Internet Journal of Food Safety, Vol.10, 2008, p. 92-96

A Survey on the Presence of Aflatoxin M_1 in Urfa Cheese

Mustafa Ardic[1*], Mustafa Atasever[2], Gulsah Adiguzel[2], Meryem Atasever[2], Yakup Karakaya[2], Cemal Unsal[3], Hisamettin Durmaz[1]

[1]*Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Harran University, Sanliurfa, Turkey*

[2]*Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Ataturk University, Erzurum, Turkey*

[3]*9th Army cops "A" Type Food Control Detachment Commander, Erzurum, Turkey*

Abstract: The presence and concentration range of aflatoxin M_1 (AFM_1) were investigated by Enzyme Linked Immunosorbent Assay (ELISA) technique in 64 samples of Urfa cheese obtained from retail outlets in Sanliurfa, Turkey. AFM_1 at detectable level (50 ng/kg) was found in 6.25% in these samples. The concentration of AFM_1 in samples ranged from 51.10 to 99.60 ng/kg. None of the cheese samples exceed the legal limit of 250 ng/kg established by Turkish Food Codex. It is concluded that, despite the widespread occurrence of AFM_1 in different cheeses, contamination levels in Urfa cheese were not a serious human health hazard.
(10 font spacing)
Key words: Aflatoxin M_1, Urfa cheese, ELISA

* Corresponding author. mailing address: Department of Food Hygiene and Technology, Harran University, Sanliurfa, Turkey, Tel: +90-414-312-8456-06, Fax: +90-414-314-4158 E-mail: mardic@harran.edu.tr

Introduction

Aflatoxins are a group of extremely toxic metabolites produced by some species of *Aspergillus* namely *A. flavus*, *A. parasiticus* and the rare *A. nomius* during the growth of these fungi on foods and feeds. *A. flavus* produces only B aflatoxins, while the other two species produce both B and G aflatoxins. The formation of aflatoxins depends on the foods on which the moulds grow and the conditions of heat and humidity during the growth, harvesting and storage of the crops (Sweeney and Dobson 1998; Creepy 2002). These toxins are a significant threat to both human and animal health because they are potent carcinogens, teratogens and mutagens. There is also economical lose due to food contamination. (Blesa et al. 2004). Among aflatoxins, aflatoxin B_1 (AFB_1) is the most commonly found in foods and feeds. It is highly toxic, in terms of both acute and chronic toxicity (Sweeney and Dobson 1998; Moss 2002; Cavaliere et al. 2006), and is so highly carcinogenic as to be classified by the International Agency for Research on Cancer (IARC) as a Group 1 human carcinogen (IARC 1993).

Aflatoxin M_1 (AFM_1) is the hydroxylated metabolite of AFB_1 forming in liver by means of cytochrome P450-associated enzymes (Cavaliere et al. 2006). It may be found in milk or dairy products obtained from livestock that have ingested contaminated feed with AFB_1. There is a linear relationship between the amount of AFM_1 in milk and AFB_1 in feed consumed by animals. It has been reported that 0.3-6.2% of AFB_1 depending upon the amount of feed ingested by animal is transformed to some AFM_1 by the hepatic microsomal mixed function oxidase system and excreted in milk (Creepy 2002; Tekinsen and Tekinsen 2005). Studies have clearly demonstrated that AFM_1 causes toxic and carcinogenic effects (Galvano et al. 1996; Cavaliere et al. 2006),

therefore this toxin, initially classified by IARC as a Group 2B human carcinogen (IARC 1993), has now been moved to Group 1 (IARC 2002).

