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Occurrence of Aflatoxin M_1 in Milk, White Cheese and Yoghurt From Ankara, Turkey Markets

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Abstract: In addition to the wide consumption of milk across age groups, cheese and yoghurt are frequently used in Turkish cuisine. Therefore, the presence of aflatoxin M_1 (AFM_1) in dairy foods is a significant problem for human health. The aim of this study was to investigate the incidence and levels of AFM_1 in milk, cheese and yoghurt samples in Ankara, Turkey. The AFM_1 levels of the samples were determined using immunoaffinity column cleanup followed by high performance liquid chromatography for separation and fluorescence detection. A total of 70 dairy products consisting of 24 UHT pasteurized milk samples, 27 white cheese samples and 19 yoghurt samples were analyzed for AFM_1 during the first 6 months of 2013. Eighty-three percent of the milk samples, 92.6% of the cheese samples and 89.5% of the yoghurt samples were contaminated with AFM_1. The levels of AFM_1 in the samples ranged from 7.3 to 107.2 ng/kg. Only 5 yoghurt samples and none of the milk or cheese samples exceeded the safety limits established by the Turkish Food Codex of 50 ng/kg for milk and yoghurt and 250 ng/kg for cheese. The survey results showed the necessity of regular monitoring for the occurrence of AFM_1 in dairy products.

Keywords: Aflatoxin M_1, diary products, HPLC-FLD, IAC

1. Introduction

Aflatoxins are a group of mycotoxins that are produced by some *species* of *Aspergillus* fungus, particularly *Aspergillusflavus* and *Aspergillusparasiticus* and, more rarely, *Aspergillus* nomius [1-5] Eighteen different aflatoxins have been identified, with the most common being aflatoxins B_1, B_2 and G_1, G_2. Both B and G aflatoxins are produced by *A. parasiticus* and *A. nomius*,while *A. flavus* produces only B aflatoxins. [2, 6] Aspergillus species are found in most soils and cause grains to decay. When grain kernels are damaged by insects, birds, mites or stresses (frost, heat or drought), aflatoxins are produced by naturally occurring fungi both in the field and during storage. [7]

Aflatoxins M_1 and M_2 are normally not found in foods unless lactating animals are fed with aflatoxin-contaminated feedstuff. Aflatoxin M_1 (AFM_1) is the 4-hydroxy derivative of aflatoxin B_1 (AFB_1). AFM_1 may be found in milk and in other dairy products obtained from livestock that have ingested contaminated feed [2]. In addition to indirect contamination (carry-over from feed to milk), residues of aflatoxins may be present in the organs, tissues and body fluids of animals that consume contaminated feed. [8-10]

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Among the highly toxic, carcinogenic, teratogenic and mutagenic aflatoxin compounds, AFB_1 is the most hepatocarcinogenic metabolite. [4, 11, 12] Aflatoxins can contaminate various agricultural products, such as nuts, cereals, spices, fat-containing crops, peanuts, dried fruits, potatoes, and oilseeds. [13-15] Because these commodities are traded internationally, aflatoxin contamination is of concern to both producing and importing countries.

When AFB_1 contaminates the feed of lactating animals, it is converted to its relatively less carcinogenic metabolite AFM_1 with a carry-over rate of 0.3 - 6.2% in the ruminant's body.[2, 16, 17] AFM_1 is formed in the liver by means of P450 cytochrome enzymes and then secreted into milk through the mammary glands of lactating animals.[2, 18-20]

AFM_1 can also cause DNA damage by the metabolite AFB-8,9-exo-epoxide, which binds to DNA to form AFB_1–DNA adducts. [8, 21] The carcinogenicity of AFM_1 is approximately 2-10% that of AFB_1.[19, 20, 22]

Aflatoxin contamination can be classified as either acute or chronic. Chronic toxicity is caused by low-dose exposure of the contaminant over a long period of time. Chronic aflatoxin toxicity can result in cancers and other irreversible health effects.Acute aflatoxin toxicity is characterized by a rapid onset and toxic response. [7] The animal LD50 for aflatoxin (lethal dose; i.e., the amount of substance required to kill 50% of the test population) is given as 1 to 15 mg/kg BW according to the sex, age and resistance of the animal species. For instance, the LD50 is 7 mg/kg for male Wistar rats, 14 mg/kg for female Wistar rats, and 2 mg/kg for male Fischer rats. [4, 23] AFM_1 is cytotoxic, and in ducklings and rats its acute and short-term toxicity is similar or slightly less than that of AFB_1. [9, 24] In mammalian cells (in vitro), AFM_1 can cause DNA damage, gene mutation, chromosomal anomalies, and cell transformation, and in insects, it causes infection with lower eukaryotes and bacteria. [9] In animals, aflatoxins cause liver damage, suppressed immunity and decreased milk production. The main target organ for toxic aflatoxins in humans is the liver, and its health effects range from acute hepatic toxicity to chronic disease and cancer. [7, 25]

In 1993, The International Agency for Research on Cancer (IARC), as part of the WHO, classified AFB_1 as a primary (Group I) and AFM_1 as a secondary (2B) group of human carcinogens. In 2002, AFM_1 was reclassified as a class 1 carcinogen (carcinogenic to humans). [2, 26, 27]

