Short communication: Aflatoxin M₁ in dairy products sold in Şanlıurfa, Turkey

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ABSTRACT

The aim of this study was to detect the presence of aflatoxin M₁ (AFM₁) in samples of raw milk ($n = 38$), UHT milk ($n = 12$), white pickled cheese ($n = 50$), and yogurt ($n = 50$) collected from the Şanlıurfa city markets and locally produced dairy products by ELISA. The mean contamination rates were 56.74 ± 40.32 , 43.1 ± 23.19 , 103.2 ± 29.13 , and 55.28 ± 12.68 ng/kg, respectively, for raw milk, UHT milk, white pickled cheese, and yogurt. According to the data, 21 (55%) raw milk, 3 (25%) UHT milk, 10 (20%) white pickled cheese, and 10 (20%) yogurt samples were contaminated with AFM₁ over the acceptable levels (≥ 50 ng/kg), ranging from 0.82 to 130.89 ng/kg. None of the white pickled cheese samples contained AFM₁ levels above the Turkish legal limit (250 ng/kg). Consequently, the AFM₁ contamination levels determined in this study in white pickled cheese were not considered to pose a serious public health hazard. However, the AFM₁ levels in raw and UHT milk and yogurt samples indicate an increased human health risk in Turkey related to high aflatoxin levels. Therefore, milk and dairy products have to be monitored by the Turkish public health authorities continuously to detect AFM₁ contamination.

Key words: aflatoxin M₁, milk, white pickled cheese, yogurt

Short Communication

The aflatoxins are a group of toxic and carcinogenic secondary metabolites produced by different *Aspergillus* species such as *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* (Ito et al., 2001). Aflatoxin M₁ (AFM₁), which is a hepatocarcinogen that is classified in group 1 (carcinogenic to humans) by the International Agency for Research on Cancer (IARC, 2002), is the main monohydroxylate derivative of aflatoxin B₁ (AFB₁), formed in the liver by means of cytochrome P450-associated enzymes (Zinedine et al., 2007), and is secreted in the milk of mammals that

have eaten contaminated foods. Although AFM₁ is considered to be less carcinogenic than AFB₁ by at least an order of magnitude, its presence in dairy products should be limited to the lowest level practicable. It is relatively stable during pasteurization, sterilization, and the preparation of various dairy products (Fallah et al., 2009). And the presence of AFM₁ in milk and milk products is considered to be undesirable (Prandini et al., 2009). According to European Commission regulations (Prandini et al., 2009) and the Turkish Food Codex (Turkish Food Codex, 2008), AFM₁ levels should not exceed 50 ng/kg as the regulatory limit in raw milk, heat-treated milk, and milk for the manufacture of milk-based products. The Turkish Food Codex recommends the maximum limit of cheese products to be 250 ng/kg (Turkish Food Codex, 2008). The regulatory limit for AFM₁ in the United States is 500 ng/kg (Van Egmond, 1989). Using ELISA for the detection of AFM₁, which has been included in the official collection of test procedures by the German Federal Board of Health, is quick, reliable and cost-effective (Kaniou-Grigoriadou et al., 2005). Because milk and dairy products are part of the main nutrition in Turkey, it is important not only to determine AFM₁ levels in certain milk and milk product samples, but also to consider routine monitoring surveys in this regard (Gürbay et al., 2006). In the current study, analyses of AFM₁ in milk and milk products in Şanlıurfa, Turkey were carried out and the incidence of AFM₁ contamination is discussed.

One hundred fifty dairy product samples were randomly purchased from the Şanlıurfa city markets and local dairies during January and February 2012 in Şanlıurfa, Turkey [raw milk (500 mL; $n = 38$), UHT milk (500 mL; $n = 12$), white cheese (100 g; $n = 50$), and yogurt (500 g; $n = 50$)]. The samples were transferred to the laboratory in sterile plastic bags at 4°C and stored at -18°C until analysis. Material samples were prepared suitably for ELISA quantification to perform AFM₁ analysis. For raw milk samples, an aliquot of unprocessed raw milk sample (2 mL) was placed at 4°C overnight to allow the fat globules to rise to the surface in a natural creaming effect. The upper cream layer was completely removed and 200 μL of the skim milk was used directly in the test. The UHT milk was