Milk and dairy products are a major nutrient for humans especially children. However, at the same time these products may be contaminated with AFM_1. The presence of AFM_1 in milk and dairy products can be a potential threat to the health of consumers (Sarimehmetoglu et al. 2004). In order to reduce this risk, many countries have regulated the levels of AFB_1 in feeds and have set or proposed maximum permissible levels of AFM_1 in milk and dairy products. The European Commission has set a limit of 50 ng/kg for AFM_1 in raw milk, heat-treated milk and milk for the manufacture of milk-based-products (Commission Regulation 2001). Turkish legal limits for AFM_1 in milk and cheese are 50 ng/l and 250 ng/kg respectively (Turkish Food Codex 2002).

When cheese is made from AFM_1 contaminated milk, the toxin can be carried over into cheese, whey and curd (Kaniou-Grigoriadou et al. 2005). The distribution of AFM_1 in each component can be ascribed to different factors such as extraction technique, methodology, type and degree of milk contamination, differences in milk quality and the cheese manufacture process (Blanco et al. 1988).

In Turkey, about 40-50 different cheese varieties have been produced. Apart from being well-known cheese varieties such as tulum, kashar and Turkish white brine cheese (Hayaloglu et al. 2002), there are many traditional cheese varieties and their production is largely based in small-scale dairies and family farms. Urfa cheese is semihard, traditional cheese produced mainly in the south-east of Turkey from either raw ovine or bovine milks or appropriate mixtures of both. Production of Urfa cheese is carried out from March to June in this region. The main stages of the production of Urfa cheese are as follows. The milk is coagulated with rennet at 30-32 ºC. The coagulum is cut into small cubes (approximately 1 cm^3). After whey draining, curd is scalded in hot water and stretched until it becomes a close knit with elastic structure. Afterwards, it is shaped with hand in small cotton bag and kept in brine for about 3-4 months. The most typical form of this cheese is oval, conical or convex-conical shape due to handling of the curd in small cotton bag during stretching. It is generally consumed after ripening, but also consumed freshly (Ardic et al. 2007). It is gaining nationwide popularity and has even been exported to Middle Eastern and Central Asian countries. Annual production of Urfa cheese have been estimated to be approximately 35 000-40 000 tones (Ozer et al. 2002; 2003).

There is very limited information available about Urfa cheese in the literature and no study has so far been carried out to determine the AFM_1 levels in this cheese. Therefore, this study was aimed to determine the presence and levels of AFM_1 in Urfa cheese produced in Sanliurfa province, Turkey.

Materials and Methods

Samples. A total of 64 Urfa cheese samples consumed in Sanliurfa province of Turkey were evaluated for the presence of AFM_1. The cheese samples taken from 200-500 g quantities were randomly collected from retail outlets between April 2006 and June 2006 and transported within an insulated container at about 4 ºC for analysis.

Method. The AFM_1 concentrations of the samples were determined by competitive ELISA method (Ridascreen AFM_1 Art no: R1101) according to the procedure described by R-Biopharm GmbH (1999). This method is quick, reliable and cost effective for the estimation of AFM_1 and has been included in the official collection of test procedures by the German Federal Board of Health. The test shows cross-reaction to AFB_1 (12.4%) but this is not relevant when analyzing AFM_1, considering that AFB_1 usually is not to be found in milk or milk products. Most of the reagents used were contained in the Ridascreen AFM_1 test kit; which included microtiter plate coated with capture antibodies, AFM_1 standard solutions (1.3 ml each 0 ppt, 5 ppt, 10 ppt, 20 ppt, 40 ppt and 80 ppt), peroxidase conjugated AFM_1, substrate (urea peroxidase), chromogen (tetramethylbenzidine) and stop solution contains 1 N sulphuric acid. Methanol, n-heptane and dichloromethane used were provided by Merck. Phosphate Buffer Solution (PBS) was prepared by mixing 0.55 g sodium dihydrogen phosphate hydrate with 2.85 g disodium hydrogen phosphate-2-hydrate and 9 g sodium chloride and filling up to 1000 ml with distilled water.