The tolerable daily intake (TDI) is defined as the highest daily dose of toxin a human can consume without suffering from adverse effects over a lifetime. For ethical reasons, tests are carried out on experimental animals to determine the No Observed Adverse Effects Level (NOAEL). To take into account the sensitivity differences between animals and humans, an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for variation between individuals) is applied. The NOAEL (mg/kg/experimental animal) value is divided by this uncertainty factor of 100 to obtain the TDI (mg/kg per human) value. Then, the TDI×BW (body weight) is divided by the milk intake (kg/day) to obtain the Maximum Residue Level (MRL - mg/kg). Milk intake values are calculated for five geographical regions, comprising the Far East, Africa, Europe, Middle East and Latin America. [4] This approach does not apply for genotoxic carcinogens such as aflatoxins. In these cases, JECFA and EC does not allocate a tolerable daily intake. Instead, they recommend that the level of the contaminant in food should be As Low As Reasonably Achievable (ALARA). The MRLs for aflatoxins are set according to the ALARA principle. [28-30]

Due to high toxicity and health concerns, many countries have set MRLs for AFM_1 to prevent or reduce the risks of aflatoxins. In Turkey, according to the Turkish Food Codex and European Commission Regulations, AFM_1 concentrations must not exceed 0.05 ug/kg in milk. [31-33]

Because it is based on the ALARA principle, the maximum allowable level for AFM_1 in milk in the EU is among the lowest in the world. [30, 34]

Several analytical methods for the measurement of AFM_1 in milk and dairy products have been developed. Routinely used methods are mainly based on immunochemical methods, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The most common immunochemical method is the enzyme-linked immuno sorbent assay (ELISA), [6] which is preferred because of its rapidity and simplicity. [35-45]

Thin layer chromatography (TLC) has also been described for aflatoxin analysis. [46, 47] However, TLC requires cumbersome procedures and large amount of solvents. Raw milk samples have been analyzed using an immunoaffinity column (IAC) followed by TLC to achieve comparable sensitivity to that of HPLC. [48] Currently, HPLC with fluorescence or mass spectrometric detection is frequently used. [13, 49-54] HPLC is a rapid, relatively simple and sensitive method. Instead of M_1 specific IAC, less expensive but non-specific cartridges are also used for extraction and cleanup purposes. [16, 55]

It is necessary to minimize the health risks resulting from the AFM_1 contamination of dairy products. It is also necessary to know the level of the precursor AFB_1 in animal feed. Although several surveys have described the occurrence of AFM_1 in dairy products in Turkey, they cannot be considered enough. Furthermore, survey data for yoghurt samples are very limited throughout the world. Regular monitoring is essential to reduce AFB_1 in feed along with AFM_1 occurrence in dairy products. Based on these facts, the aim of our research team is to collect relevant data on a regular basis.

The aim of this study was to investigate the occurrence of AFM_1 in the most frequently consumed dairy products and to compare the results with similar studies conducted by other researchers.

2. Materials and Methods

Apparatus

Quantitative analysis of AFM_1 in samples was carried out using an Agilent 1200 Series HPLC system equipped with a quaternary pump (G1311A), a degasser (G1322A), an autosampler (ALS; G1329A), a thermostated column compartment (TCC; G1316A), a fluorescence detector (FLD; G1321A) and a Zorbax Eclipse XDB-C18 (4.6x250 mm, 5 µm) analytical column (Agilent, USA). Other equipment used in this study included a Sartorius ED224S Model analytical balance with 0.1 mg precision, a Eutech pH 510 model digital pH meter supplied with an epoxy combined electrode, a vortex mixer (Labnet, S0200-230 V-EU) and a centrifuge (Nüve, NF800R).

Chemicals and Supplies

Methanol and acetonitrile were HPLC grade (Merck, Germany). Other chemicals and supplies used included an Aflatoxin M_1 standard (Supelco 46319-U, USA) and Afla M_1Immunoaffinity columns (IAC) (VICAM, Watertown, MA, USA).

The AFM_1 standard was purchased in acetonitrile at a concentration of 10 µg/mL. The deionized water used throughout the experiments was generated by purification with an ELGA Pure Lab Option DV35 followed by an ELGA Pure Lab Ultra RO Unit.

All the laboratory glassware were soaked in a diluted sulfuric acid solution (approx. 2 M) prior to use, then rinsed thoroughly with deionized water (DIW) and washed with sodium hypochlorite (household bleach) and aqueous acetone after the analyses.

Preparation of Standards

The working standard of AFM_1 was prepared in acetonitrile at a concentration of 1.0 µg/mL and was kept at -20ºC. Calibration standards were prepared immediately before use using appropriate dilutions of a working standard in the mobile phase (acetonitrile:water:methanol; 3:5:2 v/v) at concentrations of 0.2, 0.4, 0.6, 0,8, 1.0, 1.2, 1.5 and 2.0 ng/mL. Two-level spiking solutions were prepared at concentrations of 20 and 50 ng/mL.

Collection and Preparation of Samples

A total of 70 samples were purchased from supermarkets in Ankara, Turkey, for Aflatoxin M_1 determination by IAC clean up followed by HPLC-FLD. These products included 24 milk samples (12 UHT whole milk samples, 8 UHT treated skim milk samples, 4 pasteurized milk samples), 27 white cheese samples and 19 yoghurt samples (10 yoghurt samples, 6 nonfat yoghurt samples and 3 traditional (village) yoghurt samples). Approximately 1 kg of each type of sample was homogenized in a food blender (Waring) before analysis.

