Praca oryginalna *Original paper*

Aflatoxin M1 levels in different type of cheese products produced in Turkey

KURSAT YAPAR, MEHMET ELMALI*, ASIM KART, HILMI YAMAN*

Department of Pharmacology and Toxicology, *Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Kafkas University, 36100, Kars, Turkey

Yapar K., Elmali M., Kart A., Yaman H.

Aflatoxin M1 levels in different type of cheese products produced in north-east Turkey

Summary

The aim of the study was to investigate aflatoxin M1 (AFM1) levels in different types of cheese samples (old kashar cheese, fresh kashar cheese, civil cheese, white cheese, and Gravier cheese) produced in north eastern Turkey and determine the potential risk posed to human health by the consumption of these products. AFM1 was detected in 75 cheese samples (71.42%) out of 105 samples analyzed, whereas the levels of AFM1 in 40 cheese samples (38.08%) were found to exceed the limits (250 ng/kg) allowed by TFC. Of the analyzed cheese samples, AFM1 levels in 7 (28%) white cheeses, 5 (20%) old kashars, 6 (24%) fresh kashars, 20 (80%) civil cheeses and 2 (40%) gravier cheeses were above the limits set by TFC.

In conclusion, the elevated levels of AFM1 in some cheese samples found above the TFC limits could adversely affect human health. Therefore, it is important to prevent toxin production from the production stage to consumption of the cheeses as well as devising an effective detoxification process.

Keywords: aflatoxin, AFM1, cheese, ELISA

Aflatoxins (AFs) are toxic, immunosuppressive and carcinogenic metabolites produced by certain strains of *Aspergillus flavus* and *Aspergillus paraciticus* spp. (9, 10). Studies showed that AFs are capable of inducing many disorders including hepatotoxicity and liver cancer (15, 27, 28). In addition, contamination of foods with aspergilli causes several undesirable effects on foodstuff including unpleasant odor, taste, aroma and decreased shelf-life. Aflatoxins are classified in 4 different fractions including B1, B2, G1 and G2 (10, 35). The B1 and B2 fractions are metabolized by the hepatic mixed-function oxidase system to yield M1 and M2 fractions which are excreted in milk. The M1 form is reported to be as toxic as AFB1 and shows similar toxic effects in human and animal species. (9, 14, 24, 35).

AFB1 and AFM1 were classified by the International Agency for Research on Cancer (IARC) as 1st and 2nd class carcinogens, respectively (4). In addition, studies clearly indicate that AFB1 and its hydroxylated metabolite AFM1 have the greatest toxic effects (especially for the genotoxic effect) among aflotoxins (8, 32). Therefore, AFM1 levels in milk and milk products are of great public concern, especially as far as young children are concerned. Hence, the level of AFM1 in dairy products is strictly regulated by governmental agencies. According to FAO/WHO (1) and EU countries (35), the maximum allowed level of AFM1 is 50 ng/kg in milk samples. Turkish Food Codex (3) allows 50 ng/L of AFM_1 in milk and 250 ng/kg AFM_1 in cheese samples.

Igdir, Ardahan and Kars are the leading cities of animal husbandry in north-eastern Turkey. The number of sheep and cattle raised in this region accounts for an important part of Turkey's animal population, and the majority of milk produced is mainly utilized in cheese production as kashar, white cheese, cecil and gravier. These products are sold in both local markets and big cities of western Turkey and consumed by different age groups including children at a developing age. Ninety percent of the milk produced in Turkey is processed as kashar, white and cecil cheese (19).

Kashar is a type of aromatic cheese which can be consumed either immediately after its production (fresh cheese) or after a 3-4 month fermentation period at 10°C (old kashar). Cecil is a locally produced cheese which can be consumed either freshly or can be fermented in pots by mixing it with white cheese and salt to be consumed later. Gravier is a Kaskaval-like cheese produced primarily in the Balkans with a limited production in the Kars and Ardahan vicinity.

