

Aflatoxin M₁ Contamination of Ice Cream in Samsun, Turkey

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Abstract: This study was undertaken to determine the presence and levels of Aflatoxin M₁ (AFM₁) in ice cream samples consumed in the province of Samsun. For this purpose, a total of 115 samples comprising 25 vanilla ice creams, 65 fruit ice creams and 25 chocolate ice creams were used as the study materials. The ice cream samples were purchased randomly from 11 different markets. The samples were analysed by the Enzyme Linked Immunosorbent Assay (ELISA) method. Aflatoxin M₁ was detected in 30 (26.08%) of the ice cream samples, ranging from 6.12-32.15 ng kg⁻¹ whereas 85 samples (73.92%) did not reveal the presence of this toxin. The AFM₁ levels were not higher than the limits of the Turkish Food Codex (50 ng kg⁻¹ for ice cream) in all of the ice cream samples. It was concluded that the aflatoxins contained in some of the investigated samples were potential risks for public health. Therefore, milk which is the basic ingredient of ice cream must be continuously monitored for AFM₁ contamination. Furthermore, dairy cow feed should be stored in such a way that they do not become contaminated.

Key words: Aflatoxin M₁, ELISA, ice cream, public health, fruit, milk, Turkey

INTRODUCTION

Aflatoxins, a class of mycotoxins are generally produced by some competent mould strains of *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*. There are four main aflatoxin compounds found in agriculture: B₁, B₂, G₁ and G₂. Aflatoxins B₁ and B₂ are metabolized into M₁ and M₂ derivatives in the liver. Aflatoxin B₁ (AFB₁) is the most powerful natural carcinogen in mammals (Sweeney and Dobson, 1998; Aycicek *et al.*, 2005; Hussain *et al.*, 2008). The International Agency for Research on Cancer of the WHO classified AFB₁ as a group 1 human carcinogen. Also, AFB₁ and Aflatoxin M₁ (AFM₁) share first and second place in toxicity classification, respectively (IARC, 1993).

Aflatoxin M₁ is the main monohydroxylate derivative of AFB₁, formed in the liver by means of cytochrome P450-associated enzymes (Zinedine *et al.*, 2007). There is sufficient evidence to show that AFM₁ is a genotoxic carcinogen and that it is less toxic than AFB₁ (Creepy, 2002). Aflatoxin B₁ is easy to feed to farm animals and 85-90% of this toxin is excreted in the faeces and urine in the first 24 h after ingestion. When animal's consume feed stuff contaminated with AFB₁, the toxin is metabolized in

the liver and the first 12-24 h after excreted as AFM₁ in their milk. Aflatoxin M₁ is metabolized in the liver into the body of the AFB₁ and ingestion of milk begins to be taken along with feeds. A direct relationship has been observed between the amount of AFM₁ in milk and AFB₁ consumption via feedstuffs. The conversion rate of ingested AFB₁ into AFM₁ is highly variable ranging from 0.3-6.2% (Creepy, 2002; Yiannikouris and Jonany, 2002; Var and Kabak, 2009).

Moreover, as milk is the main nutrient for young children whose vulnerability is noteworthy and potentially more sensitive than that of adults, the occurrence of AFM₁ in human breast milk, commercially available milk and milk products is one of the more serious problems in food hygiene (Galvano *et al.*, 1996). Therefore, milk is always a potential risk factor for developing children in terms of AFM₁ (Kim *et al.*, 2000). In addition to its direct consumption, milk is also indirectly consumed as cheese, ice-cream and other milk products. Ice cream consumption in Turkey has increased even though the regular drinking of milk by adults is uncommon.

Aflatoxin M₁ cannot be inactivated by the heat processing used in the dairy industry i.e., pasteurization or sterilization (Prandini *et al.*, 2009). Evidence of a