Preparation of the Milk Samples

The VICAM procedure with slight modifications was applied. Samples were warmed to 37ºC in a water bath and afterwards were centrifuged at 4500 rpm for 15 min. The upper fatty part was removed, and the remaining skimmed milk was filtered through Whatman No. 4 filter paper. A 50 mL volume of the filtered portion was then loaded onto the AFM_1immuno affinity column (IAC) and passed at a rate of approximately 1-2 drops/second. The IAC was washed with 10 mL DIW twice, and AFM_1 was eluted by passing 1.25 mL acetonitrile:methanol (3:2 v/v) through the column at a rate of approximately 2-3 drops/second. Next, 1.25 mL DIW was passed, and all of the eluted sample (1.25 + 1.25 mL) was collected in a vial. Finally, 100 µL of the collected sample was injected into the HPLC.

Preparation of the Cheese Samples

Samples of 20 g homogenized cheese (paste) were weighed in a blender jar and blended at high speed for 3 min with 75 mL dichloromethane and 1 g NaCl. A 5 g quantity of Celite was placed on microfiber filter paper, and the contents of the food blender were poured over it. The filter paper was further washed with 50 mL dichloromethane. The combined filtrates were evaporated via rotary evaporator at 40ºC to dryness. A solution of 1 mL methanol, 30 mL DIW and 50 mL hexane was added to the residue, and the content was transferred to a separatory funnel. The phases were washed twice with 10 mL DIW, and the extracted aqueous phase portions were collected and loaded onto the IAC. The IAC elution procedure was similar to that described above for the milk samples. Finally, 100 µL of the collected eluate was injected into the HPLC.

Preparation of the Yoghurt Samples

Samples of 20 g homogenized yoghurt were blended with 75 mL chloroform and 2 mL saturated NaCl solution for 3 min at high speed. The samples were centrifuged for 15 min at 4500 rpm, then the fatty part was removed, the aqueous phase was discarded, and the chloroform phase was transferred into a volumetric flask. The centrifuge tube was washed with 25 mL chloroform, and the solvent of the combined extracts was evaporated to dryness in a rotary evaporator. The residue was transferred into a separatory funnel with 5 mL methanol and 50 mL PBS (phosphate buffer saline) solution. The rotary evaporator flask was washed with 45 mL PBS into the separatory funnel. The extract was washed 2-3 times with n-hexane, and the collected aqueous phases were loaded onto the IAC and passed at a rate of approximately 1-2 drops/second until air came through the column. The column was then washed twice with 10 mL DIW at the same rate. The preconcentrated and trapped AFM_1 within the column was eluted in two steps as follows: 1.25 mL acetonitrile:methanol (3:2 v/v) followed by 1.25 mL DIW were passed at a rate of 2-3 drops/sec and collected in a vial, then 100 µL of the eluate was injected into the HPLC.

3. Results and Discussion

In this study, AFM_1 contamination in milk and dairy products (yoghurt, cheese) was determined by IAC extraction prior to HPLC-FLD. Seventy milk and milk-based samples, including 24 milk samples, 19 yoghurt samples and 27 cheese samples, were analyzed during the first 6 months of 2013.

The precisions and accuracies of the recovery assays are given in Table 1. The results of the recovery study for AFM_1 added to milk, cheese, and yoghurt samples are summarized in the table. Blank samples were artificially contaminated at 25 ng/kg AFM_1. The mean recovery for spiked levels ranging from 25 to 150 ng/kg was 95.11% (the mean of triplicate analyses) for milk samples, with a standard deviation (SD) of 3.76 and a mean relative standard deviation (RSD) of 3.96%; 93.69% for cheese samples, with a SD of 3.34 and a mean RSD of 3.57%; and 95.76% for yoghurt samples, with a SD of 3.42 and a mean RSD of 3.58%. The limit of detection (LOD) (LOD=3S/b, b is the slope of the calibration curve) [17, 56] was 2.2 ng/kg, and the limit of quantification (LOQ) (almost three times the LOD) was 7.3 ng/kg. The intra-day (n=8) and inter-day (6 different days) mean coefficients of variation at a spiked level of 50 ng/L were 3.35% and 4.07%, respectively.

A small survey was conducted for the occurrence of AFM_1 in milk, cheese and yoghurt samples purchased in Ankara, the capital city of Turkey, during the first 6 months of 2013. The incidences and levels of AFM_1 in milk and in other dairy products are summarized in Table 2. According to the survey results, the incidence of AFM_1 contamination in milk, cheese and yoghurt was high: 88.5% of samples tested positive (Table 2). AFM_1 was detected in 10 (83.3%) of the UHT whole milk samples, at levels of 7.3 to 49 ng/L; in 7 (87.5%) of the UHT skim milk samples, at levels of 7.3-19.4 ng/L; in 3 (75%) of the pasteurized milk samples, at levels of 7.3-19.7 ng/L; in 9 (90%) of the yoghurt samples, at levels of 16 to 107.2 ng/kg; in 5 (83.3%) of the nonfat yoghurt samples, at levels of 16.8-42 ng/kg; in 3 (100%) of the traditional village yoghurt samples, at levels of 17-26 ng/kg; and in 25 (92.6%) of the white cheese samples, at levels of 7.3 to 84.4 ng/kg. The contamination level of AFM_1 in milk (Table 2) is not considered a serious health threat, as no milk sample was over the permissible level of 50 ng/L accepted as the EC Regulation and the Turkish legal limit. If the Turkish legal limit [33] of 250 ng/kg is considered, no cheese samples were contaminated above the legal limit. When the EC Regulation of 50 ng/kg is considered, only 2 cheese samples were above the legal limit. Five yoghurt samples were above the limit stated by EC regulation 2174/2003 [57] and the Turkish Food Codex [33] of 50 ng/kg.