Samples preparation for analysis. Preparation of samples was conducted according to the instructions of the Ridascreen kit. A representative cheese sample was triturated coarsely and thoroughly mixed, without the addition of liquid. Two g of triturated cheese samples weighed into a centrifugal glass vial and 40 ml dichloromethane was added and extracted by stirring/shaking the vial for 15 min. The suspension was filtered and 10 ml of the extract was evaporated at 60 ºC under a weak nitrogen stream. The oily residue was redissolved in 0.5 ml methanol, 0.5 ml PBS buffer and 1 ml heptane and was mixed thoroughly. After centrifugation for 15 min at 2700 g, the upper heptane-layer was completely removed. Aliquot of the lower methanolic-aqueous phase was carefully poured off using a Pasteur pipette. 100 µl of this aliquot brought up to a 10 % methanol content by addition of 400 µl Ridascreen buffer 1 and 100 µl was used per well in the test.

ELISA test procedure. A sufficient number of microtiter wells were inserted into the microwell holder for all standards and samples. 100 µl standard solutions and prepared samples were added in separate wells and incubated for 60 min at room temperature (20 ºC) in the dark. The liquid was removed from the wells and the microwell holder was tapped upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Then the wells were washed twice with 250 µl of distilled water. 100 µl of the diluted enzyme conjugate (peroxidase conjugated AFM_1) was added and incubated for 60 min at room temperature in the dark. The wells were again washed with 250 µl of distilled water as described above. In the next stage 50 µl of substrate (urea peroxidase) and 50 µl of chromogen (tetramethylbenzidine) were added to each well and mixed thoroughly and incubated for 30 min at room temperature in the dark. Then 100 µl of the stop reagent (1 N H_2SO_4) was added to each well and mixed and the absorbance was measured at 450 nm in ELISA reader (ELX-800, Bio-Tek Instruments, Winooski, VT, USA).

Evaluation. The samples were evaluated according to the Rida Soft Win computer program prepared by R-Biopharm. The lower detection limit is 50 ng/kg, the recovery rate 102% and the average coefficient of variation 11% for cheese.

Results

In this study, a total of 64 Urfa cheese samples were analysed for the presence of AFM_1. The presence and the distribution of AFM_1 concentration in various ranges in Urfa cheese samples are presented in Table 1.

Table 1 The AFM_1 levels of Urfa cheese samples

Number of samples	Positive samples[a]	Mean±SD[b]	Range	Distribution n (%)	
n	n (%)	ng/kg	ng/kg	<50 ng/kg	50-100 ng/kg
64	4 (6.25)	64.95±23.17	51.10-99.60	60 (93.75)	4 (6.25)

[a] (≥50 ng/kg)[b] Mean±SD of positive samples

The incidence of AFM_1 in Urfa cheese was quite low, since 93.75% of samples were below the detectable level of 50 ng/kg. AFM_1 was found in 4 (6.25%) of cheese samples above the detectable level. The levels of AFM_1 in Urfa cheese samples were in the range of 51.10-99.60 ng/kg (mean=64.95±23.17 ng/kg). None of the samples was over the Turkish legal limit (250 ng/kg for cheese) established by Turkish Food Codex.

Discussion

AFM_1 levels in milk and dairy products are important since many people use milk and dairy products in their diets frequently. For this reason, there are many studies concerning the presence of AFM_1 in cheese (Table 2) and numerous studies on aflatoxin in milk and dairy products have been reviewed by some authors (Galvano et al. 1996; Sweeney and Dobson 1998; Creepy 2002).

In the present study, the incidence of AFM_1 was 6.25% in Urfa cheese samples. The results indicate that the incidence of AFM_1 in the Urfa cheese samples were low. None of the AFM_1 amounts were at the risk level for human health because cheese samples did not exceed the Turkish legal limit of 250 ng/kg. These findings indicate that examined samples were manufactured from AFB_1 free milk or low levels of AFB_1 containing milk. Our results were similar to those obtained by Barbieri et al. (1994), who observed 9% of cheese samples, presenting levels of AFM_1 ranging from 35 to 190 ng/kg.

