of TRNC. The samples of halloumi were collected from dairy farmhouses and retail markets.

Cluster Sampling Method was used in selection the dairy farmhouses and retail markets. Cyprus was divided into four main clusters. Each cluster was selected to be heterogeneous as possible. Farmhouses and retail markets were selected from each cluster by using Random

Number Tables with Random Sampling Method. Thus, the randomization for sampling was completed carefully.

The size of halloumi samples were at least 200 g. The samples were preserved in their original packages or plastic bags, and during collection and transportation, samples were kept in an icebox. The samples were immediately transported to the laboratory in a cooler with ice packs and stored at -20°C until analysis. All samples were analyzed before their expiry dates. Laboratory studies of research were completed in Nutrition and Dietetics Laboratories in Eastern Mediterranean University (EMU).

Method of Analysis

The quantitative analysis of AFM_1 was performed using enzyme immunoassay: Ridascreen aflatoxin M kit (R-Bipharm AG, Germany).

The test is based on the antigen-antibody reaction. The assay was performed according to the manufacturer's recommendation and as described elsewhere [25]. The mean lower detection limit of the assay was 5 ng/l.

Extraction and ELISA Analysis

The analysis of AFM_1 in halloumi samples were performed according to the R-Biopharm Aflatoxin M_1 test kit's instructions. Determination of AFM_1 in the cyprus traditional cheese was determined using Aflatoxin M_1 Test Kit (R-Biopharm AG, Darmstadt, Germany), which is competitive enzyme immunoassay kit. One hundred micro liter of standart solutions and prepared samples were added into separate microtitre wells and incubated for 60 min at room temperature in the dark. The liquid was then poured out and the wells washed with washing buffer (250 µl) twice. In the following stage, 100 µl of the diluted enzyme conjugate was added to the wells and incubated for 60 min at room temperature in the dark. Again, the wells were washed 3 times with washing buffer. Afterwards, 50 µl of substrate and 50 µl chromogen was added, mixed gently and incubated in the dark at room temperature for 30 min. Finally, 100 µl of the stop reagent was added into the wells and absorbance was measured at 450 nm in ELISA plate reader.

Statistics

All continuous variables were presented as mean ± standard error of mean (SEM), 95% Confidence Interval (95% CI) and all categorical variables were presented as number of patients and percentages. Significant differencies between the mean values for two kind of Halloumi cheese (industrial and home made) were analyzed by using Student's t test for two independent groups. Student's t test for one population mean was also used to test the both means of AFM_1 found for industrial and home made with the mean values of the European Commission (EC) and Turkish Food Codex (TFC).

A power analysis using "Proportion Difference Power/ Sample Size Calculation" software was conducted to calculate sample size. Using previously published data for AFM_1, proportion difference of 27%, with the power of the test set to 0.85, and significance level at 0.05 resulted in sample size 128.

For all statistical analyses a P value ≤ 0.05 was considered statistically significant. The SPSS 15.0 statistical package was used to perform all statistical analyses (SPSS Inc., Chicago, IL, USA).

RESULTS

In the current study a total of 128 industrial made halloumi cheese samples including 36 industrial made and 92 home made were analysed for AFM_1 with the competitive ELISA.

The occurrence and the distribution of AFM_1 concentration in various ranges in cheese samples for industrial and home made are presented in *Table 1* and *Table 2* respectively.

The mean, standart error of mean (SEM), 95% Confidence Interval (95%CI) values of aflatoxin M_1 in halloumi cheese with industrial and home made samples were presented by *Table 3*. *Table 3* also contents the results of Student's t test and significant value.