Our results also show that AFM_1 is present more frequently in yoghurt and in cheese samples than in milk samples. Previous results have found that, in comparison to milk, AFM_1 concentrations are increased 2.4-2.8-fold in Turkish white cheese [50] 4.5-fold in Italian long-maturing cheese [58] 2.5-3.3-fold in Turkish soft cheese and 3.9-5.8-fold in hard cheese. [46]

Table 1. Recovery of AFM_1 from artificially contaminated milk, cheese and yogurt samples.

AFM_1 added to artificially contaminated samples (ng/L)	MILK[a]			CHEESE[a]			YOGURT[a]		
	Milk samples before AFM_1 addition (ng/L)	Mean AFM_1 content ng/L (%Rec) n=3	SD	Cheese samples before AFM_1 addition (ng/kg)	Mean AFM_1 content ng/L (%Rec) n=3	SD	Yogurt samples before AFM_1 addition (ng/L)	Mean AFM_1 content ng/kg (%Rec) n=3	SD
25	25.8	48.83 (96.1)	4.88	26.3	46.87 (91.4)	3.87	24.8	48.17 (96.7)	5.24
50	25.5	74.03 (98.1)	2.66	27.1	74.30 (96.4)	4.08	26.0	74.50 (98.0)	2.72
100	26.7	123.1 (97.2)	3.82	24.4	121.23 (98.6)	2.73	25.7	122.23 (97.2)	2.75
150	26.1	156.9 (89.1)	3.67	25.5	157.23 (89.6)	2.69	25.8	160.07 (91.1)	2.99
Mean ± SD[b] RSD(%)[c]	95.11±3.76 (3.96%RSD)			93.69±3.34 (3.57%RSD)			95.76±3.42 (3.58%RSD)		

[a] Blank samples contaminated at 25 ng/L AFM_1 before 25 - 150 ng/kg standard additions were measured

[b] Mean % recovery±standard deviation

[c] Relative Standard Deviation

Each sample was analyzed in triplicate

Table 2. Incidence and occurrence of AFM_1 in milk and dairy products commercialized in Ankara, Turkey.

Samples	Samples analyzed (n)	Positive samples [a] n (%)	Range of AFM_1 concentrations (ng/kg) Frequency distribution n (%)						Contamination level (ng/kg)	
			< 2.2	2.2-7.3	7.5-10	11-20	21-50	50-125	Range [b]	Mean [c]
UHT whole milk	12	10(83.3)	1(8.3)	1(8.3)	2(16.7)	3(25)	3(25)	-	7.3- 49	24.68±16.18
UHT skim milk	8	7(87.5)	1(12.5)	2(25)	1(12.5)	1(12.5)	2(25)	-	7.3-19.4	11.08±6.87
Pasteurized milk	4	3(75)	-	-	1(25)	2(50)	-	-	7.3- 19.7	11.26±7.95
Yogurt	10	9(90)	-	-	-	1(10)	4(40)	4(40)	16-107.2	47.92±33.59
Yogurt - nonfat	6	5(83.3)	-	-	-	2(33.3)	2(33.3)	1(16.7)	16.8-42	25.47±9.68
Yogurt - village	3	3(100)	-	-	-	2(66.7)	1(33.3)	-	17-26	21±4.58
White Cheese	27	25(92.6)	3(11.1)	3(11.1)	3(11.1)	7(25.9)	7(25.9)	2(7.41)	7.3-84.4	22.11±22.05
TOTAL	70	62(88.57)	5(7.14)	6(8.57)	7(10)	18(25.7)	19(27.1)	7(10)	7.3 – 107.2	23.36±13.89

[a]above LOD positive AFM_1

[b]min-max;

[c]mean of positive samples±standard deviation

Table 3 summarizes the selected results of similar studies conducted in other countries between 2003 and 2013. The average AFM_1 contamination of 51% is relatively high, which can be explained by the heat resistivity of AFM_1 during heat treatments, such as UHT and pasteurization processes.[35, 59]

This AFM_1 incidence is very high considering that milk and dairy products are consumed daily at every age.

Based on the published contamination levels of milk and other dairy products, AFM_1 incidence ranges from 3.3 to 100%. This wide range may be explained by seasonal changes, as it is reported [35, 39] that the level of AFM_1 contamination in milk in winter is higher than that in summer. Due to climate conditions, feed is used more often in winter.

Table 4 compares the present study results with previous investigations of AFM_1 contamination in milk, cheese and yoghurt from Turkish markets.

In 2000, 90 milk samples were analyzed in the Van province of Turkey. [46]

AFM_1 was detected in 79 (87.77%) of the milk samples, at levels from 12.5 to 123.6 ng/kg. Among the positive samples, 35 (44.30%) were above the legal limits. This contamination range is higher than that determined in the present study.

In a different survey, [60] AFM_1 was found at low levels of 10-20 ng/L in 29.6% of 27 examined milk samples obtained in Ankara, Turkey, and 25.9% of the milk samples had levels ranging from 21 to 50 ng/L. This contamination range is lower than that found in the 2001 survey conducted in Van [46] and is similar to the findings of the present study given in Table 2.

