Turk J Vet Anim Sci
28 (2004) 527-530

Research Article

Occurrence of Aflatoxin M_1 in Some Cheese Types Sold in Erzurum, Turkey

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Received: 24.12.2002

Abstract: Sixty-three samples of cheese consisting of 23 White cheeses, 14 Kaşar cheeses, 11 Tulum cheeses, 9 Civil cheeses and 6 Lor cheeses (whey-curd), were analyzed for the occurrence of aflatoxin M_1 (AFM_1) using enzymatic immunoassay. In 28 of 63 samples (44.44%), the presence of AFM_1 was detected in concentrations between 7 ng/kg and 202 ng/kg. The mean levels of AFM_1 were 28.08 ng/kg in White cheeses, 22.80 ng/kg in Kaşar cheeses, 74.05 ng/kg in Tulum cheeses, 12.32 ng/kg in Civil cheeses and 15.95 ng/kg in Lor cheeses.

Key Words: Aflatoxin M_1 (AFM_1), cheese, enzymatic immunoassay (EIA)

Erzurum İlinde Satılan Bazı Peynir Çeşitlerinde Aflatoxin M_1 Varlığı

Özet: Bu araştırmada, 23 Beyaz peynir, 14 Kaşar peyniri, 11 Tulum peyniri, 9 Civil peynir ve 6 Lor peyniri olmak üzere toplam 63 peynir örneği aflatoxin M_1 (AFM_1) varlığı bakımından enzimatik immunoassay tekniği ile (EIA) analiz edilmiştir. Sadece 28 peynir örneğinde AFM_1 tespit edilmiş ve miktarları 7 ile 202 ng/kg arasında değişmiştir. Ortalama AFM_1 seviyesi Beyaz peynirlerde 28,08 ng/kg, Kaşar peynirlerinde 22,80 ng/kg, Tulum peynirlerinde 74,05 ng/kg, Civil peynirlerinde 12,32 ng/kg ve Lor peynirlerinde ise 15,95 ng/kg olarak belirlenmiştir.

Anahtar Sözcükler: Aflatoksin M_1 (AFM_1), peynir, enzimatik immunoassay (EIA)

Introduction

Aflatoxins are carcinogenic, highly toxic secondary metabolic products of some Aspergillus spp. (*Aspergillus flavus* and *Aspergillus parasiticus*). They easily occur on foods and feeds during growing, harvest or storage (1-3). Aflatoxin M_1 (AFM_1) is produced as a metabolite of aflatoxin B_1 (4-6). It is secreted with the milk after the feedings of aflatoxin B_1 containing foodstuffs to lactating cows (7-9). AFM_1 is stable in raw and processed milk products. Pasteurization and the processing of milk into cheese result in negligible destruction of AFM_1 (10,11). AFM_1 is associated with the casein in milk and then concentrated in the cheese (12,13).

Many chromatographic methods of analysis have become available for the determination of AFM_1 in milk and milk products. However, these methods are very expensive and lengthy. Nowadays, immunological methods are preferred to chromatographic methods in routine and survey work. In addition, enzymatic immunoassay (EIA) to determine AFM_1 in milk products is fairly cheap, sensitive and quick (14).

The presence of AFM_1 was determined in some cheese types made from different types of milk (cow, ewe, goat and mixed) sold in Erzurum province in Turkey. Each sample was analyzed in duplicate (n: 2) and the mean of the results was determined.

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Materials and Methods

Solvents

Dichloromethane (27, 056-3), chloroform (27, 063-6), methanol (27, 047-4) and heptane (27, 051-2) were used. All the solvents were analytically pure and purchased from Sigma.

Samples

A total of 63 cheeses; 23 White cheeses, 14 Kaşar cheeses, 11 Tulum cheeses, 9 Civil cheeses and 6 Lor cheeses sold in Erzurum, Turkey, were investigated. The samples of cheese were randomly purchased from 21 retail markets and transferred to the laboratory in plastic bags under refrigeration and stored at 2-4 °C until being analyzed.

Determination of AFM_1 by EIA

The presence of AFM_1 in cheese samples was detected with EIA (Ridascreen®Aflatoxin M_1 Art. no.: R1101, R-Biopharm GmbH, Germany) as described by Lopez et al. (15). Two grams of sliced cheese were weighed into a glass vial and 40 ml of dichloromethane was added and the resulting mixture was shaken for 15 min. The suspension was filtered and 10 ml of the extract was evaporated at 60 °C under a N_2 stream. The residue was dissolved in 0.5 ml of methanol, 0.5 ml of buffer phosphate (Na_2HPO_4: 1.427% w/v, K_2HPO_4: 0.907% w/v, ratio 8:2, pH 7.2) and 1 ml of heptane. After centrifugation at 2700 x g for 15 min, the upper heptane-layer was completely removed. The lower methanol-water phase was carefully poured off using a Pasteur pipette; 100 µl of this aliquot was diluted with 400 µl of phosphate buffer.

Following the R-Biopharm (Ridascreen® Aflatoxin M_1) instructions, 100 µl of the standard solutions and prepared sample were added to microtiter wells and incubated for 60 min in the dark at room temperature. The liquid was completely poured off the wells. The wells were filled with 250 µl of distilled water and the liquid poured out again. Then, 100 µl of the diluted enzyme conjugate was added to the wells and incubated for 60 min in the dark at room temperature. The wells were washed 3 times with 250 µl of distilled water; 50 µl of substrate and 50 µl of chromogen were added to the wells. After incubation for 30 min in the dark at room temperature, a stop reagent was added to each well. The wells were mixed and the absorbance measured at 450 nm using an EIA reader (Stat Fax, Neogen Corporation) within 60 min of the addition of the stop solution.

