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A survey of aflatoxin M1 levels in Kahramanmaraş cheese

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Abstract: Aflatoxin M1 (AFM1) levels were measured in 46 Kahramanmaraş semi-hard cheese samples (22 from bovine milk, 18 from goat milk, and 6 from ovine milk) using high-pressure liquid chromatography (HPLC). None of the ovine milk cheese samples contained AFM1. AFM1 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 AFM1, respectively. With the exception of 2 bovine milk cheese samples (one contained 1.2 ng g^{-1} of AFM1, the other contained 0.25 ng g^{-1} of AFM1), the other samples (96%) had levels of AFM1 below the acceptable limit for cheese (0.25 ppb) set forth by the Turkish Alimentarus Codex.

Key words: Aflatoxin M1, bovine cheese, goat cheese, ovine cheese, HPLC

Kahramanmaraş peynirinde aflatoxin M1 oranlarının belirlenmesi üzerine bir çalışma

Özet: Çalışmada HPLC (High pressure liquid chromatography) ile 46 adet yarı sert peynir örneğinde (22 adet inek, 18 adet keçi, 6 adet koyun sütünden yapılmış) Aflatoxin M1 (AFM1) oranları belirlenmiştir. Koyun peynirlerinde AFM1 tespit edilemezken, inek ve keçi peynirlerinin 32 tanesinde 0,069-1,2 ng g^{-1} ve 0,06-0,22 ng g^{-1} oranında AFM1 tespit edilmiştir. İnek peynirlerinin analiz sonuçları % 96 oranında Türk Gıda Kodeksi peynirde AFM1 sınırı olan 0,25 ppb değerine uygunluk gösterirken 1 tanesi 1,2 ng g^{-1}, diğeri 0,25 ng g^{-1} AFM1 olarak tespit edilmiştir.

Anahtar sözcükler: Aflatoxin M1, inek peyniri, keçi peyniri, koyun peyniri, HPLC

Introduction

Aflatoxins (AF) are a group of highly toxic secondary metabolic products of some *Aspergillus* spp., mainly *A. flavus* and *A. parasiticus*, that occur during growth on feeds and/or foods (1,2), and, more rarely, *A. nomius*, *A. pseudotamarii* (3), *A. bombycis* (4), *A. tamarii*, and *A. ochraceoroseus* (5,6). Aflatoxin M1 (AFM1) and M2 (AFM2) are oxidative metabolic products of aflatoxin B1 (AFB1) and B2 (AFB2) produced by animals, and are usually excreted in the milk, urine, and feces of dairy cattle and other mammalian species that have ingested aflatoxin-

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contaminated food (7). Aflatoxins have toxic and carcinogenic effects on humans and animals, and AFM1 is classified in Group 2 as a probable human carcinogen (8).

The tolerance limits in Turkey for AFM1 in milk and cheese are 0.05 and 0.25 ppb, respectively (9). Turkey produces many traditional cheese varieties, largely in small-scale dairies and family farms. Maraş cheese is a traditional cheese produced primarily in Kahramanmaraş, a city in the southeastern region of Turkey, and is made from raw ovine, goat, or bovine milk, or appropriate mixtures of them. The manufacturing process and chemical compounds used are similar to those used for mozzarella cheese. The production and consumption of cheese, especially Kahramanmaraş semi-hard cheese, are widespread in the southeastern region of Turkey. This study aimed to determine AFM1 levels in Kahramanmaraş cheese using high-pressure liquid chromatography (HPLC).

Materials and methods

The study included 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. The cheese samples were transferred to the laboratory in iceboxes. All samples were collected between January and April 2007.

The chloroform, hexane, Celite 545, and acetonitrile used in the study were HPLC grade and purchased from Merck (Darmstadt, Germany). All other chemicals and solvents were also purchased from Merck (Darmstadt, Germany). Vicam immunoaffinity columns (Watertown, MA, USA) were used to clean the samples. Standards for AFM1 were obtained from the Community Bureau of Reference BCR (RM 423). Stock solutions and standards were prepared and assayed according to Sharman et al. (10).

AFM1 was extracted from each 20-g cheese sample into a 250-mL beaker with 75 mL of chloroform, a saturated 1-mL NaCl solution, and 5 g of Celite 545. The mixture was homogenized for 2-3 min to produce a slurry, and then was filtered through Whatman no. 4 filter paper. The beaker was washed with 50 mL of chloroform, which was then filtered, followed by the filter paper being squeezed against the funnel to obtain the maximum yield of extract. The chloroform extract was evaporated to dryness under vacuum at 30 °C, and 1 mL of methanol, 30 mL of water, and 50 mL of hexane were added to the residue. After gentle swirling the mixture was transferred to a separating funnel with washing (2 ´ 10 mL of water), and was then shaken for 10-15 s. The lower layer was collected and used in the affinity column stage of the sample clean up procedure (10).

The immunoaffinity column was washed with 10 mL of distilled water, using a syringe and a flow rate of about 2-3 mL min^{-1}, and then a 50-mL sample was extracted, followed by washing with 10 mL of distilled water. AFM1 was then slowly eluted from the column with 2 mL of acetonitrile into a glass vial. The solvent was evaporated to near dryness with a gentle nitrogen stream and was re-dissolved in 1:1 acetonitrile-water; the extract was finally filtered before HPLC analysis (10). The volume of injection into HPLC was 100 µL.