In another study, samples were collected between January and February 2005 in Central Anatolia, Turkey. [44] A total of 129 UHT milk samples were analyzed, and 58.1% of the samples were contaminated. Furthermore, 47% of the contaminated samples were over the permissible level. This contamination range is higher than those found in the other studies mentioned above.

In a survey conducted in Adana, Turkey, 20 milk and 20 white cheese samples were analyzed. [45] AFM_1 was found in all 20 milk samples analyzed in the range of 10-80 ng/kg. Only 15% of the samples were above the legal limit of 50 ng/kg. This rate of incidence of AFM_1 in milk samples is similar to that found in the present study; however, the contamination levels are nearly twice as high as those measured in the present study, as shown in Table 2. Among the 20 cheese samples, AFM_1 was found in 16 (80%) of the samples in the range of 54-263 ng/kg. When EC regulation is considered, all of the samples would be above the legal limit of 50 ng/kg. According to the Turkish Food Codex limit of 250 ng/kg for cheese, only 1 (5%) of the samples was above the legal limit. The rates of incidence and contamination levels of AFM_1 in cheese samples found in this survey are lower than those in our findings; however, the range of the toxin levels detected in the present study is lower.

In another study, a total of 100 milk samples were collected between September 2007 and January 2008 from 5 large cities in Turkey. It was shown that AFM_1 was found in 67 (67%) samples in the range of 10-630 ng/kg. [43] Among the 67 positive samples, 31 (31%) of them were above the legal limit of 50 ng/kg. Although the incidence in this study is lower than that in our findings, the contamination levels and the number of samples exceeding the contamination limits are nearly 6 and 4 times higher, respectively, than those in our study.

Table 3. The incidence and levels of AFM_1 contamination in milk, white cheese and Yogurt reported between 2003 and 2013 in various other countries

Sample	Country (Year of sampling)	No. of samples	Incidence n (%)	Range (ng/L)	Exceed legal limit [a] n (%)	Method (LOD ng/L)	Ref
Milk, White cheese, yogurt	Turkey 2013	24 27 19	20 (83.3) 25 (92.6) 17 (89.5)	7.3-49 7.3-84.4 16–107.2	-- 2 (7.41) 5 (26.3)	HPLC(2.2)	present study
Milk,Milk powder	Argentina 1999	77	18 (23%)	10-20	--	ELISA(10)	[39]
Milk	Spain 2000-01	92	5 (5.43%)	14-24.9		ELISA(10)[b] HPLC(10)[b]	[64]
Yogurt	Portugal	96	18 (18.8)	19-98	6 (6.25%)	HPLC(10)	[65]
Milk	Morocco 2006	54	48 (88%)	1-117	4 (7.4)	HPLC (1)	[20]
Cheese	Portugal	128	8 (6.25%)	5-50		(HPLC(5)	[54]
Milk(buffalo & cow)	Pakistan 2007	480	360 (42.5%) 120 (52.5%)	2-174	67 (13.9%)	HPLC(4)	[51]
Milk, Milk powder	Brazil 2006	125	119 (95.2%)	10-500	33 (26.4%)	HPLC (3)	[66]
Milk	Iran 2008	196	196 (100%)	19-126	158 (80.6%)	ELISA(5)	[41]
Milk	Iran 2007-08	210	116 (55.2%)	5-200	70 (33.3%)	ELISA ((5)	[67]
Cheese	Italy 2010	102	85 (83%)	25-250	58 (57%)	ELISA(25)	[35]
Milk	Greece 2010-11	234	43 (18.4%)	5-149	4 (1.76%)	ELISA(5)HPLC	[68]
Milk	Greece 2009-10	196	91 (46.5%)		2	ELISA	[19]
Milk, Milk powder	Brazil	83	51 (61%)	8-215 20-760	25 (30%)	HPLC(3)	[59]
Milk, yogurt	Pakistan 2011-12	188 96	74 (39%) 32 (33%)	4-890 4-880	46 (62%) 21 (66%)	HPLC(4)	[52]

[a] Based on EC Regulation 50 ng/L for milk, cheese and yogurt. [b]LOQ was given.

Table 4. The incidence and levels of AFM_1 contamination in milk, white cheese and Yogurt reported between 2001 and 2011 in Turkey

