

DETERMINATION OF AFLATOXIN M₁ LEVELS IN CHEESE SOLD IN İZMIT PROVINCE

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Summary

This study was conducted to determine the the AFM₁ levels of Turkish white and kashar cheese purchased from different markets and sellers in İzmit province. AFM₁ was determined by ELISA technique in total of 185 cheese samples including 102 Turkish white and 83 kashar cheese. The aflatoxin clearance was carried out with r-biofarm Rida®Aflatoxin (Art.No. R5001/R5002) immunoaffinity column, AFM₁ analyses were performed with r- biofarm Rida®Aflatoxin M1 test kits (Art.No 1101). Of the total of 185 cheese samples, 123 (66.5%) were found AFM₁ positive. 75 (73.5%) of positive samples were white cheese samples while 48 (%57.8) of which were kashar cheese. The mean AFM₁ levels were 189.47 ng/kg and 229.79 ng/kg for white and kashar cheese respectively. 16 samples belonging to each of white and kashar cheese, exceeded the corresponding maximum permissible limit (250 ng/kg) laid down in Turkish Food Codex.

Key words: Aflatoxin, cheese, ELISA

Human, has long been using some moulds for ripening their foods. However several researchs are reported concerning the health risks of some mould species. It is well known that the moulds cause significant health risks by their metabolites. These metabolites produced by moulds are colled as mycotoxins (Şanlı, 1989). Moulds affect the human health by two different ways: the disease occurs by direct contact is colled mixis and the disease come out as intoxication with mycotoxins are colled mycotoxicosis. The most hazardous mycotoxins are known be the aflatoxins (Howel, 1982; Clarke et al, 1985). Aflatoxins are commonly found in all type of stored feed, feeding materials and food and are toxic metabolites mainly produced by toxigenic *Aspergillus flavus*, *Aspergillus*, *Penicillium* and *Rhizopus spp.* (Kaya et al, 1985). The major aflatoxins of concern are designated B₁, B₂, G₁ and G₂. Among these, AFB₁ is the most toxic metabolite. Aflatoxins M₁ (AFM₁) is monohydroxi derivate of AFB₁ produced by animals and it is secreted into milk from mammary glands by dairy cattle that have consumed AFB₁contaminated feed (De Longh et al, 1964). Aflatoxins are effective by their epoxide derivates formed as a result of some metabolic alterations mediated by

liver microsomal enzymes (Kaya, 1989). Aflatoxins are actually acute toxic, carcinogenic, teratogenic, mutagenic, immunosuppressive compounds. The main target organ for the toxicity and the carcinogenicity is liver (Smith, 1997; Peraica et al, 1999).

AFM₁ is a significant public health problem to which human exposure can arise from consumption of milk and milk products. The excessive consumption of milk and milk products especially by the teenagers increases the significance of this problem. AFM₁ is relatively stable in milk and is unaffected by pasteurization process. Therefore the main target to obtain AFM₁ negative milk and milk products must be to avoid the ingestion of contaminated feed by dairy cattle as well as to avoid the exposure of end products with aflatoxins (Özkaya et al, 2002). In our country, the cheese, manufactured by small plants under poor hygienic conditions without any supervision are highly risky foods for their aflatoxin contents. This situation not only causes the lack of quality for the product but also causes potential risk for public health. The cheese consumption, especially white and kashar cheeses is widespread in Turkey (Tekinşen, 2000). Limited research is available concerning the aflatoxin levels and indicating the preventive measures in Turkey.

This study was conducted to determine the AFM₁ levels of white and kashar cheeses purchased from different markets and sellers in İzmit province.

Materials and methods

A total of 185 cheese samples, 102 white cheese and 83 kashar cheese were collected randomly from different markets and sellers in İzmit between November 2004- July 2005, and were used as materials in this study. The collected cheese samples were transferred to the laboratory in ice boxes at temperatures of 6 and 10°C.

ELISA technique was used to detect the AFM₁ levels of white and kashar cheeses. Immunoaffinity columns (r-biofarm Rida®Aflatoxin column Art.No. R5001/R5002) were used for samples clean up prior to the analyses. AFM₁ analyses were performed with r-biofarm Rida®Aflatoxin M₁ test kits (Art.No 1101). The samples were measured at an absorbance of 450 nm and the detection limit was < 100 ng/kg (R-Biopharm: Rida Aflatoxin Column, 2005; R-Biopharm: Ridascreen Aflatoxin M₁, 2005).

Results and discussions

The numbers of AFM₁ positive cheese samples, the maximum, minimum and the average values of AFM₁ levels are shown in Table 1. AFM₁ levels and the incidence of positive samples are given in Table 2. The comparison of the results with the Turkish legal limits are presented in Table 3.