It is known that AFM1 in milk is not affected during the process of cheese production, and the level of AFM1 in cheese remains same as in the milk (13, 20, 25, 34). The aim of this study was to investigate AFM1 levels in cheese samples (old kashar, fresh kashar, cecil, white and gravier cheese) produced in the above region and determine the potential risk posed to human health by the consumption of these cheese products.

Material and methods

Samples of 25 Turkish-type white cheese, 25 old kashar, 25 fresh kashar, 25 civil and 5 Kars gravier cheese (approximately 50 g for each sample) were collected from the local stores in Kars, Ardahan and Igdir and stored at 4°C until they were analyzed. The quantitative analysis of aflatoxin M1 (AFM1) in cheese samples were carried out by competitive enzyme immunoassay test procedures as described by R-Biopharm (29) GmBH, Germany (Ridascreen® Aflatoxin M1 30/15, Art. No.: R1101).

Preparation of samples for analysis:

Preparation of samples and ELISA test procedure were carried out according to the test booklet obtained along with a commercially available kit (Ridascreen®). Two grams of cheese sample were weighed into a centrifugal glass vial. Forty ml of dichloromethane (Merck) were added to the samples and manually shaken for 15 minutes. The mixture was filtered through Whatman filter paper. Dichloromethane were evaporated at 60°C from 10 ml of the extract. The residue was mixed with the addition of 0.5 ml methanol, 0.5 ml PBS buffer and 1 ml heptane (Merck). Following the centrifugation of the mixture at 2700 × g for 15 minutes, the upper heptane layer was completely removed. An aliquot of 100 µL of the lower layer (methanolic-aqueous phase) was taken using a Pasteur pipette. The aliquot was then mixed with 400 µL Buffer 1, and 100 µL of this mixture was used for the analysis.

A hundred µL of standard solution and the previously prepared samples were added to micro titer wells and then incubated in a dark room at room temperature for 30 minutes. At the end of incubation, the liquid in the wells was poured out, and the micro well holder was tapped upside down on an absorbent paper to remove the remainder of the liquid. The wells were washed twice with 250 µL of washing buffer, and then the liquid in them was poured out. A hundred µL of the diluted enzyme conjugate was added to each well, and they were gently shaken. The wells were incubated for 15 minutes at room temperature in a dark room. Following the addition of 100 µL of the stop reagent to each well, absorbance was measured spectrophotometrically at 450 nm (Spectra Max 384 Plus). The data obtained from the standards and samples were evaluated using a computer program (Ridawin, Food, Mycotox, AflaM1. met, RIDA®SOFT Win) for Windows.

Results and discussion

The levels of AFM1 in different type of cheese samples were presented in tab. 1. Of the total 105 cheese samples analyzed, AFM1 was detected in 71.42% (75/105) of the samples whereas 38.08% (40/105) of the total number of cheese samples was found to exceed the maximum allowed limit (250 ng/kg) set by The Turkish Food Codex (TFC) (3). Amongst the cheese products, the AFM1 levels in 7 white cheeses (28%), 5 old kashars (20%), 6 fresh kashars (24%), 20 civil cheeses (80%) and 2 gravier cheeses (40%) were not in accordance with the TFC limits.

Milk and other dairy products are always at risk of being contaminated with aflatoxin M1. Parallel to the increasing amount of milk and dairy product consumption, studies on the presence of AFM1 in cheese products have been increasing globally as well as in Turkey.