Sample	Province (Year of sampling)	No of samples	Incidence n (%)	Range (ng/L)	Exceed legal limit [a] n (%)	Method (LOD ng/L)	Ref
Milk, White cheese, yogurt	Turkey 2013	24 27 19	20 (83.3) 25 (92.6) 17 (89.5)	7.3-49 7.3-84.4 16–107.2	-- 2 (7.41) 5 (26.3)	HPLC(2.2)	present study
Milk	Van	90	79 (87.77%)	12.5 to 123.6	35 (44.30%)	TLC(12.5)	[46]
Milk	Ankara	27	16 (59.3%)	10-50.5	1 (3.7%)	(HPLC(10)	[60]
Milk	Central Anatolia 2005	129	75 (58.1%)	0-543.64	61 (47%)	ELISA (10)	[44]
Milk, White cheese	Adana	20 20	20 (100%) 16 (80%)	10-80 54-263	3 (15%)[a] 5 (25%)[b]	ELISA(10) (50)[c]	[45]
Milk	5 cities 2007-08	100	67 (67%)	10-630	31 (31%)	ELISA(10)	[43]
White Cheese	Ankara 2001-02	100	82 (82%)	50-800	100 (100%)[a] 27 (27%)[b]	ELISA	[61]
White Cheese	Ankara 2002-03	94	86 (91.49%)	1-250	69 (73%)[a] 12 (12.76%)[b]	ELISA(<10)	[25]
White Cheese	Bursa 2001-02	200	10 (5%)	100-600	10 (5%)[a] 2 (1%)[b]	ELISA (100)	[62]
Cheese	Erzurum 2006	193	159 (82.4%)	52-860	51 (26.4%)[b]	ELISA(50)	[36]
Cheese, yogurt	Ankara 2004	39 40	11 (28.21%) 32 (80%)	50-188.44 60-365.64	11 (28.20%)[a] 32 (80%)[a]	ELISA(50) (60)[c]	[60]
Milk White cheese yogurt	Kayseri 2010	50 20 50	43(86%) 14(23%) 28(56%)	1-30 15.6-154.6 2.5-78	- - 7(14%)	ELISA(5) (50)[c]	[37]

[a] Based on EC Regulation of 50 ng/L for milk, cheese and yogurt [b] Based on Turkish legal limit of 250 ng/kg for cheese.[c]LOD of ELISA kit for cheese

In Turkey, cheese consumption is widespread, and white cheese is the most popular cheese variety. The occurrence of AFM_1 in the white cheese consumed in Turkey has been investigated by several researchers.

According to the results of a survey conducted in Ankara, Turkey between September 2001 and July 2002, AFM_1 was found in 82% of 100 white cheese samples in the range of 50-800 ng/kg. [61] According to the Turkish legal limit of 250 ng/kg for cheese, 27 white cheese samples were above the limit. When the EC Regulation of 50 ng/kg is considered, the entire sample batch was above the limit.

Between September 2002 and September 2003, [25] 94 white cheese samples were analyzed in Ankara, and AFM_1 was detected in 86 (91.49%) of them. Among the positive samples, 12 (12.76%) were above the maximum permissible limit established by the Turkish Food Codex (250 ng/kg) for cheese.

The incidence rates of AFM_1 in white cheese samples in the above two surveys (Table 4) are nearly the same as that in the present study. However, the levels obtained in the first study [61] are ten times higher than that in the present study (Table 2). When the EC Regulation of 50 ng/kg is considered, almost 80% of the samples [25] were above the permissible limit.

From January 2001 to February 2002, 200 white cheese samples were examined in the Bursa province of Turkey. [62] AFM_1 was detected in 10 (5%) of the cheese samples in the range from 100 to 600 ng/kg. Among the positive samples, when the Turkish Food Codex of 250 ng/kg is considered, 2 (1%) of the samples were above the legal limit. Although the incidence of AFM_1 in white cheese is lower than that in the present study (Table 2), the method had a very high LOD (100 ng/kg), and both the levels of the positive samples and the number of results exceeding the maximum permissible level are higher than that in the present study.

To determine the occurrence and levels of AFM_1 in white cheese samples in the province of Erzurum, between April 2006 and June 2006, 193 samples were analyzed, and AFM_1 was found in 82.4% of the samples in the range of 52 – 860 ng/kg. [36] Of the 159 positive samples, 42 (26.4%) exceeded the Turkish legal limit of 250 ng/kg. The incidence rates and contamination levels are well above those identified in our study.

Yoghurt is extensively used in Turkish cuisine. Thus, the regular monitoring of mycotoxin levels is very important, and the number of surveys held to date is not adequate.

A study was conducted in February 2004 in the province of Ankara, in which 39 cheese samples and 40 yoghurt samples were analyzed for AFM_1 occurrence. [63] According to the survey results, the incidence of AFM_1 contamination in cheese and yoghurt were as follows: AFM_1 was detected in 11 (28.21%) of the cheese samples at levels ranging from 78.20 to 188.44 ng/kg. According to the Turkish legal limit of 250 ng/kg, no cheese samples were contaminated above the maximum permissible limit. However, when the EC Regulation of 50 ng/kg is considered, all 11 positive samples were above the legal limit. Thirty-two (80%) of the yoghurt samples were contaminated by AFM_1 at levels from 61.61 to 365.64 ng/kg. According to the EC Regulation of 50 ng/kg for yoghurt, 32 samples had AFM_1 levels higher than the legal limit.

A survey was conducted between January 2010 and March 2010 in the Central Anatolia province of Kayseri, Turkey, for the occurrence of AFM_1 in milk, cheese and yoghurt samples. [37] Fifty milk, 50 yoghurt and 20 white cheese samples were analyzed. The incidence of AFM_1 was 71%. AFM_1 was detected in 43 (86%) of the milk samples at levels ranging from 1-30 ng/kg; in 28 (56%) of the yoghurt samples at levels ranging from 2.5-78 ng/kg; and in 14 (23%) of the white cheese samples at levels ranging from 15.6-154.6 ng/kg. According to the Turkish Food Codex (2008) limit of 50 ng/kg, only 7 yoghurt samples were contaminated above the legal limit. The AFM_1 incidence in that survey was higher than that in our findings; however, the contamination levels and the number of samples that exceeded the maximum permissible limit were similar.

