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Aflatoxin M_1 Levels in Surk Samples, a Traditional Turkish Cheese from Southern Turkey

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Abstract In this study, aflatoxin M_1 (AFM_1) contamination was investigated in Surk cheese, a traditional Turkish cheese consumed particularly in southern Turkey. For this purpose, 120 Surk cheese samples were collected from different retail markets and analysed by enzyme-linked immunoassay. The level of AFM_1 varied from 16 to 1,043 ng/kg in 72 of the Surk samples (60%), 16 of which (13.3% of 120 samples) contained AFM_1 amounts exceeding the maximum tolerance limit (250 ng/kg) established in Turkey. The results indicated that the occurrence of AFM_1 in Surk cheese samples may be considered as a possible risk for consumer health.

Keywords Aflatoxin M_1 · Surk · Cheese · ELISA

Aflatoxins are mainly produced by moulds species, *Aspergillus flavus*, *A. parasiticus* and rarely *A. nomius* during growth on feeds and/or foods. Aflatoxin M_1 (AFM_1) is a hydroxylated metabolite of aflatoxin B_1 (AFB_1) and can be found in the milk of animals that are fed with AFB_1 contaminated feed. About 0.3%–6.2% of the ingested AFB_1 can pass into milk (Cathey et al. 1994; Creppy 2002; Moss 2002). AFM_1 is relatively stable in raw and processed milk products and cannot be inactivated by heat treatments like pasteurization during processing into cheese (Bakirci 2001; Govaris et al. 2001; Moss 2002). AFM_1 can also be present in cheese manufactured from contaminated milk because the toxin bind to the casein fraction (Galvano et al. 1996; Lopez et al. 2001).

Aflatoxins have potential toxic, carcinogenic, and/or teratogenic effects for human and animals. It is well established that the toxicity of AFM_1 is about one order of magnitude less than that of AFB_1. The International Agency for Research on Cancer (IARC) classified AFB_1 as a class 1 and AFM_1 as a class 2B (or probable) human carcinogen (Cathey et al. 1994; Galvano et al. 1996; Creppy 2002; Moss 2002). For this reason, the presence of AFM_1 in milk and milk products is a potential public health concern. The maximum acceptable level of AFM_1 in cheeses in some European countries including Switzerland, France, Austria (Kaniou-Grigoriadou et al. 2005; Pietri et al. 1997) and Turkey (Anonymous 2002) has been established as 250 ng/kg.

Surk cheese is a traditional dairy product and is one of the most commonly consumed cheese in the southern part of Turkey, particularly in Antakya (Antioch). Surk is manufactured from skim milk cheese (cokelek), made from diluted yoghurt (ayran) through boiling, which was then put under pressure in a cotton bag for 5–6 h to release its water content. Various spices including peppermint, thyme, cumin, black pepper, cinnamon, ginger, (0.1%–0.3% each), chili pepper (2%) and salt (5%) are added into skim milk cheese. Garlic can also be added into the cheese, at a ratio of 1%. The mixture is given a pear-like shape after

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kneading. These balls can be consumed either fresh or after storage for 20–25 days at ambient temperature for making mouldy. The moisture and salt contents and pH value of the mouldy Surk cheese samples have been reported to be 44.59%, 5.88% and 5.31%, respectively (Aygun et al. 2007). Currently, there is no study carried out on the occurrence of AFM_1 in mouldy Surk cheese. The aim of the present study was to evaluate the presence of AFM_1 in mouldy Surk cheese concerning public health.

Materials and Methods

One hundred-twenty mouldy Surk cheese samples (~200 g each) were randomly collected between June and November 2006 from different retail markets of Antioch. The samples were transported to the laboratory under refrigeration and kept at −20°C until being analyzed.

Both the preparation and the measurements of the AFM_1 quantity in Surk cheese samples were performed by enzyme linked immunoassay (EIA) using the RIDASCREEN test kit according to its instructions (Ridascreen, Aflatoxin M_1, Art. No.: R1101, R-Biopharm AG, Darmstadt, Germany). Two grams of the triturated Surk cheese were homogenized and then extracted with 40 mL dichloromethane for 15 min. After that, the suspension was filtered. Ten millilitres of the extract were evaporated at 60°C under a stream of nitrogen. The oily residue was dissolved in 0.5 mL methanol, 0.5 mL phosphate buffered solution (PBS-buffer: 0,55 g sodium dihydrogen phosphate hydrate, 2,85 g disodium hydrogen phosphate dihydrate and 9 g sodium chloride were filled up to 1000 mL with distilled water) and 1 mL *n*-heptane. After centrifugation for 15 min at 2700 rpm and 15°C, the upper heptane-layer was completely removed. Aliquot of the lower methanolic-water phase was carefully poured off using a Pasteur pipette and 100 µL of this aliquot was diluted by addition of 400 µL Ridascreen buffer 1. A 100 µL of this solution was used per well in the test. After the preparation of samples, 100 µL of the standard solutions or prepared sample were added to separate duplicate wells, mixed gently and then incubated for 60 min at room temperature in the dark. The liquid was removed from the wells and then the wells were washed twice with 250 µL of washing buffer. After that, 100 µL of the diluted enzyme conjugate (peroxidase conjugated AFM_1) was added, mixed gently and then incubated for 60 min at room temperature in the dark. The wells were again washed twice with 250 µL of washing buffer. Following that 50 µL of substrate (urea peroxidase) and 50 µL of chromogen (tetramethylbenzidine) were added to each well, they were mixed thoroughly and incubated for 30 min at room temperature in the dark. After that, 100 µL of the stop reagent (1 N H_2SO_4) was added to each well and mixed gently and the absorbance was then measured at 450 nm in ELISA reader (Organon Teknika). The evaluation of the samples were performed according to the Rida Soft Win computer program prepared by R-Biopharm. The lower detection limit is 50 ng/kg and the recovery rate in cheese is 102%.

