OCCURRENCE OF AFLATOXIN M₁ IN YOGURT SAMPLES FROM AFYONKARAHISAR, TURKEY

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Abstract

The present study was aimed to analyse the presence and levels of aflatoxin M₁ (AFM₁), in 177 yogurt samples consisting of 104 samples of ordinary yogurt, 21 of fruit yogurt, and 52 of strained (Torba) yogurt. The competitive ELISA method was used to determine the aflatoxin. The highest AFM₁ concentration was 150 ng/kg in strained yogurt, 100 ng/kg in ordinary yogurt as well as in fruit yogurt. The incidence of AFM₁ contamination in yogurt samples were 65.38% (68 samples) of all ordinary yogurt samples, 33.33% (7 samples) of all fruit yogurt samples, and 55.77% (29 samples) of all strained yogurt samples. In addition, 11.53% of ordinary yogurt (12 samples), 9.52% of fruit yogurt (2 samples), and 21.15% of strained yogurt (11 samples) had higher AFM₁ levels, than the acceptable levels for the Turkish Food Codex (50 ng/kg for yogurt). The results of this study imply that more emphasis should be given to the routine AFM₁ inspection of milk and dairy products in the Afyonkarahisar region. Furthermore, both farmers and dairy companies should be informed on the importance of AFM₁, and the consequences of the presence of the aflatoxin in dairy products.

Key words: yogurt, aflatoxin M₁, Turkey.

Aflatoxins (AF) are mycotoxins produced by certain fungi, especially Aspergillus flavus. They display strong carcinogenicity (1). Therefore, aflatoxins are dangerous food contaminants, and many countries have set stringent regulatory demands on the level of aflatoxins permitted in imported and traded commodities (1, 19). Human exposure to one of the aflatoxins - aflatoxin B₁ (AFB₁), may arise from direct consumption of contaminated commodities, and the milk of farm animals previously exposed to AFB₁ in their feed (5). The ingestion of AFB₁ by dairy cattle leads to the biotransformation of that substance, which is eliminated via milk as aflatoxin M₁ (AFM₁). AFM₁ Furthermore, it is relatively stable in raw and processed milk products, and is unaffected by pasteurization or processing into cheese. Thus, if raw milk contains AFM₁, yogurt made from such milk will also contain the aflatoxin (3, 14).

Mycotoxins, when present at high levels in the diet, cause acute and/or chronic adverse health effects in animals and humans. These compounds may affect many target organs and systems, notably the liver, kidneys, nervous system, endocrine system, immune system, and blood (4, 9, 11, 13, 16). There is a positive correlation between the incidence of primary liver cancer and average dietary concentration of AF. Since milk has the greatest demonstrated potential for introducing AF residues from foods of animal origin into the human diet, and is, also the main nutrient for infants and children, the occurrence of AFM₁ in commercially available milk and dairy products is of an equal concern. However, the percentage of consumption could be changed depending on the economic level of people. On the other hand, to produce good quality milk and dairy products, it is essential to keep feeds free from contamination by AFB₁. In Turkey, although the pasture is widely available depending on the season, the feeding of dairy cattle with commercial feedstuffs is more common, especially at large dairy farms. Therefore, it is important to determine not only AFM₁ levels in certain milk products, but also routine-monitoring surveys should be considered in this regard.

Considering all this information, the present study was undertaken to gain some data on the presence of AFM₁ in various types of yogurt samples, consumed in Afyonkarahisar, Turkey.

Material and Methods

A total of 177 yogurt samples, consisting of 104 samples of ordinary yogurt, 21 of fruit yogurt, and 52 of strained yogurt, were purchased at random from
different groceries, markets, and supermarkets in Afyonkarahisar, Turkey. The samples were analysed for the presence of AFM$_1$ with the competitive ELISA, using specific antibody coated microtitre strips (R-Biopharm GmbH) (2). The samples were analysed between March and July 2005.

The analyses of the samples were performed according to the following procedure: all yogurt samples were heated to 80°C for 3 min, in order to inactivate living yogurt bacteria and then pasteurized. Then, the samples were cooled down to a room temperature, and diluted in 1:10 in PBS-buffer (pH 7.2). Afterwards, they were homogenized by stirring. The diluted samples were used directly in the test (14). Microtitre wells were used for the standards, and the samples were inserted into the microwell holder; then 100 µl of a standard solution, and the prepared samples were added to separate wells and incubated for 60 min at room temperature in the dark. The liquid was poured out of the wells, and the microwell holder was tapped upside down vigorously (3 times in succession) against absorbent paper to ensure the complete removal of liquid from the wells. All the wells were filled with 250 µl of distilled water, and then the liquid was poured out again. The washing procedure was repeated once. A 100 µl volume of the enzyme conjugate was added and incubated for 60 min at room temperature in the dark. The washing sequence was repeated 3 times. A 50 µl volume of substrate and 50 µl of chromogen were added to each well, mixed thoroughly, and incubated for 30 min at room temperature in the dark. A 100 µl volume of the stop reagent were added to each well and mixed. The absorbance at 450 nm was measured against an air blank.