In this work, the number of cheese samples contaminated with AFM1 were found to be higher (71.42%) than in previously reported studies by many authors (11, 12, 16, 19, 22, 23). Previous studies monitoring the AFM1 levels of cheese samples produced in Turkey showed that cheese samples contain AFM1 levels in excess of the allowed limits. Yaroglu et al. (36) reported that 5% of the analyzed cheese samples (30/600) was contaminated with AFM1, and 1% of all the analyzed samples (6/600) were above the limits of TFC (> 250 ng/kg). In addition, Sarimehmetoglu et al. (30) found that 81.75% of cheese samples had AFM1 (327 contaminated out of 400 samples), and 27.5% of the samples (110/400) exceeded the allowed limits according to the TFC. In another study, Oruc and Sonal (26) reported that 89.5% of the analyzed samples had AFM1 levels between 0-810 ng/kg. Similarly, Baskaya et al. (7) reported that AFM1 levels in different types of cheese samples (kashar, white and processed cheese) were above the TFC limits in 22.04% of the analyzed samples (80 out of 363). In another study performed on 108 cheese samples (31), it was found that 91.81% of cheese samples contained AFM1, but 6% of all the analyzed samples exceeded the TFC limits. Aycicek et al. (5) studied AFM1 levels in cream cheese, kashar and white cheese samples. AFM1 were detected in 89.9% of cream cheese, 91.49% of white cheese and 88.68% of kashar cheese samples. While AFM1 levels in cream cheese samples were within the allowed levels of TFC, 12.76% of white cheese and 13.20% of kashar cheese samples were above the TFC limits. In another study, Aycicek et al (6) showed that 65% of white cheese samples (121 out of 186) contained AFM1, and 19% of all samples (35/186) were above the TFC limit. Gurses et al. (17) found that AFM1 in different type of cheese products were at 7-202 ng/kg levels in 28 samples out of 63.

The percentage of cheese samples contaminated with AFM1 in the present study were found to be lower than that of previous reports of Yaroglu et al. (36) and Aycicek et al. (6), but higher than that other authors (5, 26, 30, 31).

Karaioannoglu et al. (21) reported that while the presence of AFM1 was detected in 4 out of 99 raw milk samples collected from different regions of Greece, no AFM1 was found in Feta and Teleme cheese samples in Greece. Stoloff and Wood (33) found that average the

Tab. 1. Levels of Aflatoxin M1 in some cheese types produced in North Eastern Part of Turkey

Type of cheese analyzed	Number of sample	< 50 ng/kg	51-250 ng/kg	251-400 ng/kg	> 400 ng/kg
White cheese	25	8	10	7	–
Old kashar	25	11	9	4	1
Fresh kashar	25	7	12	4	2
Civil cheese	25	4	1	20	–
Gravier cheese	5	–	3	2	–
Total	105 (100 %)	30 (28.57%)	35 (33.33%)	37 (35.23%)	3 (2.85%)

AFM1 level was 0.08 ng/kg in 399 cheese samples in the USA.

The rate of AFM1 detection in cheese samples varies in relation to the year. This variation could be due to the fact that the techniques used detecting AFM1 in the earlier years were not sensitive enough. The use of high performance liquid chromatography (HPLC) and ELISA techniques in particular over the last decade may have contributed to the frequency of AFM1 detection in cheese samples. Indeed, the frequency of AFM1 detection in cheese samples has increased in studies performed over the last 5 years.

Since AFM1 is a metabolite of AFB1 excreted in milk, detecting highly increased concentrations of AFM1 in cheese samples implies the presence of very high AFB1 levels in feed. Kars, Ardahan and Igdir are located in the north eastern part of Turkey with very harsh winter seasons. Farmers in this part of Turkey harvest hay in the summer and feed it to the cattle during the winter. Fungi present in haystacks may easily produce toxins in inappropriate storage conditions. Following the consumption of highly contaminated feed with AFB1, conversion of AFB1 to AFM1 takes place in the liver and leads to elevated levels of AFM1 in the milk. It is known that pasteurizing milk has no effect on the level of AFM1. Therefore, it is important to reduce the occurrence of toxins (AFB1) in feedstuff and take prophylactic measures to prevent factors enhancing toxin production. These factors include environmental temperature, humidity, and moisture content of the feed as well as pH and mechanical damage to the grain affecting mold production. Hence, management practices in harvest and storage regarding the aforementioned factors could decrease AF occurrence in feed.

Alternatively, the addition of propionate and gentian violet to the feed as the mycotoxin binding agent has been studied as a way of reducing mycotoxin levels in contaminated feeds (2). Therefore, effective methods can be utilized to prevent, reduce or minimize the risk AF in animal feed and cheese products in human diet.

In conclusion the presence of AFM1 was detected in 75 cheese samples (71.42%) out of 105 samples analyzed, whereas the levels of AFM1 in 40 cheese samples (38.08%) were found to exceed the limits (250 ng/kg) allowed by TFC. The elevated levels of AFM1 in some cheese samples found which were above the TFC limits could adversely affect human health.