Table 1. *Distribution of aflatoxin M_1 contents in various range in halloumi cheese with industrial made samples*
Tablo 1. *Endüstri yapımı hellim peyniri örneklerinde aflatoksin M_1 dağılımı*

Samples	AFM1 (ng/kg)	Percent (%)	Total Percent (%)	Min-Max (ng/kg)
26	0.00 (None)	72.2(None)	72.2(None)	0.00-4.63
8	1.00-3.90	22.2	28.8	
2	4.00-6.90	6.6		
36 (Total)		100.0	100.0	

Table 2. *Distribution of aflatoxin M_1 contents in various range in halloumi cheese with home made samples*
Tablo 2. *Ev yapımı hellim peyniri örneklerinde aflatoksin M_1 dağılımı*

Samples	AFM_1 (ng/kg)	Percent (%)	Total Percent (%)	Min-Max (ng/kg)
72	0.00 (None)	78.3(None)	78.3(None)	0.00-16.66
8	1.00-3.90	8.7	21.7	
6	4.00-6.90	6.4		
3	7.00-9.90	3.3		
2	10.00-12.90	2.2		
0	13.00-15.90	0.0		
1	16.00-18.90	1.1		
92 (Total)		100.0		

Table 3. *Descriptive statistics and test results for industrial and home made of halloumi cheese*
Tablo 3. *Endüstriyel ve ev yapımı hellim peyniri için tanımlayıcı istatistikler ve test sonuçları*

Halloumi	n	X AFM_1 (ng/kg)	SEM	95%CI	t	p
Industrial made	36	0.84	0.24	0.35-1.35	0.81	0.422
Home made	92	1.28	0.32	0.63-1.93		

x: *Mean,* ***SEM:*** *Standard Error of Mean; 95%CI: 95% Confidence Interval,* ***t:*** *Student's t test was analyzed two different means*

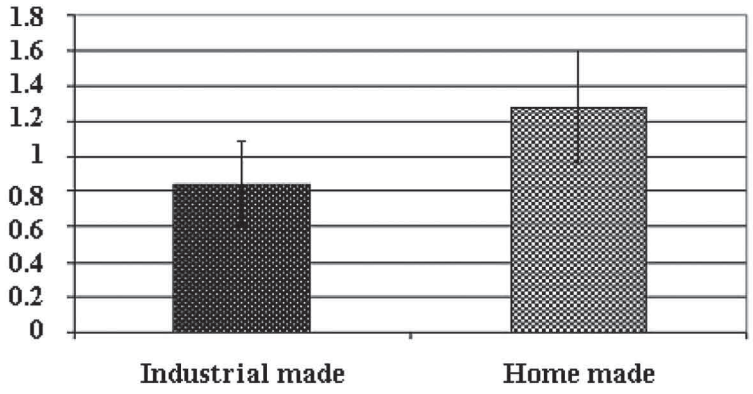

Fig 1. The distribution of mean and SEM values of industrial and home made halloumi cheese

Şekil 1. Endüstriyel ve ev yapımı hellim peynirinin ortalama ve standart hata dağılımı

According to the results of *Table 3*, there was not found significant differencies between the mean values for two kind of Halloumi (P=0.422).

Both means of AFM_1 found for industrial (0.84 ng/kg) and home made (1.28 ng/kg) were analyzed with the mean values of the European Commission (EC) (250 ng/kg) and Turkish Food Codex (TFC) (500 ng/kg) by using Student's t test for one population mean. The results were found significantly difference (P<0.001).

Fig. 1 represents the distribution of mean and SEM values in two kind of Halloumi cheese.

DISCUSSION

The percent of AFM contamination in halloumi cheese was found to be low, since 28.8% (0-4.63 ng/kg) of the samples were positive in industrial made and 21.7% (0-4.63 ng/kg) in home made. Both persentages were not significantly difference and found very low from the limits of European Commission (EC) (250 ng/kg) [15] and Turkish Food Codex (TFC) (500 ng/kg) [16].

Filazi et al.[24] reported that AFM_1 in cheeses may be hazardous to human, particularly children. For this reason, there are many studies concerning the presence of AFM_1 in dairy products [26-28]. The presence of AFM_1 was detected in concentrations between 20-2000 ng/kg in 14 of 50 samples (28%). Altogether, 5 cheese samples (10%) were found to have levels that exceed the legal limits of 250 ng/kg established by the Turkish Food Codex. It was therefore concluded that, widespread occurrence of AFM_1 in ewe's milk cheese samples produced in Urfa city were considered to be possible hazards for human health.