The mean values of the absorbance were obtained for the standards, and the samples were evaluated according to standard RIDAWIN.EXE programme, prepared by R-Biopharm.

## Results

As shown in Table 1, AFM$_1$ was found in 68 (65.38%) samples of ordinary yogurt, 7 (33.33%) samples of fruit yogurt, and in 29 (55.77%) samples of strained yogurt. Twenty (11.53%) samples of ordinary yogurt, 2 samples (9.52%) of fruit yogurt, and 11 (21.15%) samples of strained yogurt had higher AFM$_1$ levels than the acceptable levels of the Turkish Food Codex (50 ng/kg for yogurt).

### Discussion

Aflatoxins are highly toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic compounds. The main target organ for their toxicity and carcinogenicity is the liver. Milk and milk products, are a major nutrient for humans, especially children. For this reason, AFM$_1$ in milk and dairy products should be controlled systematically, because some previous studies reported high levels of AFM$_1$ in cheese samples (6, 7, 12, 14, 15, 17, 18).

Galvano et al. reported (8) that 80% of all yogurt samples in Italy were contaminated with AFM$_1$. To our knowledge, there is no study reporting the AFM$_1$ levels in yogurt samples in Afyonkarahisar region. In a previous study, Sarimehmetoglu et al. (14) reported that most of the yogurt samples (62.88%) purchased at different markets in Ankara was free of AFM$_1$. However, in our study the incidence of AFM$_1$ contamination in yogurt samples was quite high, since 65.38% of ordinary yogurt samples, 33.33% of fruit yogurt samples, and 55.77% of strained yogurt samples contained the aflatoxin. Such a high contamination was probably caused by only a few contaminated milk samples entering the bulk milk supply. This high contamination levels may be a serious problem for public health, since all these products are consumed by all age groups, including infants and children, consume these products worldwide. The occurrence of AFM$_1$ in strained yogurt was not as high as in ordinary yogurt. However, the concentration of AFM$_1$ in strained yogurt was remarkably higher than that in ordinary yogurt. Since AFM$_1$ is associated with casein, it may depend on the casein occurring in high concentrations in strained yogurt.

### Table 1

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Tested (n)</th>
<th>Not detected ng/kg. n (%)</th>
<th>Positive Samples ng/kg. n (%)</th>
<th>Turkish legal limit &gt;50 ng/kg. n (%)</th>
<th>1-30 ng/kg. n (%)</th>
<th>31-50 ng/kg. n (%)</th>
<th>51-100 ng/kg. n (%)</th>
<th>101-150 ng/kg. n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordinary yogurt</td>
<td>104</td>
<td>36 (34.62)</td>
<td>68 (65.38)</td>
<td>12 (11.53)</td>
<td>40 (38.46)</td>
<td>16 (15.38)</td>
<td>12 (11.54)</td>
<td>-</td>
</tr>
<tr>
<td>Fruit yogurt</td>
<td>21</td>
<td>14 (66.66)</td>
<td>7 (33.33)</td>
<td>2 (9.52)</td>
<td>3 (14.29)</td>
<td>2 (9.52)</td>
<td>2 (9.52)</td>
<td>-</td>
</tr>
<tr>
<td>Strained yogurt</td>
<td>52</td>
<td>23 (44.23)</td>
<td>29 (55.77)</td>
<td>11 (21.15)</td>
<td>12 (23.08)</td>
<td>6 (11.54)</td>
<td>6 (11.54)</td>
<td>5 (9.61)</td>
</tr>
</tbody>
</table>
There is currently not known procedure for destroying AFM₃ in milk without destroying the milk. For all practical purposes, AFM₃ is stable in unprocessed milk and processed milk products, and is unaffected by pasteurization or processing of milk into cheese or yogurt. For this reason, milk and dairy products have to be inspected continuously for AFM₃ contamination, at least twice a year. On the other hand, to produce milk of good quality, it is essential to keep feeds free from contamination by AFB₁. In Turkey, although the pasture is widely available depending on seasons, feeding dairy cattle with commercial feedstuffs is more common, especially at large dairy farms. Therefore, it is important to inform producers and consumers about the toxicity potential of aflatoxins, in order to reduce their potential health risk and economic loss. In this regard, organization of official training programmes should be considered by the government. Furthermore, increasing intake of antioxidants and vitamins with the diet, in order to prevent carcinogenesis should be involved in the prevention strategies. Researchers aim to develop effective prevention management and decontamination technologies to minimize the toxic effects of AF (4, 9, 10).

In conclusion, the results of this study imply that more emphasis should be given to the routine AFM₃ inspection of milk and dairy products in Turkey. Furthermore, governmental agencies need to inform both farmers and dairy companies about the importance of AFB₁ and AFM₃, and the consequences of the presence of AFM₃ in products produced by them. Since there is not enough study done in Turkey, regarding the AFM₃ contamination of yogurt, further studies need to be done.

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References