potentially hazardous human exposure to AFM₁ through the consumption of milk and milk derivatives has been documented in several studies (Kamber, 2005; Tekinsen and Ucar, 2008; Ghanem and Orfi, 2009; Fallah, 2010). In recent years, different levels of AFM₁ in milk and milk products (cheese, butter and yoghurt) have been reported in Turkey (Gurbuz *et al.*, 1999; Bakirci, 2001; Oruc and Sonal, 2001; Aycicek *et al.*, 2002; Aycicek *et al.*, 2005; Celik *et al.*, 2005; Baskaya *et al.*, 2006; Unusan, 2006) as well as around the world (Stoloff *et al.*, 1981; Karaioannoglu *et al.*, 1989; Galvano *et al.*, 1996; Barrios *et al.*, 1997; Saitanu, 1997; Kim *et al.*, 2000; Lopez *et al.*, 2003; Martins and Martins, 2004; Kamkar, 2008). However, there are very few studies documenting the levels of AFM₁ in ice cream. Atanda *et al.* (2007) reported that out of six samples of ice cream in Nigeria, one (16.7%) contained AFM₁ with a level of 2.23 µg L⁻¹. Fallah (2010) reported that out of 36 samples of ice cream in Iran, 25 (69.4%) contained AFM₁ with levels ranging from 15-132 ng kg⁻¹. In another study, Mutlu *et al.* (2010) reported that out of the 16 ice cream samples analysed in Turkey, 6 (37.5%) samples were contaminated with AFM₁ at a mean level of 6.06 ng kg⁻¹.

Turkish laws regarding the Maximum Residue Level (MRL) for aflatoxins are harmonized with the European Legislation (EC, 2006). The maximum permitted amount of AFM₁ in milk and ice cream is 0.05 µg kg⁻¹ (50 ng L⁻¹).

Very few data have been published on the occurrence of AFM₁ in ice cream in Turkey in the scientific literature. Therefore, the aim of this study was to determine the occurrence of AFM₁ in popular open ice cream samples offered for sale in Turkey.

MATERIALS AND METHODS

Materials: In the period between May and October 2009, 120 open ice cream samples (25 vanilla ice creams, 65 fruit ice creams and 25 chocolate ice creams) were analysed. The samples were collected from 11 different small patisseries in Samsun. The ice cream samples (approximately 200 g) were taken and transferred under refrigeration to the laboratory where they were analysed.

Methods: The samples were analysed for AFM₁ using the competitive ELISA (RIDASCREEN Aflatoxin M₁, R-Biopharm, Germany, Product No: RI 101) procedure as indicated by R-Biopharm GmbH (Biopharm, 2006).

Preparation of the ice cream samples: Preparation of the samples was conducted according to the instructions of the RIDASCREEN test kit. The samples were diluted by half and centrifuged at

3500 g at 10°C for 10 min in order to separate the fat. Finally, the fatty layer on top was removed and the rest was acquired by a pasteur pipet and used for analysis.

Test procedure: The AFM₁ standards and the blanks and ice cream samples were analysed in duplicate. They were placed onto microlitre plates coated with antibodies for AFM₁, mixed gently and then incubated for 30 min at room temperature in the dark. At the end of incubation, the liquid in the wells was poured out and the microwell holder was tapped upside down onto a Whatmann paper to remove the remainder of the liquid. The wells were washed twice with 250 µL washing buffer. After washing, 100 µL of the conjugated enzyme was added to the microwells and these were incubated in the dark at room temperature for 15 min. After this procedure, the wells were washed 3 times with 250 µL washing buffer. Then, 50 µL of the enzyme substrate and 50 µL of the chromogene were added to each well and incubated for 15 min at room temperature in the dark. Finally, 100 µL of the stop reagent was added to each well and the absorbance was measured at 450 nm on an ELISA reader (International Immuno-Diagnostics, Foster city, CA, USA, TKA-544).

The detection limit of the ELISA kit was 5 ng L⁻¹. The levels of AFM₁ in the samples were determined by data analysis using RIDA SOFT WIN software (r-Biopharm AG, Germany) and interpolation from the standard curve obtained by analysing five internal standards, ranging from 5-80 ng L⁻¹.

RESULTS

In this study a total of 115 ice cream samples including 25 vanilla ice creams, 65 fruit ice creams and 25 chocolate ice creams were analysed for the presence of AFM₁ using competitive ELISA. The results are shown in Table 1 and 2.