Previous studies have reported different levels of AFM_1 in cheese samples (Table 2). While some researchers have reported high or low levels of AFM_1 in different cheese samples, others have reported the absence of AFM_1 at detectable level in cheese samples. The occurrence of AFM_1 in Urfa cheese is lower than the AFM_1 incidence in different types of cheese reported by Sarimehmetoglu et al. (2004), Tekinsen and Tekinsen (2005), Kamhar (2005) and Baskaya et al. (2006). These authors detected higher percentiles in samples contaminated with AFM_1, ranging from 76.36% to 93.66% of the samples analysed. On the other hand, the levels of AFM_1 observed by Sarimehmetoglu et al. (2004), Tekinsen and Tekinsen (2005), Yaroglu et al. (2005), Kamhar (2005) and Baskaya et al. (2006) in different cheese samples were higher than values observed in the present study, ranging from 50 to 4100 ng/kg. The present results are not in agreement with those obtained by Kivanc (1990), Taguchi et al. (1995) and Kaniou-Grigoriadou et al. (2005), who did not detected AFM_1 in cheese samples. These differences may be due to not only the use of different analytical methods and the variety of cheese, but also the geographic region where cheese is produced and the possible presence of aflatoxin B in the feed (Pittet 1998). In addition, The AFM_1 level in the milk was significantly affected by the geographical region,

the country and the season (Galvano et al. 1996). In summary, the levels found in this survey indicate that the natural occurrence of AFM_1 in Urfa cheese does not represent a significant health risk for the consumer.

Table 2. Presence and level of AFM_1 in various cheeses

References	Country	Cheese variety	No of samples	Positive (%)*	Range (ng/kg)
Kivanc (1990)	Turkey	White brine	25	ND	
		Van otlu	25	ND	
Barbieri et al. (1994)	Italy	Cheese	200	9	35–190
Taguchi et al. (1995)	Japan	Cheese	41	ND	
Sarimehmetoglu et al. (2004)	Turkey	White brine	100	81.75	51->800
		Kashar	100		
		Tulum	100		
		Processed	100		
Tekinsen and Tekinsen (2005)	Turkey	Van otlu	60	76.36	100-726
		White brine	50		
Yaroglu et al. (2005)	Turkey	White brine	200	5	100-800
		Kashar	200		
		Cream	200		
Kaniou-Grigoriadou et al. (2005)	Greece	Feta cheese	54	ND	
Kamhar (2005)	Iran	Feta cheese	80	82.5	150-2410
Baskaya et al. (2006)	Turkey	White brine	131	93.66	50-4100
		Processed	132		
		Kashar	100		

*: indicates percentage of total samples ND: Not determined

In conclusion, the data of present study revealed that AFM_1 is not common contaminant of Urfa cheese and AFM_1 levels have not been constituted a human health risk. However, because AFM_1 is considered a potent hepatocarcinogen, further studies are necessary to evaluate the risk to human health due to the ingestion of this toxin in milk and dairy products. Also, more samples should be analysed and surveillance programs must be continuous and widespread in feeds and foods.

References

Ardic M, Kav K, Guner A, Dogruer Y. 2007. Identification of enterobacteriaceae in Urfa cheese. Acta Aliment. 36: 483-488.

Barbieri G, Bergamini C, Ori E, Reska P. 1994. Aflatoxin M_1 in parmesan cheese: HPLC determination. J. Food Sci. 59: 1313-1331.

Baskaya R, Aydin A, Yildiz A, Bostan K. 2006. Aflatoxin M_1 levels of some cheese varieties in Turkey. Med. Weter. 62: 778-780.

Blanco JL, Domingues L, Gomez-Lucia E, Garayzabal, JEE, Goyache J, Suarez G. 1988. Behavior of aflatoxin during the manufacture, repining and storage of Manchego-type cheese. J. Food Sci. 53: 1373-1376.