These results suggest that it is important to prevent toxin production in these products from the production stage to consumption as well as creating effective detoxification processes.

References

1. *Anon.*: FAO/WHO News Archive.: Codex Committee on Food Additive Contaminant, Thirtienth Session, The Hague, Netherlands 9-13 March 1998.
2. *Anon.*: Mikotoksinler ve toksin bağlayicileri. Kanatli Ar-ge yayinleri, seminerler. Thermal Otel, Bolu, Turkey 2000. (in Turkish).
3. *Anon.*: Turkish Food Codex (TFC).: Gida Maddelerinde Belirli Bulasanlarin Maksimum Seviyelerinin Belirlenmesi Hakkinde Teblig. 16.10.2002 tarihli 24908 sayili resmi gazete. 2002. (in Turkish).
4. *Anon.*: World Health Organization (WHO), International Agency For Research On Cancer (IARC). IARC monographs on the evaluation of carcinogenic risksto humans, some naturally occuring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. 2007 volume 56.
5. *Aycicek H., Aksoy H., Saygi S.*: Determination of aflatoxin levels in some dairy and food products which consumed in Ankara, Turkey. Food Control 2005, 16, 263-266.
6. *Aycicek H., Yarsan E., Sarimehmetoglu B., Cakmak O.*: Aflatoksin M_1 in white cheese and butter consumed in Istanbul, Turkey.Vet. Human Toxicol. 2002, 44, 295-296.
7. *Baskaya R., Aydin A., Yildiz A., Bostan K.*: Aflatoxin M1 levels of some cheese varieties in Turkey. Medycyna Wet. 2006, 62, 778-780.
8. *Canton J. H., Kroes R., Van Longten M. J., Van Schothorst M., Stavenuiter J. F. C., Verhulsdonk C. A. H.*: The carcinogenicity of aflatoxin M1 in rainbow trout. Food Cosmetics Toxicol. 1975, 13, 441-443.
9. *Castegnaro M., Mcgregor D.*: Carcinogenic risk assesement of mycotoxins. Rev. Vet. Med. 1998, 149, 671-678.
10. *Concon J. M.*: Contaminants and Additives. Food Toxicology, Part B, Marcel Dekker, Inc., New York 1988, pp. 667-743.
11. *Demirer M. A.*: Sut ve mamullerinde Aflatoksin M1 ve B1 aranması uzerine arastirmalar. Ankara Univ. Vet. Fak. Derg. 1973, 20, 2-3, 421-443.
12. *Demirer M. A., Dincer B., Kaymaz S., Alperden I., Yalcin S.*: Bazı gida maddelerinde mikroflora ve mikotosin analizleri. Ankara Univ. Vet. Fak. Derg. 1989, 36, 1, 85-107. (in Turkish).
13. *Galvano F., Galofaro V., De Angelis A., Galvano M., Bognanno M., Galvano G.*: Survey of the occurrence of aflatoxin M_1 in dairy products marketed in Italy. J. Food Prot. 1998, 61, 738-741.
14. *Galvano F., Galofaro V., Galvano G.*: Occurrence and stability of Aflatoxin M1 in milk and milk products: A Worldwide Review. J. Food Prot. 1996, 59, 1079--1090.
15. *Galvano F., Piva, A., Ritieni A., Galvano G.*: Dietary strategies to counteract the effects of mycotoxins: A review. J. Food Protec. 2001, 64, 120-131.
16. *Gurbuz U., Nizamlioglu M., Nizamlioglu F., Dinc I., Dogruer Y.*: The investigation of Aflatoxin B1 and M1 in some meat, dairy products, and spices. Veterinarium 1999, 10,1, 34-41.
17. *Gurses M., Erdogan A., Cetin B.*: Occurrence of Aflatoxin M1 in some cheese types sold in Erzurum, Turkey. Turk. J. Vet. Anim. Sci. 2004, 28, 527-530.