Table 4 summarizes the selected results of similar studies conducted in Turkey between 2001 and 2011. The average value of 69.6% AFM_1 contamination is very high. Because AFM_1 is resistant to heat, it remains stable during pasteurization or ultra-high temperature treatment during cheese and yoghurt production processes. [35, 59] The AFM_1 incidences ranged from 3.3 to 100%.

4. Conclusion

According to the reports of the Turkish Ministry of Food, Agriculture and Livestock and the Chamber of Food Engineers, Turkey ranks 15th among the world's milk producers. However, the average consumption of milk in Turkey, which is reported as 24-26 L/person/year, is low compared to European, Latin American and Middle Eastern diets, as estimated by the JECFA. [4] To encourage children to drink milk, the Turkish government launched the "school milk project". People of all ages consume milk, and cheese and yoghurt are also used frequently in Turkish cuisine. Therefore, the AFM_1 presence in dairy foods is a real risk to human health. Milk and other dairy products should be regularly monitored for AFM_1 contamination, and feed should be monitored for AFB_1 contamination. According to the results of regular evaluations and due to the favorable climatic and geographical conditions in Turkey, pasture feeding should be encouraged by official authorities to reduce mycotoxin occurrence.

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References

[1]. P. D. Andrade, J. L. G. da Silva, E. D. Caldas, J. Chromatogr. A., 1304, 61-68 (2013).

[2]. Creppy, E. E. Toxicol. Lett.127, 19–28 (2002).

[3]. F. Galvano, V. Galofaro, A. Ritieni, M. Bognanno, A. De Angelis, G. Galvano, Food Addit. Contam.18(7), 644–646 (2001).

[4]. Joint FAO/WHO Expert Committee on Food Additives (JECFA). 56th Meeting Food Additives Series 47.Geneva, Switzerland; WHO. (2001).

[5]. V. Siddappa, D. K. Nanjegowda, P. Viswanath, Food Chem. Toxicol.50, 4158-4162 (2012).

[6]. S.C. Pei, Y. Y. Zhang, S. A. Eremin, W. J. Lee, Food Control. 20, 1080–1085 (2009).

[7]. B.I. Agag, Assiut Univ. Bull. Env.Res.7(1), 173-205 (2004).

[8]. L. Afsah-Hejri, S. Jinap, P. Hajeb, S. Radu, Sh. Shakibazadeh, Compr. Rev. Food Sci. F. 12, 629-651 (2013).

[9]. A. Prandini, G. Tansini, S. Sigolo, L. Filippi, M. Laporta, G.Piva, Food Chem. Toxicol.47, 984–991(2009).

[10]. World Health Organization (WHO). 11, 1-127 (1979).

[11]. R. Bhat, R. V. Rai, A. Karim, Compr. Rev. Food Sci. F. 9, 57–81(2010).

[12]. D. Stagos, G. Amoutzias, A. Matakos, A. Spyrou, A. Tsatsakis, D.Kouretas, Food Chem. Toxicol.50, 2155–2170 (2012).

[13]. M. R. Asi, S. Z. Iqbal, A. Ariño, Hussain, A. Food Control. 25, 34-38 (2012).

[14]. N. A. Lee, S. Wang, R. D. Allan, I. R. Kennedy, J. Agric.Food Chem. 52, 2746-2755 (2004).

[15]. S. Nawaz, K. A. Scudamore, S. C. Rainbird, Food Addit. Contam.14, 249–62 (1997).

[16]. B. Skrbic, J. Zivancev, I. Antic, M. Godula, Food Control.40, 113-119 (2014).

[17]. M. Tabari, G. Karim, M. Ghavami, M.Chamani, Toxicol. Ind. Health, 27(7), 629–635 (2011).

[18]. E. P. Gallagher, L. C. Wienkers, P. L. Stapleton, K. L. Kunze, D. L. Eaton, Cancer Res. 54, 101–108 (1994).

[19]. I. N. Tsakiris, M. N. Tzatzarakis, A. K. Alegakis, V. I. Vlachou, E. A. Renieri, A. M.Tsatsakis, Food Chem. Toxicol. 56, 261–265 (2013).

[20]. A. Zinedine, L. González-Osnaya, J. M. Soriano, J. C. Molto, L.Idrissi, J. Manes, Int. J. Food Microbiol. 114, 25–29 (2007)

[21]. O. Gursoy-Yuzugullu, H. Yuzugullu, M. Yilmaz, M. Ozturk, Liver Int. 31(4),561–571(2011).

[22]. A. Zinedine, J. Mañes, Food Control. 20, 334-344 (2009).

[23]. P. M. Newberne, W.H.Butler, Cancer Res. 29, 236-250 (1969).

[24]. Caloni, F. Stammati, A. Frigge, G. De Angelis, I. Toxicon. 47, 409-415 (2006).

[25]. H. Aycicek, A. Aksoy, S. Saygi, Food Contro. 16, 263-266 (2005).

[26]. IARC. Monograph.Lyon, France Vol 56. pp: 19-23 (1993).

[27]. IARC. Monograph. Lyon, France Vol 82. pp:173-245 (2002).

[28]. J. Alexander, D. Benford, A. Boobis, M. Eskola, J. Fink-Gremmels, P. Fürst, C. Heppner, J. Schlatter, R. van Leeuwen, EFSA J. 10(10),1004 (2012).

[29]. M. Broberg, Danish Institute for International Studies (DIIS) Working Paper,09.ISBN 978-87-7605-325-3 (2009).