The numerical results of chemical analysis were given as mean, minimum, maximum and standard deviations (SD) with n being number of samples, using SPSS software (SPSS for Windows, 9.05 program).

Results and Discussion

In this study, the presence of AFM_1 was investigated in a total of 120 mouldy Surk cheese samples. The analysis showed that 60% (72/120) of Surk cheese samples were positive in terms of AFM_1. The levels of AFM_1 in 16 (13.3%) of the 120 Surk cheese samples exceeded the maximum acceptable level (250 ng/kg) for Turkey (Anonymous 2002). The occurrence and the frequency distribution of AFM_1 concentrations in Surk cheese samples are presented in Table 1, which showed great variations among the samples.

A number of studies on the occurence of aflatoxins in cheeses have been reported from many countries including Turkey. In some studies carried out on various types cheeses from Turkey, 65% (121/186) of white cheese samples (Aycicek et al. 2002), 86.7% (52/60) of Van otlu (herb) cheese samples and 62% (31/50) of white pickle cheese samples (Tekinşen and Tekinşen 2005) and 5% (30/

Table 1 Occurrence of AFM_1 in 120 Surk cheese samples (ng/kg)

	AFM_1 contamination				Frequency distribution					
	Positive samples	>250[a]	Average[b] ± SD	Range[b]	<10[c]	10–50	51–150	151–250	251–800	800<
n	72	16	221.3±202.9	16–1,043	48	1	38	18	13	3
%	60	13.3	–	–	40	0.8	31.7	14.2	10.8	2.5

[a] Turkish tolerance limit

[b] Avarage/range of positive cheese samples

[c] Negative samples

600) of several types of cheeses samples (Yaroglu et al. 2005) have been found to be AFM_1-positive. The ratio of the samples exceeding the Turkish maximum acceptable level have been given as 19% (35/186) in white cheese samples (Aycicek et al. 2002), 80% (48/60) in Van otlu (herb) cheese samples, 40% (20/50) in white pickle cheese samples (Tekinşen and Tekinşen 2005) and 1% (6/600) in several types of cheese (Yaroglu et al. 2005). The ratios of AFM_1 positive cheese samples (60%) and those exceeding the Turkish maximum acceptable level (13.3%) in the current study were lower than those of white cheese samples (Aycicek et al. 2002), Van otlu (herb) cheese samples and white pickle cheese samples (Tekinşen and Tekinşen 2005), but they were higher than those of several types of cheese (Turkish white, kashar and cream cheeses) samples (Yaroglu et al. 2005). The incidence of AFM_1-positive samples (60%) in the present study was lower than those reported by Pietri et al. (1997) for Grana Padano cheese from Italy (98.2%) and Elgerbi et al. (2004) for white cheese from Libya (75%), but higher than those reported by Barrios et al. (1996) for cheeses from Spain (45.71%), De Sylos et al. (1996) for cheese from Brazil (0%) and Kaniou-Grigoriadou et al. (2005) for Feta cheese from Greece (0%).

According to the reports cited above, the presence of AFM_1 in cheeses appears to be much variable. It was emphasized that these variable AFM_1 concentrations in various cheeses may be the result of several factors such as the AFM_1 contamination of milk (Bakirci 2001; Galvano et al. 1996), manufacturing and ripening procedures (Govaris et al. 2001; Lopez et al. 2001), the chemical composition of the cheeses (Lopez et al. 2001), different analytical methods and seasons (Galvano et al. 1996). It was also principally mentioned that the animal feeds contaminated with aflatoxigenic moulds stored under unsuitable conditions could also result in high AFM_1 concentrations in milk and cheeses (Galvano et al. 1996; Elgerbi et al. 2004; Kaniou-Grigoriadou et al. 2005). In the current study, AFM_1 contamination of Surk cheese may also be possibly resulted from the milk containing AFM_1 obtained from milking animals fed on feed with AFB_1. Therefore, we suggest that feed of milking animals should be free from AFB_1 in order to minimize AFM_1 contamination in cheese. Good manufacturing practice and good storage practices can reduce concentration of AFB_1 in feed (Creppy 2002). It has to be noted that the AFM_1 is heat stabile, therefore cannot be reduced by heat treatments of milk (Bakirci 2001; Lopez et al. 2001) and it was not influenced by the cheese environment for long periods (Govaris et al. 2001). However, it is mentioned also that some lactic acid bacteria have a detoxificating effect on AFB_1 and AFM_1 (Pierides et al. 2000; Haskard et al. 2001).

The findings of the present study indicate that mouldy Surk cheese may be considered to be a risk for consumer health because AFM_1 concentrations of 13.3% of the samples (16/120) exceeded the maximum acceptable level (250 ng/kg) established in Turkey. To be cautious, feeds used for feeding milking animals should be kept away from fungal and/or AFB_1 contaminations particularly during storage and controlled periodically in terms of AFB_1 content. Moreover, milk and dairy products have to be inspected regularly for AFM_1 contamination. Additionally, further investigations are necessary to determine mould contamination and levels of other mycotoxins including AFB_1 to estimate safety of mouldy Surk cheese for human health.

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