18. *Kamber U.*: Aflatoxin M1 contamination of some commercial Turkish cheese from markets in Kars, Turkey. Fresenius Environ. Bull. 2005, 14, 1046-1049.
19. *Kamber U.*: Traditional Anatolian Cheese. Miki matbaacilik, 1st ed., Ivedik, Ankara, Turkey 2005 (in Turkish).
20. *Kamkar A.*: A study on the occurrence of aflatoxin M_1 in Iranian Feta cheese. Food Control 2005, 16, 593-599.
21. *Karaioannoglu P., Mantis A., Koufidis D., Koidis P., Triantafillou J.*: Occurrence of aflatoxin M1 in raw and pasteurized milk and Feta and Teleme cheese samples. Milchwissensch. 1989, 44, 746-747.
22. *Kardes E.*: Turk silahlı kuvvetlerine bagli askeri birliklere alinan peynirlerde aflatoksin B1 ve M1 varliginin ve seviyelerinin saptanmasi. (PhD Thesis). Ankara University, Institu of Health Sciences. Ankara 2000 (in Turkish).
23. *Kivanc M.*: Mold growth and the presence of aflatoxin in some Turkish cheese. J. Food Safety 1990, 10, 287-294.
24. *Lafont P., Siriwardana M., Lafont J.*: Genotoxicity of hydroxy-aflatoxins M_1 and M_4. Microb. Aliment. Nutr. 1989, 7, 1-8.
25. *Oruc H. H., Cibik R., Yilmaz E., Kalkanli O.*: Distribution and stability of Aflatoxin M1 during processing and ripening of traditional white pickled cheese. Food Add. Contam. 2006, 23, 2, 190-195.
26. *Oruc H. H., Sonal S.*: Determination of aflatoxin M1 levels in cheese and milk consumed in Bursa. Vet. Human Toxicol. 2001, 43, 292-293.
27. *Peraica M., Radic B., Lucic A., Pavlovic M.*: Toxic effects of mycotoxins in humans. Bulletin of the World Health Organization 1999, 77, 754-766.
28. *Pittet A.*: Natural occurrence of mycotoxins in foods and feeds. An updated review. Revue Med. Vet. 1998, 149, 479-492.
29. R-Biopharm.: Enzyme immunoassay for the quantitative analysis of aflatoxin M1. GmBH, Germany (Ridascreen® Aflatoxin M1 30/15, Art. No.: R1101) 2006.
30. *Sarimehmetoglu B., Kuplulu O., Celik T. H.*: Detection of Aflatoxin M1 in cheese samples by ELISA. Food Control 2004, 15, 45-49.
31. *Seyrek K.*: Turk silahli kuvvetlerine agli birliklrde tuketilen beyaz peynirlerdeki aflatoxin M1 seviyesinin ELISA (Enyzmbe linked immunosorbent assay) metodu ile saptanması. Vet. Hek. Der. Derg. 2001, 72, (1, 2, 3, 4), 55-58.
32. *Sinnhuber R. O., Lee D. J., Wales J. H., Landers M. K., Keyl A. C.*: Hepatic carcinogenesis of aflatoxin M1 in rainbow trout. J. Natl. Cancer Inst. 1974, 53, 1285-1288.
33. *Stoloff L., Wood G.*: Aflatoxin M1 in manufactured dairy products produced in the United States in 1979. J. Dairy Sci. 1981, 64, 2426-2430.
34. *Stubblefield R. D., Shannon G. M.*: Aflatoxin M_1: Analysis in dairy products and distribution in dairy foods made from artificially contaminated milk. J. AOAC 1974, 57, 847-851.
35. *Van Egmond H. P.*: Current situation on regulations for mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis. Food Add. Contam. 1989, 6, 139-188.
36. *Yaroglu T., Oruc H. H., Tayar M.*: Aflatoxin M1 levels in cheese samples from some provinces of Turkey. Food Control 2005, 16, 883-885.

Author's address: Dr. Kursat Yapar, Department of Farmacology and Toxicology, Faculty of Veterinary Medicine, Kafkas University, 36100, Kars, Turkey; e-mail: k_yapar@hotmail.com