[30]. EFSA Opinion of the scientific panel. EFSA J. 39, 1-27 (2004).

[31]. European Commission Directive 2003/100/EC of 31 October OJEU. L285, 33-37 (2003).

[32]. European Commission Regulation (EU) No 1881/2006 of 19 December OJEU. L364, 5-24 (2006).

[33]. Turkish Food Codex. Official Gazette Number 26879, 3-4 (2008).

[34]. S. C. Duarte, A.M. Almeida, A. S. Teixeira, A. L. Pereira, A. C. Falcão, A. Pena, C. M.Lino, Food Control. 30, 411-417 (2013).

[35]. L. Anfossi, C. Baggiani, C. Giovannoli, G. D'Arco, C. Passini, G. Giraudi, Food Control. 25, 125-130 (2012).

[36]. M. Ardic, Y. Karakaya, M. Atasever, G. Adiguzel, Food Control. 20,196-199 (2009).

[37]. N. Ertas, Z. Gonulalan, Y. Yildirim, F. Karadal, Food Control. 22, 1956-1959 (2011).

[38]. J. Kos, J. Levic, O. Duragic, B. Kokic, I. Miladinovi, Food Control. 38, 41-46 (2014).

[39]. C. E. Lopez , L. L. Ramos, S. S. Ramadan, L. C. BulacioFood Control. 14, 31-34 (2003).

[40]. E. Rahimi, M. Bonyadian, M. Rafei, H. R. Kazemeini, Food Chem. Toxicol. 48, 129-131(2010).

[41]. A. M. Sani, H. Nikpooyan, R. Moshiri, Food Chem. Toxicol.48,2130-2132 (2010).

[42]. K. K. Tekinsen, G.Ucar, Food Control. 19, 27-30 (2008).

[43]. K. K. Tekinsen, H. S. Eken, Food Chem. Toxicol, 46, 3287-3289 (2008).

[44]. N. Unusan, Food Chem. Toxicol. 44, 1897-1900 (2006).

[45]. I. Var, B. Kabak, Int. J. Dairy. Technol 6, 15-18, (2008).

[46]. Bakırcı, I. Food Control, 12, 47-51 (2001).

[47]. L. Lin, J. Zhang, P. Wang, Y. Wang, J. Chen, J Chromatogr. A, 815, 3-20 (1998).

[48]. L. Shundo, M. Sabino, Braz. J Microbiol, 37,164-167 (2006).

[49]. C. Chen, W. Li, K. Peng, J. Agric. Food Chem, 53(22), 8474-8480 (2005).

[50]. O. Deveci, Food Control., 18, 1103-1107 (2007).

[51]. I. Hussain, J. Anwar, M. A. Munawar, M. R. Asi, Food Control. 19, 1126-1129 (2008).

[52]. S. Z. Iqbal, M. R. Asi, Food Control., 30, 235-239 (2013).

[53]. S. Z. Iqbal, M. R. Asi, S. Jinap, Food Control. 34,714-718 (2013).

[54]. H. M. Martins, S. A. Magalhães, I. Almeida, M. Marques, M. M. Guerra, F. Bernardo, Rev. Port. Cienc. Vet. 102(563-564),321-325 (2007).

[55]. Y. Wang, X. Liu, C. Xiao, Z. Wang, J. Wang, H. Xiao, L. Cui, Q. Xiang, T. Yue, Food Control. 28, 131-134 (2012).

[56]. D.Y. Sarıca, A.R. Turker, Clean – Soil, Air, Water.40(5), 523-530 (2012).

[57]. European Commission Regulation (EC) No. 2174/2003 amending Regulation (EC) No. 466/2001. OJEU. L 326, pp. 12-15 (2003)

[58]. A.C. Manetta, M. Giammarco, L. D. Giuseppe, I. Fusaro, A. Gramenzi, A. Formigoni, G. Vignola, L. Lambertini, Food Chemistry.,113, 595-599 (2009).

[59]. M. H. Iha, C. B. Barbosa, I. A. Okada, M. W. Trucksess, Food Control, 29, 1-6 (2013).

[60]. A. Gurbay, S. Aydın, G. Girgin, A. B. Engin, G. Sahin, Food Control.17, 1-4 (2006).

[61]. B. Sarımehmetoglu, O. Kuplulu, T. H. Celik, Food Control. 15, 45-49 (2004).

[62]. T. Yaroglu, H. H. Oruc, M. Tayar, Food Control. 16, 883-885 (2005).

[63]. A. Gurbay, A. B. Engin, A. Caglayan, G. Sahin, Ecol.FoodNutr. 45, 449-459 (2006).

[64]. M. L. R. Velasco, M. M. C. Delso, D. O. Escudero, Food Addit.Contam. 2003, 20(3), 276-280.

[65]. M. L. Martins, H. M. Martins, Int J Food Microbiol, 91, 315- 317(2004).

[66]. L. Shundo, S.A. Navas, L. C. A. Lamardo, V. Ruvieri, M. Sabino, Food Control, 20, 655-657 (2009).

[67]. A. Heshmati, J. M. Milani, Food Control. 21, 19-22 (2010).

[68]. E. Malissiova, A. Tsakalof, I. S. Arvanitoyannis, A. Katsafliaka, A. Katsioulis, P. Tserkezou, M. Koureas, A. Govaris, C. Hadjichristodoulou, Food Control., 34,241-248 (2013).