Table 1
AFM₁ positive cheese samples, the maximum, minimum and the average values of AFM₁ levels in white and kashar cheeses (ng/kg)

Samples	N	n (%)	Minimum	Maximum	Mean
White cheese	102	75 (73.5)	12.31	760.45	189.47±13.56
Kashar cheese	83	48 (57.8)	14.25	675.93	229.79±17.24

N : Number of total samples

n : Number of AFM₁ positive samples

Table 2
Incidence and levels of AFM₁ in cheese samples (ng/kg)

Samples	Aflatoxin M ₁ levels				Total
	0-250 n (%)	250-500 n (%)	500-750 n (%)	750-100 n (%)	
White cheese	59 (78.7)	11 (14.7)	4 (5.3)	1 (1.3)	75
Kashar cheese	32 (66.7)	10 (20.8)	6 (12.5)	-	48

Table 3
The comparison of AFM₁ levels of analysed white and kashar cheese with the Turkish legal limits

Samples	n	n ₁ (%)	n ₂ (%)
White cheese	75	59 (78.7)	16 (21.3)
Kashar cheese	48	32 (66.7)	16 (33.3)

n : AFM₁ positive samples

n₁: Samples below the limits of Turkish Food Codex (250 ng/kg)

n₂: Samples exceeding the limits of Turkish Food Codex (250 ng/kg)

Of the total of 185 white and kashar cheese samples, 123 (66.5%) were found positive for AFM₁. Distribution of positive samples by cheese types and the AFM₁ levels are shown in table 1 and 2 respectively. As seen in Table 3, 16 (21.3 %) of the 75 AFM₁ positive white cheese and 16 (33.3%) of the 48 AFM₁ positive kashar cheese samples exceeded the corresponding permissible limits (250 ng/kg) established by Turkish Food Codex (Anonymus, 2002).

Günşen and Böyükyörük (Günşen and Böyükyörük, 2003), detected AFM₁ contamination from 86 (68.8%) of 125 kashar cheese samples at an average of 206.23 ng/kg. However Oruç and Sonal (Oruç and Sonal, 2001) found the average AFM₁ content of kashar cheese consumed in Bursa as 16 ng/kg. Alkan et al. (Alkan et al., 2006), reported that the average AFM₁ levels of white cheese sold in Amasya province was found to be 115.53 ng/kg. Ayçiçek et al., (Ayçiçek et al., 2002), stated that AFM₁ levels in 19% of 186 white cheese samples were higher than the maximum acceptable limits of Turkish Food Codex. Gürses et al., (Gürses et al., 2004) expressed that non of 63 cheese samples exceeded the concerning legal limit. Dağoğlu et al., (Dağoğlu et al, 1995) reported that 45.2% of 75 cheese samples contained AFM₁ levels at an average of 273 ng/kg, whereas Hisada et al.

(Hisada et al., 1984) stated that 53% of 276 cheese samples had 100-430 ng/kg of average AFM₁ concentrations.

The highest AFM₁ level in the studies conducted in some European countries (Finoli and Vecchio, 2003; Prado et al, 2000; Sharman et al, 1989), is found to be 80 ng/kg. This value is below the legal limits set by European countries. However in some other researchs (Galvano et al, 1998; Piva et al, 1988; Peitri et al, 1997) on the AFM₁ levels of milk and milk products in European countries, limited numbers of samples are reported to exceede the legal permissible limits.

The results obtained in this study match well with the results of Günşen and Böyükhörük, 2003. However our results concerning the mean concentrations and the number of samples exceeding the acceptable limits are found to be higher than some studies (Alkan et al., 2006; Oruç and Sonal, 2001; Ayçiçeği et al.,2002; Gürses et al., 2004) but lower than the others (Dağoğlu et al., 1995; Hisada et al., 1984). The AFM₁ levels of cheeses found in this study are higher than the studies carried out in European countries (Finoli and Vecchio, 2003; Prado et al, 2000; Sharman et al, 1989; Galvano et al, 1998; Piva et al, 1988; Peitri et al, 1997) .

Conclusions

In conclusion, the results obtained in this study indicate that the white and kashar cheeses sold in this province could provide a potential risk for consumer health in respect to their AFM₁ content. To prevent aflatoxicosis in human consuming milk and milk products, initial approach must be to control the AFB₁ contamination of animal feed. Hygienic conditions need to be improved in cheese manufacture and storage conditions. Widespread and continuous training and surveillance programs must be arranged for both the producers and consumers.

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