Blesa J, Soriano JM, Molto JC, Manes J. 2004. Limited survey for the presence of aflatoxins in foods from local markets and supermarkets in Valencia, Spain. Food Addit. Contam. 21: 165-171.

Cavaliere C, Foglia P, Pastorini E, Samperi R, Lagana A. 2006. Liquid chromatography/tandem mass spectrometric confirmatory method for determining aflatoxin M_1 in cow milk comparison between electrospray and atmospheric pressure photoionization sources. J. Chromatogr. A 1101: 69-78.

Commission Regulation (EC). 2001. No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Commun. L 77: 1-13.

Creppy EE. 2002. Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol. Lett. 127: 19-28.

Galvano F, Galofaro V, Galvano G. 1996. Occurrence and stability of aflatoxin M1 in milk and milk products: A worldwide review. J. Food Prot. 59: 1079-1090.

Hayaloglu AA, Guven M, Fox PF. 2002. Microbiological, biochemical and technological properties of Turkish White cheese 'Beyaz Peynir'. Int. Dairy J. 12: 635-648.

IARC. 1993. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC monographs on the evaluation of carcinogenic risks to humans Volume 56. Lyon: IARC Scientific Publication, pp. 19-23.

IARC. 2002. Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. IARC monographs on the evaluation of carcinogenic risks to humans Volume 82. Lyon: IARC Scientific Publication, pp. 9-13.

Kamkar A. 2006. A study on the occurrence of aflatoxin M_1 in Iranian Feta cheese. Food Control 17: 768-775.

Kaniou-Grigoriadou I, Eleftheriadou A, Mouratidou T, Katikou P. 2005. Determination of aflatoxin M_1 in ewe's milk samples and the produced curd and Feta cheese. Food Control 16: 257-261.

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

Moss MO. 2002. Risk assessment for aflatoxins in foodstuffs. Int. Biodeterior. Biodegradation 50: 137-142.

Ozer B, Atasoy F, Akin S. 2002. Some properties of urfa cheese (a traditional white-brined Turkish cheese) produced from bovine and ovine milks. Int. J. Dairy Technol. 55: 94-99.

Ozer BH, Robinson RK, Grandison AS. 2003. Textural and microstructural properties of urfa cheese (a white-brined Turkish cheese). Int. J. Dairy Technol. 56: 171-176.

Pittet A. 1998. Natural occurrence of mycotoxins in foods and feeds. An updated review. Rev. Med. Vet. 149: 479-492.

R-Biopharm GmbH. 1999. Enzyme immunoassay for the quantitative analysis of aflatoxin M1 Ridascreen Aflatoxin M_1 Art. No.: R1101. R-Biopharm GmbH, Darmstadt, Germany.

Sweeney MJ, Dobson ADW. 1998. Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species. Int. J. Food Microbiol. 43: 141-158.

Sarimehmetoglu B., Kuplulu B, Celik H. 2004. Detection of aflatoxin M_1 in cheese samples by ELISA. Food Control 15: 45-49.

Taguchi S, Fukushima S, Sumumoto T, Yoshida S, Nishimune T. 1995. Aflatoxins in food collected in Osaka, Japan from 1988 to 1992. J. AOAC Int. 78: 325-327.

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

Turkish Food Codex. 2002. Türk Gıda Kodeksi Gıda Maddelerinde Belirli bulaşanların maksimum seviyelerinin belirlenmesi hakkinda tebliğ. (Legislation about determination of maximum levels of certain contaminant in foods.) Resmi Gazete, 23 Eylül 2002, Sayı 24885. Ankara: Başbakanlık Basımevi.

Yaroglu T, Oruc HH, Tayar M. 2005. Aflatoxin M_1 levels in cheese samples from some provinces of Turkey. Food Control 16: 883-885.