Atasever et al.[22] have examined in terms of AFM_1 in 304 cheese samples 85 white cheese, 75 kashar cheese, 62 civil cheese, 82 cream cheese) put up for sale in various places in Erzurum. The AFM_1 ontent 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_1, 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_1 level determined in some cheese types is an important problem threatening the public health in Turkey.

In the study of Turgay et al.[20] have also selected 46 cheese samples that were obtained from various markets located in Kahramanmaraş. In all, 22 of the 46 samples were made from bovine milk, 6 were made from ovine milk, and 18 were made from goat milk. None of the ovine milk cheese samples contained AFM_1. AFM_1 was present in 32 samples (69.6%) of bovine and goat cheese. Bovine milk cheese and goat milk cheese samples contained 0.069-1.2 ng g^{-1} and 0.06-0.22 ng g^{-1} of AFM_1, respectively. With the exception of 2 bovine milk cheese samples (one contained 1.2 ng g-1 of AFM_1, the other contained 0.25 ng g of AFM_1), the other samples (96%) had levels of AFM_1 below the acceptable limit for cheese (0.25 ppb)

set forth by the Turkish Alimentarus Codex.

Tekinsen and Tekinsen [29] reported in 60 samples of Van otlu (herb) and 50 white pickle cheese samples obtained from retail outlets in Van and Hakkari, Turkey. The rate of AFM_1 in Van otlu and white pickle cheese samples ranged from 0.16 to 7.26 µg kg and from 0.10 to 5.20 µg kg^{-1} respectively. In all, 80% of Van otlu cheese and 40% of white pickle cheese samples exceeded the maximum acceptable level. However in the study of Kivanc [30] the absence of AFM_1 was found at detectable levels in Van otlu and white cheese samples in Van, Turkey.

The result of studies for AFM_1 were examined for the cheeses produced outside of Turkey were presented as follows. In the results of Rubio et al.[10], Aflatoxin M_1 distribution in curd, whey, Manchego cheese, the traditional Spanish whey cheese Requesón and Requesón whey, and its stability during two different cold treatments, have been studied. At the end of study the contamination AFM_1 in Manchego cheese was found at the EU limit level (50 ng/kg).

In the study of Oliveira et al.[31], 24 samples of Minas Frescal cheese and 24 samples of Minas Padrao cheese produced in the North-east region of the state of Sao Paulo, Brazil, were analyzed for aflatoxin M_1. AFM_1 was detected in 13 (27.1%) samples at concentrations ranging from 0.037 to 0.313 ng g^{-1}. The mean concentrations of high incidence of AFM_1 in positive samples of Minas Frescal and Minas Padrao cheese were 0.142±0.118 and 0.118±0.054 ng g^{-1}, respectively. In another study, fresh cheese produced in Argentina from artificially contaminated milk with AFM_1 at levels of 1.7-2.0 ng mL^{-1} had 60% of AFM_1 in the whey and 40% in cheese [32].

The mean values of AFM_1 found in the present study in halloumi were found lower than the results of all cheeses reported above. This result is noteworthy for human exposure to this toxin.

Aflatoxicosis is primarily a hepatic disease. The susceptibility of individual animals to aflatoxins varies considerably depending on species, age, sex and nutrition. In fact, aflatoxins cause liver damage, decreased milk and egg production, recurrent infection as a result of immunity suppression, in addition to embryo toxicity in animals consuming low dietary concentrations. While the young of a species are most susceptible, all ages are affected but in different degrees for different species. Clinical signs of aflatoxicosis in animals include gastrointestinal dysfunction, reduced reproductivity, reduced feed utilization and efficiency, anemia and jaundice. Nursing animals may be affected as a result of the conversion of aflatoxin B_1 to the metabolite aflatoxin M excreted in milk of dairy cattle. Aflatoxin B_1, M_1, and G_1 have been shown to cause various types of cancer in different animal species. Aflatoxin develops in the field when grains are exposed to severe environmental conditions. Management practices that improve plant health strongly discourage aflatoxin development which timely planting, adequate fertility, good weed and insect control, supplemental irrigation, suitable plant population, and hybrid selection should help reduce aflatoxin potential [33].

