Aflatoxins are generated by contamination of food and feed products by three *Aspergillus* spp.: *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (8). Aflatoxin B1, B2, G1 and G2 are known as the chief aflatoxins. Aflatoxins are acute toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic factors. Liver is the most affected organ by carcinogenic and toxic effects of aflatoxins (18). While *Aspergillus flavus* produces type B aflatoxins, other two produces both B and G types (27). Aflatoxin M1 (AFM1) and M2 (AFM2) are the hydroxylated metabolites of aflatoxin B1 and aflatoxin B2 (oxidative metabolic products). Aflatoxin B1 can easily transferred to the milk of the dairy animals within 24-48 hours following feeding of the animals with AFM1 contaminated fodder (22, 26). Aflatoxin M1 has also similar carcinogenic and toxic effect in comparison to AFB1 (15, 20).

Aflatoxin may not only exist in milk but also in milk products. Because of the toxin’s affinity to the casein it exists widely in cheese (12, 16). Several ratios on the amount of AFM1 transferred from milk to cheese had been reported and it is found that AFM1 level in cheese is directly related to the technology, type and the eliminated water during the process (5-7, 16, 17, 19, 23).

Numerous studies have been carried out on the AFM1 level in cheeses sold in Turkey and in the other country. In addition to being inconsistent, generally a high concentration of AFM1 level was determined (1, 2, 9, 12-14, 21, 24). The many milk products, produced in the other region (Edirne, Tekirdağ, Balıkesir, Bursa etc.), are usually marketed in Istanbul, the biggest city of Turkey. Hence it gives Istanbul to represent the overall of Turkey. The present study has been performed in order to determine the AFM1 levels in white cheese, processed cheese and kashar cheese in Istanbul.

**Material and methods**

A total number of 131 white, 132 processed, 100 kashar, totally 363 packaged cheese samples brought to No 1. Food Control and Analysis Laboratory between January 2002 and December 2004 were used as material.

The quantitative analysis of AFM1 in cheese samples was performed by competitive ELISA method (Ridascreen, AFM1 No: R1101, R-Biopharm AG, Germany). Preparation of samples and ELISA test procedure were performed according to the instructions of the test kit. Cheese samples (2 g each) were homogenized (Ultraturrax, Junke&Kunkel, Germany) and extracted with 40 ml dichloromethane. The suspension was filtered and 10 ml of the filtrate was evaporated under 60°C under nitrogen. The oily residue was redissolved in 0.5 ml methanol, 0.5 ml PBS buffer and 1 ml n-heptane and was mixed thoroughly. After centrifugation for 15 min. at 2700 rpm, 100 µl of the methanic phase was brought up to a 10% methanol concentration by addition of 400 µl Ridascreen buffer 1. An aliquot of this solution was used in the test (100 µl/well).
Aflatoxin M₃ standards or the prepared sample solutions were added to microtitre wells in duplicate. 100 µl standard solutions and prepared samples in separate wells were added and incubated for 60 min. at room temperature in the dark. The liquid was then removed completely from the wells, which were washed twice with distilled water (250 µl). Subsequently, enzyme substrate (urea peroxide, 50 µl) and chromogen (tetramethyl-benzidine, 50 µl) were added to each well and incubated for 30 min. at room temperature in the dark. Then the addition 100 µl of the stop reagent (1 M H₂SO₄) and the absorbance was measured at 450 nm in ELISA reader (ELX 800, Bio-tek Inst., USA).

The mean values of the absorbances for the standards and the samples were evaluated according to the Rida® Soft Win program (RIDAVIN.EXE) distributed by Rida-screen (R-Biopharm, Germany). The lower detection limit for AFM₃ is 0.05 µg/kg, average recovery ratio for cheeses is 102% and average variation factor is 11%.

**Results and discussion**

AFM₃ level in milk and milk products are important since many people use milk and dairy products in their diets frequently, especially babies and children need milk and dairy products. Maximum AFM₃ limits were regulated by both International (10) and local standards (25). The maximum acceptable limits are 0.05 µg/kg for milk and 0.25 µg/kg for cheese in Turkey (25).