Results

The levels of aflatoxin standards used were 0, 5, 10, 20, 40 and 80 ng/kg. A standard curve was obtained from the values of absorbance (%) for these standards at 450 nm. This standard curve is presented in the Figure.

The presence of AFM_1 was detected in 28 (44.44%) of the 63 samples at concentrations between 7 and 202 ng/kg. On comparing the average number of positive samples, the highest value of AFM_1, 201.3 ng/kg, was found in a sample of Tulum cheese. Next were the White cheeses with 105.5 ng/kg. The Kaşar cheeses displayed lower levels, 67.7 ng/kg (Table).

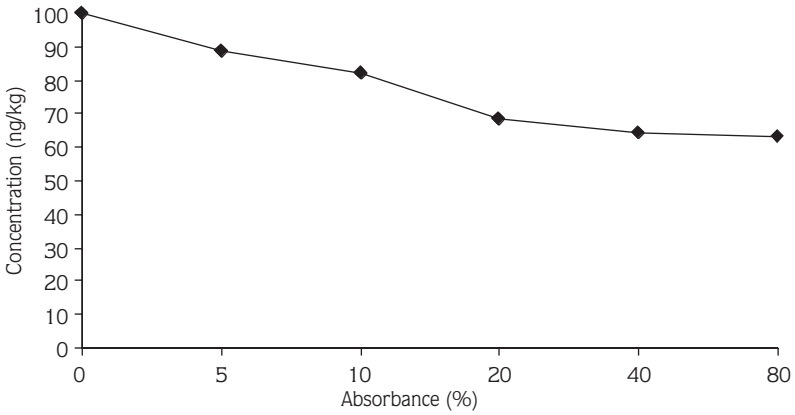

Figure. The standard curve for aflatoxin M_1 standards (Ridascreen® Aflatoxin M_1 kit).

Table. Occurrence of aflatoxin M_1 in some cheese types sold in Erzurum, Turkey.

Types of cheese	Number of Samples			Contamination (ng/kg)	
	Tested	Positive	Negative	Level[a]	Range
White	23	9	14	28.08	11-106
Kaşar	14	6	8	22.80	7-68
Tulum	11	7	4	74.05	11-202
Civil	9	4	5	12.32	12-18
Lor	6	2	4	15.95	13-19
Total	63	28(44.44%)[b]	35 (55.56%)		

[a] Means values for positive samples, [b] Percentage of positive samples

Discussion

There have been many studies regarding the presence of AFM_1 in milk and milk products. Kiermeier and Buchner (16) and Polzhofer (17) pointed out that all the types of cheese investigated exhibited very high contamination by AFM_1, between 34% and 100%. Stoloff and Wood (18) stated that one of the 399 cheese samples produced in the USA, Cheddar and Cottage cheeses contained AFM_1 levels equivalent to 0.08 ng/ml. Stoloff (4) stated that of the 190 samples of Cheddar cheese and 208 samples of Cottage cheeses, AFM_1 was present in one sample and at a level close to only 0.30 ng/kg. Truckses and Page (19) detected AFM_1 at levels of 0.1 and 1.0 ng/kg in only 8 out of 118 samples (6-8%). Blanco et al. (20) indicated that 14 of 47 samples (29.8%) of commercial ultra high temperature-treated milk were positive for AFM_1. Karaioannoglou et al. (21) reported that AFM_1 was detected at levels of 0.10 to 0.13 µg/kg in 4 of 99 samples of raw milk collected from northern and central Greece but was not detected in any Feta and Teleme cheese samples. Kıvanç (22) did not find any AFM_1 in some Turkish cheeses. Dragacci and Freum (23) reported that 3 out of 28 milk products marketed in France exhibited AFM_1 levels of 0-200 µg/kg. Barrios et al. (24) reported that AFM_1 was found in concentrations between 20 and 200 ng/kg in 16 out of 35 samples (45.71%) including fresh, ripened and semiripened cheeses produced in the south of Spain. Galvano et al. (25) reported that AFM_1 was determined in 136 (86%) milk samples, in 81 (84%) dry milk samples, and in 91 (80%) yogurt samples.

In our study, AFM_1 was determined in all the types of cheese investigated. However, the levels of AFM_1 in Tulum cheese were much higher than those in the other cheese types. This may be due to proteolysis of the casein during the ripening of Tulum cheese. The proteolysis of casein may increase the recovery of AFM_1 from cheese (12). The Table shows that the levels of AFM_1 detected in all of the cheese samples were below the maximum limits permitted for AFM_1 in cheese in some European countries such as Switzerland (250 ng/kg = 0.250 µg/kg), Russia (500 ng/kg) (26), and Turkey (250 ng/kg) (27). This situation is positive for the microbial quality of some cheese types produced in Turkey.

In conclusion, the contamination risk of AFM_1 in milk and cheese may increase with feeds being stored in insufficient conditions in the long cold Erzurum winters. This risk can be significantly reduced by using aflatoxin uncontaminated feeds, or mixing aflatoxin-contaminated feeds with uncontaminated feeds for milking animals. In addition, it is important that feeds be frequently analyzed for aflatoxins before feeding.

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