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Aflatoxin M_1 levels of some cheese varieties in Turkey

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Summary

The present study was undertaken to determine the aflatoxin M_1 (AFM_1) levels in various cheese samples in Istanbul. A total of 363 samples of cheese, 131 samples of white cheese, 132 samples of processed cheese, 100 samples of kashar cheese brought to the Istanbul Military Food Control Laboratory were analysed from 2002 to 2004. The quantitative detection of AFM_1 levels in the samples was performed by competitive enzyme linked immunoassay (ELISA) method. AFM_1 levels in 283 (77.96%) of 363 cheese samples were found to be lower than 0.25 µg/kg, determined as the maximum acceptable limit by the Turkish and International Standards and 80 (22.04%) of those were higher. The samples of unacceptable level rates were also quite different depending on the years. The highest rate (40.19%) was determined in 2002 and the rates in 2003 and 2004 were 15.39% and 13.49%, respectively. The results have indicated that high level of AFM_1 in cheese samples can provide a potential risk for consumer health, but the decreasing level of AFM_1 in the latest years may be evaluated as positive progress.

Keywords: AFM_1, cheese

Aflatoxins are generated by contamination of food and feed products by three *Aspergillus spp.*: *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (8). Aflatoxin B_1, B_2, G_1 and G_2 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 M_1 (AFM_1) and M_2 (AFM_2) are the hydroxylated metabolites of aflatoxin B_1 and aflatoxin B_2 (oxidative metabolic products). Aflatoxin B_1 can easily transferred to the milk of the dairy animals within 24-48 hours following feeding of the animals with AFM_1 contaminated fodder (22, 26). Aflatoxin M_1 has also similar carcinogenic and toxic effect in comparison to AFB_1 (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 AFM_1 transferred from milk to cheese had been reported and it is found that AFM_1 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 AFM_1 level in cheeses sold in Turkey and in the other country. In addition to being inconsistent, generally a high concentration of AFM_1 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 AFM_1 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 AFM_1 in cheese samples was performed by competitive ELISA using AFM_1 test kit (Ridascreen, AFM_1 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 methanolic 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 M_1 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 H_2SO_4) 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 Ridascreen (R-Biopharm, Germany). The lower detection limit for AFM_1 is 0.05 µg/kg, average recovery ratio for cheeses is 102% and average variation factor is 11%.

Results and discussion

AFM_1 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 AFM_1 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. AFM_1 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 AFM_1 contaminated cheese was the highest with 40.19%, then 15.39% and 13.49% in the following 2 years. In other studies in Turkey, different results from another were reported. Ayçiçek et al. (2), detected AFM_1 in 121 (65%) of the samples and similar to our research pointed out that 35 (19%) of the samples were found to exceed the Turkish Food Codex maximum acceptable limits. Dağoğlu et al. (9) reported AFM_1 presence in white cheese at a lower rate with 48%. They also reported that 16% of the samples contained 0-0.20 µg/kg, 8% contained 0.20-0.30 µg/kg, 16% contained 0.30--0.50 µg/kg and 4% contained > 0.50 µg/kg of AFM_1. AFM_1 was detected in 86 (68,8%) of the 126 kashar samples from Bursa and the 28 of the positive samples were found to exceed the Turkish Food Codex maximum acceptable limits (13). Similar AFM_1 rate (23%) was also determined in our study. Tekinsen and Tekinsen (24) analysed 50 samples of white pickled cheese and AFM_1 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, Sarımehmetoğlu et al. (21) examined white, kashar and processed cheeses, 100 from each, and detected AFM_1 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 AFM_1. Compared with our results, there seems to be a similarity in white cheese in terms of the ratio of the samples contaminated with AFM_1 more than limits, and a relatively high in kashar cheese and processed cheese. Ayçiçek 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 AFM_1 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 AFM_1 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 AFM_1 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 Sarımehmetoğlu et al. (21) with 79.85%. Ayçiçek et al. (1), Günşen and Büyükyörük (13) and Sarımehmetoğlu et al. (21) similar results with ours were found. On the other hand, Ayçiçek et al. (1) and Yaroğlu et al. (28) found relatively lower ratios. Differences between these results may be from various factors such as regional (milks and indirectly cheeses of different regions contain different amount of AFM_1), seasonal (season is also an important factor affecting the AFM_1 content of milk) and analytical (use of different analytical methods) factors.

Tab. 1. The AFM_1 levels of detected cheese varieties

AFM_1 levels	Total (n = 363)	White cheese (n = 131)	Processed cheese (n = 132)	Kashar cheese (n = 100)
≤ 0.25 mg/kg	283 (77.96%)	98 (74.81%)	108 (81.82%)	77 (77.00%)
> 0.25 mg/kg	80 (22.04%)	33 (25.19%)	24 (18.18%)	23 (23.00%)
0.26-0.50 µg/kg	50 (13.77%)	27 (20.61%)	9 (6.81%)	14 (14.00%)
0.51-1.00 µg/kg	14 (3.86%)	1 (0.76%)	8 (6.06%)	5 (5.00%)
1.01-4.10 µg/kg	16 (4.41%)	5 (3.81%)	7 (5.30%)	4 (4.00%)
Average	27.77 µg/kg	24.64 µg/kg	28.91 µg/kg	30.38 µg/kg

AFM_1 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 security in terms of AFM_1. 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 AFM_1 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 AFM_1 can be explained by the relatively high moisture and low solid content of white cheese.

Alteration of AFM_1 amount during ripening process of cheese have been studied by many researchers. Marshaly et al. (17) reported during the storage of Karish cheese was a gradual decrease in AFM_1 level. On the other hand, Blanco et al. (5), Brackett and Marth (6), Brackett and Marth (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 AFM_1 content of the milk used in cheese higher concentration of AFM_1 in kashar can be an evidence for the fact that ripening process has no decreasing effect on the AFM_1 content.

Another factor affecting the AFM_1 level in milk and milk products is the season. In winter AFM_1 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 B_1, 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 AFM_1 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 AFM_1 and it means that dairy animals had been fed by AFM_1 contaminated fodder more. It can be thought that the seasonal effects may have taken part in this difference.

In conclusion, this result suggests that AFM_1 levels in the cheese in Turkey were relatively high and it can threat the public health. In the other hand AFM_1 levels in samples analysed in Istanbul decrease year by year and this can be thought as a fortunate fact for the public health.

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