Totally 363 cheese samples were analysed. AFM₃ levels were below the detectable level (0.05 µg/kg) in 23 (6.34%) of the samples, below the maximum acceptable limit (0.25 µg/kg) in 283 (77.96%) of the samples and between 0.26 and 4.10 µg/kg in the 80 (22.04%) of them (tab. 1). The products in unacceptable levels, ratio was the highest in white cheese with 25.19% and the lowest in processed cheese with 18.18%. Looking at years, in 2002 ratio of the AFM₃ contaminated cheese was the highest with 40.19%, then 18.18%. Looking at years, in 2002 ratio of the AFM₃ contaminated cheese was the highest with 40.19%, then 18.18%. The AFM₃ presence between 0.10-5.20 µg/kg in 62% of the samples was shown and 40% of the samples were found to have levels that exceed the maximum acceptable limit (0.25 µg/kg). In other study, Sarımehtemoglu et al. (21) examined white, kashar and processed cheeses, 100 from each, and detected AFM₃ in 82, 85 and 79 of these samples respectively. The 27 white cheeses, the 34 kashar and 25 processed cheeses were also under the maximum tolerable limit for AFM₃. Compared with our results, there seems to be a similarity in white cheese in terms of the ratio of the samples contaminated with AFM₃, more than limits, and a relatively high in kashar cheese and processed cheese. Ayciçek et al. (1) were analysed a number of 223 milk products sold in Ankara and found that 7 kashar cheese, 12 white cheese, totally 19 cheeses (8.52%) contained AFM₃ more maximum acceptable limits. Similarly, Yaroğlu et al. (28) analysed 600 kashar, white and processed cheeses taken from different regions of Turkey between 2001-2002. In 10 (5%) of the white cheese, 12 (6%) of the kashar cheese and 8 (4%) of the processed cheese AFM₃ was detected and only 2 (1%) of those were exceeded the Turkish Food Codex maximum acceptable limits. The results of the last two studies are quite different from Turkey. In present study 340 (93.66%) of the 363 samples were found to have levels that exceed the minimum detectable limits of 0.05 µg/kg and this AFM₃ positive sample rate was the highest among other studies. The results similar to ours in terms of ratio of the contaminated samples were obtained by Sarımehtemoglu et al. (21) with 79.85%. Ayciçek et al. (1), Gümüşen and Bıyıkyörük (13) and Sarımehtemoglu et al. (21) similar results with ours were found. On the other hand, Ayciçek et al. (1) and Yaroğlu et al. (28) found relatively lower ratios. Differences between these results may be from various factors such as regional (milk and indirectly cheeses of different regions contain different amount of AFM₃), seasonal (season is also an important factor affecting the AFM₃ content of milk) and analytical (use of different analytical methods) factors.

AFM₃ is known to be not considerably affected from heating processes during cheese production such as pasteurisation of milk and boiling of cheese (4). Consequently, pasteurisation of the milk as raw material of cheese provide insufficient

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<th>Tab. 1. The AFM₃ levels of detected cheese varieties</th>
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<td>AFM₃ levels</td>
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<td>≤ 0.25 mg/kg</td>
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<td>&gt; 0.25 mg/kg</td>
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security in terms of AFM₁. In our study processed cheese that is normally heated during its production had the lowest ratio of contamination exceeded the limit value with 18.18%, Kashar cheese whose curd is heat treated was second with 23% and the white cheese that has no heating process during its production has the highest ratio with 25.19%. However highest average AFM₁ level was detected in Kashar cheese with 30.38 µg/kg then in processed cheese and in white cheese with 28.91 and 24.64 µg/kg respectively. The fact that white cheese is less contaminated in terms of AFM₁ can be explained by the relatively high moisture and low solid content of white cheese.

Alteration of AFM₁ amount during ripening process of cheese have been studied by many researchers. Marshal et al. (17) reported during the storage of Karish cheese was a gradual decrease in AFM₁ level. On the other hand, Blanco et al. (5), Brackett and Marriott (6), Brackett and Marriott (7) and Galvano et al. (11) reported that it is stabilized during storage and ripening process in Brick, Limburger, Camembert, Tilsit, Cheddar, Gouda, Manchego, Parmesan and Mozzarella cheeses. Ripening period of white cheese is shorter than that of Kashar cheese. Although we don’t know the AFM₁ content of the milk used in cheese higher concentration of AFM₁ in kashar can be an evidence for the fact that ripening process has no decreasing effect on the AFM₁ content.

Another factor affecting the AFM₁ level in milk and milk products is the season. In winter AFM₁ levels in milk is higher than the summer’s level. It can be explained by the fact that animals are generally fed by dry fodder, potential source of Aflatoxin B₁, in winter and that is supported by green grass that doesn’t carry any aflatoxin in summer (3, 12, 14). In our study results of year 2002 shows a great difference with the results of the 2003 and 2004. In 2002 40.19%, in 2003 15.39% and in 2004 13.47% of the samples were found to contain more AFM₁ that exceed the maximum acceptable limits by Turkish Food Codex (25). Therefore it can be suggested that in 2002 as a raw material of cheese, milk contains relatively high concentrations of AFM₁ and it means that dairy animals had been fed by AFM₁ contaminated fodder more. It can be thought that the seasonal effects may have taken part in this difference.

In conclusion, this result suggests that AFM₁ levels in the cheese in Turkey were relatively high and it can threat the public health. In the other hand AFM₁ levels in samples analysed in Istanbul decrease year by year and this can be thought as a fortunate fact for the public health.

References


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