A liquid chromatographic system (model 1100, Agilent, USA) equipped with a ODS-2 (5 µm, 250 ´ 4.6 mm i.d.) column (Hicrom, Reading, UK) was used for AFM1 measurement. The mobile phase was delivered to the column at a rate of 1mL/min. A fluorescence detector was used with an excitation wavelength of 365 nm and an emission wavelength of 435 nm. Each of the experiments was replicated 4 times and the results were reproducible. A series of calibration solutions were prepared using AFM1 stock solution at concentrations of 0.2, 0.5, 1.0, 2.5, 5.0, and 7.5 ng mL^{-1}. Calibration curves were constructed by plotting the peak area for each calibration solution against the mass of AFM1 injected ($r = 0.999$). The detection limit was 0.05 ng g^{-1} and recovery ranged between 99% and 100%.

Results

The AFM1 level in 46 samples of Kahramanmaraş semi-hard cheese was measured using HPLC (Table). None of the ovine milk cheese samples contained AFM1. With the exception of 2 samples, the bovine milk and goat milk cheese samples contained 0.069-1.2 ng g^{-1} of AFM1 and 0.06-0.22 ng g^{-1} of AFM1,

Table. Determination of AFM1 levels in Kahramanmaraş cheese.

	AFM1 levels of Kahramanmaraş cheese (ng g^{-1})		
Milk used in cheese production	Bovine	Ovine	Caprine
Number of cheese samples	22	2	18
Mean of four replication	0.24	UDL	0.18
Range	UDL-1.2	UDL	UDL-0.22
Number of samples ≥0.25 ng/g	2	-	-
Positive samples	18	-	14

UDL: under detection limit, -: not detected

respectively. AFM1 was present in 32 of the 46 samples (69.6%). With the exception of 2 bovine milk cheese samples (1 contained 1.2 ng g^{-1}, the contained 0.25 ng g^{-1}), the samples (96%) had levels of AFM1 below the acceptable limit for cheese (0.25 ppb) set forth by the Turkish Alimentarus Codex.

It is known that milk and milk products are a major source of nutrients for humans, especially children; however, at the same time AFM1 in milk and dairy products may pose a health hazard to consumers. Consequently, many researchers have studied AFM1 and reported varying results. The variation in reported results could be due to differences in cheese manufacturing procedures, milk contaminants, the type of cheese, cheese-ripening conditions, and the analytical method employed (11,12). In addition, the level of AFM1 in milk is significantly affected by geographical region and season. It was reported that summer milk had less contamination than milk produced in winter (11). The occurrence of AF in cheese can be due to the following: 1. AFM1 may be found in the milk of animals fed food containing AFB1; 2. The synthesis of AF (B1, B2, G1, and G2) by *A. flavus* and *Aspergillus parasiticus* growing on cheese (13); 3. the occurrence of these toxins in dried milk used to enrich the milk used to make cheese (14). Thin-layer chromatography (TLC), HPLC, and enzyme-linked immunosorbent assay (ELISA) are the most common techniques for detecting AFM1 in milk and dairy products.

Tekinşen and Tekinşen (15) reported that the incidence of AFM1 in Van otlu and white pickle cheese samples ranged from 0.16 to 7.26 μg kg^{-1} 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 of 0.25 μg of AFM1/kg of cheese set forth by Turkish government, but Kıvanç (16) reported the absence of AFM1 at detectable levels in Van otlu and white cheese samples in Van, Turkey. Gürbüz et al. (17) also reported the absence of AFM1 in kashar cheese.

Kamkar (18) reported that AFM1 levels in Iranian feta cheese in May, August, November, and February samples ranged from 0.17 to 1.30, 0.15 to 2.41, 0.16 to 1.11, and 0.19 to 2.05 μg kg^{-1}, respectively, while the mean values were 0.41, 0.35, 0.36, and 0.52 μg kg^{-1}, respectively. Additionally, 60.6% of their contaminated samples had AFM1 levels exceeding the acceptable level in Turkey of 0.25 μg kg^{-1}. Sarımehmetoğlu et al. (19) reported that kashar, white, tulum, and processed cheese samples contained significant amounts of AFM1 (usually higher than 250 ng kg^{-1}).

In conclusion, 96% of the Kahramanmaraş semi-hard cheese samples observed in the present study had AMF1 levels within the legal limit (9); however, contamination with aflatoxins in the field is very difficult to control because it is influenced primarily by such climatic conditions as relative humidity and temperature (1). Soil moisture and insect damage are also important factors in the contamination of commodities. The highest concentrations of aflatoxins are also associated with post-harvest growth of Aspergillus molds on poorly stored food (20). Therefore, animal feed should be checked regularly for AFB1 and feed storage conditions must be under strict control. At the same time, surveillance programs for feed and milk must be ongoing and widespread. Milk and dairy products that contain high levels of AFM1 are prohibited for human consumption.

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