Naturally, the diet of the cow has a major impact on its health. The type of grass, greatly affects the nutrition profile of the cow. The reasons of low level AFM_1 in Halloumi cheese, sheep and cows grazing on fields of luscious green grass in Cyprus. This is the main reason reduses the AFM_1 level in cheese.

In order to prevent and reduce the negative implications of these mycotoxins in cheese production, it is necessary to create both global and national strategies to reduce the amount of mycotoxins in grain. In order to prevent from introduction of aflatoxin M_1 into cheese industry cycle, hygienic conditions, appropriate storage and control of livestock feed at all stages of planting and requires system that makes aflatoxin control are necessary.

REFERENCES

1. Hellim Tanıtım Grubu: Hellim/Halloumi. http://hellimhalloumi.org, *Accessed:* 10 March 2014.

2. Darsanaki RK, Mohammadi M, Kolavani MH, Issazadeh K, Aliabadi MA: Determination of aflatoxin M_1 levels in raw milk samples in Gilan, Iran. *Adv Stud Biol,* 5 (4): 151-156, 2013.

3. Motawee MM: Reduction of aflatoxin M_1 content during manufacture and storage of Egyptian Domaiti Cheese. *Int J Vet Med Res Rep,* 2013, 1-11, 2013.

4. Ayoub MM, Mahmoud KSA, Amal AR: Evaluation of aflatoxin M_1 in raw, processed milk and some milk products in Cairo with special reference to its recovery. *Researcher,* 3 (9): 5-11, 2011.

5. Mohamadi SMA, Khezri M, Moradnia H: Determination of aflatoxin M_1 in milk by ELISA technique in Mashad (Northeast of Iran). *Int Schol Res Network ISRN Toxicolog,* 2012, 1-4, 2012.

6. Liu Y, Wu F: Global burden of aflatoxin-ınduced hepatocellular carcinoma: A risk assessment. *Environ Health Persp,* 118, 818-824, 2010.

7. World Health Organization (WHO): The Global Burden of Disease: 2004 Update. Geneva: 2008. http://www.who.int/healthinfo/global_burden_disease/2004_report_update/en/index.html. *Accessed:* 10 March 2014.

8. Wild CP, Gong YY: Mycotoxins and human disease: A largely ignored global health issue. *Carcinogenesis,* 31 (1): 71-82, 2010.

9. Manetta AC, Giammarco M, Di Giuseppe L, Fusaro I, Gramenzi A, Formigoni A, Vignola G, Lambertini L: Distribution of aflatoxin M_1 during Grana Padano cheese production from naturally contaminated milk. *Food Chemistry*, 113 (2): 595-599, 2009.

10. Rubio R, Moya VJ, Berruga MI, Molina MP: Aflatoxin M_1 in the intermediate dairy products from Manchego cheese production: Distribution and stability. *Mljekarstvo*, 61 (4): 283-290, 2011.

11. Anfossi L, Baggiani C, Giovannoli C, D'Arco G, Passini C, Giraudi G: Occurrence of aflatoxin M_1 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 (1): 125-130, 2012.

12. Grace D: Animals and aflatoxins. **In,** Unnevehr L, Grace D (Eds): Aflatoxins Finding Solutions for Improved Food Safety. 16-18, Int. Food Policy Res Inst, USA, 2013. http://www.ifpri.org/sites/default/files/publications/focus20.pdf, *Accessed:* 10 March 2014.

13. Taniwaki MH, Hocking AD, Pitt JI, Fleet GH: Growth of fungi and mycotoxin production on cheese under modified atmospheres. *Int J Food Microbiol*, 68 (1-2): 125-133, 2001.

14. Hocking AD, Faedo M: Fungi causing thread mould spoilage of vacuum packaged cheddar cheese during maturation. *Int J Food Microbiol,* 16 (2): 123-130, 1992.

15. 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.

16. Türk Gıda Kodeksi (TGK): Gıda maddelerinde belirli bulaşanların maksimum seviyelerinin belirlenmesi hakkında tebliğ. *Resmi Gazete,* Sayı: 26879, 17 Mayıs 2008.