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used directly in the ELISA. To prepare cheese and yogurt samples according to the method outlined in the ELISA kit for cheese and yogurt, 1 g of finely grated cheese or yogurt sample was added to 5 mL of absolute methanol in a capped tube and mixed for 5 min. The tube was clarified by centrifugation ($5,000 \times g$ for 5 min) and the supernatant was removed; 0.5 mL of this supernatant was transferred to a glass tube and the methanol evaporated by a stream of air. This procedure resulted in the deposition of a semisolid viscous material on the inside of the tube; 0.5 mL of the provided blank skim milk was added to the tube and vortexed vigorously for 1 min. After allowing the tube to stand for a further 5 min, 200 μ L of this milk extract was used in the test. Following preparation of the samples, AFM₁ analysis was carried out based on the manual procedure from the AFM₁ ELISA kit (Aflatoxin M₁ Assay, 961AFLM01M-96; Helica Biosystems Inc., Santa Ana, CA). The measurement was made photometrically at 450 nm on an ELISA Reader (MR-96A; Mindray Medical Germany GmbH, Bensheim, Germany). The absorbance values found for the standards and samples were evaluated according to the mycotoxin evaluation program prepared by Helica Biosystems Inc. According to the manufacturer, the mean lower detection limit of the Mindray AFM₁ test is 2 ng/kg. The corresponding dilution factor for milk and yogurt is 1 and 5 for cheese. To validate our method, a recovery study was performed by spiking known amounts of AFM₁ (50 ng/kg) into homogenized milk, cheese, and yogurt samples just before the test. The mean recovery score in spiked cheese samples was 59.5% with a coefficient of variation of 5.0%, and in spiked milk samples, it was 98.7% with a coefficient of variation of 4.1%. According to the kit instructions, the recovery rate in cheese is approximately 60.5% with a mean coefficient of variation of 5.5%, and in milk it is approximately 100% with a mean coefficient of variation of 4.2%. The recovery rates and coefficients of variation in yogurt samples were evaluated as in the cheese samples. All statistical analyses were performed using SPSS software (version 16; SPSS Inc., Chicago, IL) and the data were expressed as mean $\pm$ standard deviation.

According to ELISA quantification, the occurrences of AFM₁ in raw milk, UHT milk, white pickled cheese, and yogurt samples are shown in Table 1. Although AFM₁ was found in 94.7% of raw milk samples and in 100% of UHT milk, yogurt, and white pickled cheese samples, the results showed that 21 (55.3%) raw milk, 3 (25%) UHT milk, 10 (20%) white pickled cheese, and 10 (20%) yogurt samples were contaminated with AFM₁ over the acceptable levels (≥ 50 ng/kg), ranging from 0.82 to 130.89 ng/kg. The AFM₁ levels of the white

pickled cheese samples were below the Turkish legal limit (250 ng/kg). Only 18% of dairy product samples in Şanlıurfa were contaminated above the legal limits. Almost all of our 150 dairy materials, except 2 raw milk samples (98.6%), were contaminated with AFM₁; however, the percentage of contamination over legal limits of the Turkish Food Codex (2008) and European Community was 19.3% (29/150), but it was still below the US criteria (AFM₁ levels of all dairies below 500 ng/kg). Ertas et al. (2011) found AFM₁ contamination in 68.1% of 160 dairy samples (50 milk, 50 yogurt, and 60 cheese) by ELISA, whereas de Sylos et al. (1996) found no contamination in their 163 materials (133 milk and 30 yogurt) by thin-layer chromatography. Different and controversial numerous reports about AFM₁ contamination in milk samples were presented as well. According to the European Food Safety Authority surveys performed in more than 10 European countries between 1999 and 2001 (Prandini et al., 2009), AFM₁-positive results in dairy milk samples reached 100%, although almost more than 90% were below 10 ng/kg, whereas a few samples obtained from Greece and Portugal were beyond the legal limits of 50 ng/kg. In the present study, we found 55.3% of raw milk samples to be over the Turkish and European legal limits. In the last 10 yr, Elgerbi et al. (2004; 69.4%), Ruangwises et al. (2011; 64.6%), Ghanem and Orfi (2009; 54.4%), and Delialioğlu et al. (2010; 73.5%) declared higher percentages over legal limits; in contrast, Nuryono et al. (2009), Cano-Sancho et al. (2010), Ertas et al. (2011), Kaniou-Grigoriadou et al. (2005), and Rahimi and Ameri (2012) found legal values. Of these, Kaniou-Grigoriadou et al. (2005) and Rahimi and Ameri (2012) studied raw milks of ewes and goats, respectively. Delialioğlu et al. (2010) found markedly lower rates over legal limits in raw goat milk (10%) than in cow milk (73.5%). Different authors emphasized that goats and sheep herds were fed silage for limited times compared with cows, resulting in lower incidences of aflatoxin B₁ (Kamkar, 2006; Prandini et al., 2009; Rahimi and Ameri, 2012). Our samples comprising cow milk might explain the higher contamination rates in the present study. On the other hand, our UHT milk contamination levels were lower than those in raw milk. In fact, due to idea that heating or storing at lower temperatures would not cause an appreciable change in the amount of AFM₁, we predicted higher levels in UHT milk samples (Prandini et al., 2009). With regard to cheese samples, although all of our white pickled cheese samples were found contaminated and the highest contamination rate was reported in the current study, none of the values were over the legal limits of the Turkish Food Codex and the European Community (< 250 ng/kg). In another white