Aflatoxin M₁ was detected in 30 (26.08%) of the ice cream samples, ranging from 6.12-32.15 ng kg⁻¹ whereas 85 samples (73.92%) did not reveal the presence of this toxin. Out of the 25 vanilla ice creams tested, one sample (4%) was contaminated with 5-10 ng kg⁻¹; two samples (8%) were contaminated with 11-20 ng kg⁻¹; two samples (8%) were contaminated with 21-30 ng kg⁻¹ and one sample (4%) was contaminated with 31-50 ng kg⁻¹ of AFM₁. Out of the 65 fruit ice creams tested, five samples (7.69%) were contaminated with 5-10 ng kg⁻¹; eight samples (12.30%) were contaminated with 11-20 ng kg⁻¹ and two samples (8%) were contaminated with 21-30 ng kg⁻¹ of AFM₁. Out of the 25 chocolate ice creams

Table 1: Occurrence of AFM₁ (ng kg⁻¹) in ice cream samples

Type of ice cream	No. positive/tests (%)	No. and percentage of samples with AFM ₁ in the ng kg ⁻¹ range				
		<5	5-10	11-20	21-30	31-50
Vanilla	6/25 (24.00)	19 (76.00)	1 (4.00)	2 (8.00)	2 (8.00)	1 (4.00)
Fruit	15/65 (23.07)	50 (76.93)	5 (7.69)	8 (12.30)	2 (3.08)	-
Chocolate	9/25 (36.00)	16 (64.00)	4 (16.00)	4 (16.00)	1 (4.00)	-
Total	30/115 (26.08)	85 (73.92)	10 (8.69)	14 (12.18)	5 (4.35)	1 (0.86)

Table 2: Average aflatoxin M₁ (ng kg⁻¹) contents in ice cream: statistical data

Type of ice cream (No. of samples)	Aflatoxin M ₁		
	Positive	Range ^a	Mean ^b
Vanilla (25)	6	6.67-32.15	19.65±8.91
Fruit (65)	9	6.12-22.38	13.97±5.33
Chocolate (25)	15	6.13-22.18	12.51±5.29
Total (115)	30	6.12-32.15	14.67±6.48

^amin-max; ^bmean of positive samples±SD (Standard Deviation)

tested, four samples (16%) were contaminated with 5-10 ng kg⁻¹; four samples (16%) were contaminated with 11-20 ng kg⁻¹ and one sample (4%) was contaminated with 21-30 ng kg⁻¹ of AFM₁ (Table 1 and 2). The levels of AFM₁ found were not higher than the limits of the Turkish Food Codex (50 ng kg⁻¹ for ice cream) in all of the ice cream samples.

DISCUSSION

In related studies, Atanda *et al.* (2007) reported that out of six samples of ice cream in Nigeria, one (16.7%) contained AFM₁ with a level of 2.23 µg L⁻¹. Fallah (2010) reported that out of 36 samples of ice cream in Iran, 25 (69.4%) contained AFM₁ with levels ranging from 15-132 ng kg⁻¹. In another study, Mutlu *et al.* (2010) reported that out of the 16 ice cream samples analysed in Turkey, 6 (37.5%) samples were contaminated with AFM₁ at a mean level of 6.06 ng kg⁻¹. Compared with the findings, those of Atanda *et al.* (2007) and Fallah (2010) are higher while the findings of the Mutlu *et al.* (2010) are in parallel. The variations in these results may be partly explained by several reasons such as the different numbers of samples, the different levels of milk contamination, the different types of ice cream and the types of analytical methods employed (Wiseman and Marth, 1983; Galvano *et al.*, 1996).

The maximum permissible level for AFM₁ in milk is 50 ng L⁻¹ although, it has been proposed that infant formulae and follow-on formulae, including infant milk and dietary foods for special medical purposes intended for infants and young children should not exceed 25 ng kg⁻¹ (Creepy, 2002). In the present study, levels of AFM₁ over 25 ng kg⁻¹ were detected in two (8%) vanilla ice cream samples.

CONCLUSION

The results of the present study indicated that the levels of AFM₁ in ice cream consumed in Turkey were not higher than the limits of the Turkish Food Codex but they could be a potential hazard for all age groups particularly infants and children. For this reason, milk and milk products must be continuously monitored for the presence of AFM₁ contamination. It is also extremely important to deal with this problem by reducing AFB₁ concentration in animal feed by improving processing and storage practices. For this reason, storage conditions of feeds should be improved and animal feeds should be regularly monitoring for AFB₁. Finally, milk and dairy products containing high levels of AFM₁ must be prohibited for human consumption.

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