17. Motawee MM, McMahon DJ: Fate of aflatoxin M_1 during manufacture and storage of feta cheese. *J Food Sci*, 74 (5): 42-47, 2009.

18. Prado G, Oliveira MS, Lima AS, Moreira APA: Occurrence of aflatoxin M_1 in parmesan cheese consumed in Minas Gerais, *Brazil Ciênc,* 32 (6): 1413-1418, 2008.

19. Rubio R, Moya VJ, Berruga MI, Molina MP, Molina A: Aflatoxin M_1 in the intermediate dairy products from Manchego cheese production: Distribution and stability. *Mljekarstvo*, 61 (4): 283-290, 2011.

20. Turgay O, Aksakal DH, Sunnetci S, Celik AB: A survey of aflatoxin M_1 levels in Kahramanmaraş cheese. *Turk J Vet Anim Sci*, 34 (6): 497-500, 2010.

21. Oruc HH, Cibik R, Yilmaz E, Kalkanli O: Distribution and stability of aflatoxin M_1 during processing and ripening of traditional white pickled cheese. *Food Addit Contam*, 23 (2): 190-195, 2006.

22. Atasever MA, Adiguzel G, Atasever M, Ozturan K: Determination of aflatoxin M_1 levels in some cheese types consumed in Erzurum - Turkey. *Kafkas Univ Vet Fak Derg*, 16 (Suppl-A): S87-S91, 2010.

23. Rahimi E, Anari MM, Alimoradi M, Rezaei P: Aflatoxin M_1 in pasteurized milk and white cheese in Ahvaz, Iran. *Global Veterinaria*, 9 (4): 384-387, 2012.

24. Filazi A, Ince S, Temamogullari F: Survey of the occurrence of aflatoxin M_1 in cheeses produced by dairy ewe's milk in Urfa city, Turkey. *Ankara Üniv Vet Fak Derg*, 57, 197-199, 2010.

25. Lopez C, Ramos L, Ramadan S, Bulacio L, Perez J: Distribution of aflatoxin M_1 in cheese obtained from milk artificially contaminated. *Int J Food Microbiol*, 64, 211-215, 2001.

26. Aksoy A, Yavuz O, Guvenc D, Das YK, Terzi G, Celik S: Determination of aflatoxin levels in raw milk, cheese and dehulled hazelnut samples consumed in Samsun province, Turkey. *Kafkas Univ Vet Fak Derg*, 16 (Suppl-A): S13-S16, 2010.

27. Atasever MA, Adiguzel G, Atasever M, Ozlu H, Ozturan K: Occurrence of aflatoxin M_1 in UHT milk in Erzurum-Turkey. *Kafkas Univ Vet Fak Derg*, 16 (Suppl A): S119-S122, 2010.

28. Karakaya Y, Atasever M: Aflatoxin B_1 in corn silage and its probability passing in milk. *Kafkas Univ Vet Fak Derg*, 16 (Suppl-A): S123-S127, 2010.

29. Tekinsen KK, Tekinsen OC: Aflatoxin M_1 in white pickle and Van otlu (herb) cheeses consumed in southeastern Turkey. *Food Control*, 16 (7): 565-568, 2005.

30. Kıvanç M: Mold growth and presence of aflatoxin in some Turkish cheeses. *J Food Safety*, 10 (4): 287-294, 1990.

31. Oliveira CAF, Franco RC, Rosim RE, Fernandes AM: Survey of aflatoxin M_1 in Minas cheese from the North-East region of São Paulo, Brazil. *Food Addit Contam: Part B: Surveillance,* 4 (1): 57-60, 2011.

32. López C, Ramos L, Ramadan S, Bulacio L, Perez J: Distribution of aflatoxin M_1 in cheese obtained from milk artificially contaminated. *Int J Food Microbiol,* 64 (1-2): 211-215, 2001.

33. Talebi E, Khademi M, Rastad A: An over review on effect of aflatoxin in animal Husbandry. *Asian J Exp Biol Sci*, 2 (3): 754-757, 2011.