Table 1. Aflatoxin M₁ (AFM1) concentration in raw milk, UHT milk, white pickled cheese, and yogurt samples determined by ELISA

Sample category	No. of samples	AFM1 contamination (ng/kg)		No. of AFM1 concentrations above 50 ng/kg
		Range	Mean $\pm$ SD	
Raw milk	38	0.82–125.70	56.74 $\pm$ 40.32	21 ¹
UHT milk	12	18.93–91.27	43.1 $\pm$ 23.19	3 ¹
White pickled cheese	50	40.41–130.89	103.2 $\pm$ 29.13	5 ²
Yogurt	50	40.62–72.04	55.28 $\pm$ 12.68	5 ¹

¹Turkish Food Codex (2008) limits in raw milk, heat-treated milk, and milk for the manufacturing of milk-based products.

²Cheese samples of AFM1 concentration above 50 ng/kg but below 250 ng/kg (Turkish and legal limit of the European Community for cheese).

pickled cheese survey that took place in the same province (Şanlıurfa, Turkey), Kav et al. (2011) presented a lower contamination rate [36/127 (28.3%)], 13 of them being higher than 250 ng/kg (10.23% of all samples) and the maximal contamination rate was more than 5 times higher than our maximal value (130.89 vs. 770.97 ng/kg). The detection limit was 50 ng/kg in Kav et al. (2011) and some other studies (Ardic et al., 2008, 2009; Amer and Ibrahim, 2010; Ertas et al., 2011), and was 100 ng/kg in Yaroglu et al. (2005), whereas it was 10 ng/kg for cheese in our study. In those studies, the sensitivity of detecting lower contamination rates might have been weaker. In our study, we found that all yogurt samples were contaminated with AFM1, but only 10% of them were over the legal limit of 50 ng/kg. This contamination rate is the highest ever reported in the literature. Among the studies, Aydemir Atasever et al. (2011) and Ertas et al. (2011) reported an AFM1 incidence of 87.5 and 56.0%, respectively. Two successive Italian yogurt surveys reported contamination rates of 79.9% (Galvano et al., 1998) and 61.0% (Galvano et al., 2001). According to the results of the present study, if a 70-kg woman consumed a glass of milk (200 mL), a bowl of yogurt (200 g), and a portion of white pickled cheese (30 g) per day, calculating from mean values we obtained for each entity, she would be exposed to approximately 0.33 ng of AFM1/kg per day. This ratio would exceed 1.5 times the tolerable daily intake of 0.2 ng/kg per day calculated by Kuiper-Goodman (1990). This calculation puts forward the importance of prevention measures against aflatoxins. Even this simple calculation, which depends on the mean values obtained in the present study, must prompt health authorities to take action and implement strict measures targeting AFB1 and AFM1 control in dairy products. We suggest that the AFM1 levels in white cheese samples do not indicate a severe public health problem. However, high levels of AFM1 in raw and UHT milk and yogurt samples threaten public health in Şanlıurfa city.

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