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Short communication

Occurrence of aflatoxin M_1 in white cheese samples from Tehran, Iran

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ABSTRACT

This study was undertaken to determine the occurrence of aflatoxin M_1 (AFM_1) in 50 white cheese samples from 2 dairy factories in summer 2008 and winter 2009. Enzyme-linked immunosorbent assay (ELISA) method was used for analysis of the samples. Aflatoxin M_1 was found in 60% of the cheese samples, ranging from 40.9 to 374 ng/kg. Toxin levels in 6% of the samples exceeded the Iranian national standard limit i.e. 200 ng/kg. Considering seasonal variability, mean concentration of AFM_1 in the samples collected in winter was significantly ($P < 0.03$) higher than those collected in summer. Therefore, high occurrence of AFM_1 in cheese samples could be a potential hazard for public health.

1. Introduction

Mycotoxins are secondary toxic metabolites of fungi produced in various agricultural products under appropriate conditions of temperature and humidity (Zinedine & Manes, 2009). Aflatoxins are the best known and most intensively researched mycotoxins world-wide (Mozaffari Nejad, 2008). They are produced by *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus bombycis*, *Aspergillus ochraceoroseus*, *Aspergillus nomius*, and *Aspergillus pseudotamari* (Bennet & Klich, 2003; Cheraghali et al., 2007). Presence of aflatoxins in food and feed is of a great concern due to their detrimental effects such as liver damage, liver cirrhosis, tumor induction, mutagenic and immunosuppressive effects (Zinedine & Manes, 2009).

Four common types of aflatoxins are B1, B2, G1 and G2. Among them, aflatoxin B1 (AFB1) is notoriously the most common produced mycotoxin; and has been reported to be the most powerful natural carcinogen in mammals (Creppy, 2002). Aflatoxin M_1 (AFM_1) is the hydroxylated metabolite of AFB_1 found in milk of lactating animals which consumed AFB_1 contaminated feed (Fallah, 2010b; Kamkar, 2008b). The International Agency for Research on Cancer (IARC) of World Health Organization (WHO) included AFB_1 as primary and AFM_1 as secondary groups of carcinogenic compounds (Kamkar, 2008a; Riazipour, Tavakkoli, Razzaghi Abyane, Raf'ati, & Sadr Momtaz, 2010; Unusan, 2006). Aflatoxin M_1 could be detected in milk 12–24 h after the first ingestion of aflatoxin B_1. When the intake of the contaminated source is stopped, concentration of the toxin in milk decreases to an undetectable level within 72 h (Gurbay, Aydin, Girgin, Engin, & Sahin, 2006).

Many countries have carried out studies about the occurrence of AFM_1 in milk and dairy products and proposed permissible levels for this mycotoxin (Creppy, 2002; Kamkar, 2005). The US Food and Drug Administration (US FDA, 1996, p. 219) has established a maximum admissible level of 500 ng/l for AFM_1 in milk. However, the Institute of Standards and Industrial Research of Iran (ISIRI, 2002) has accepted 50 ng/l as the action level for AFM_1 which is the same as European Commission permitted level (European Commission, 2001). In addition, according to ISIRI (2002), the maximum admissible level of AFM_1 is 200 ng/kg in cheese.

The most common analytical techniques for determination of AFM_1 in dairy products are thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). ELISA is the most useful technique

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due to its rapidity, sensitivity, ease of application and cheapness. The method has been included in official collection of test procedures by the German Federal Board of Health (Kamkar, 2006; Unusan, 2006).

The aim of this study was to investigate the occurrence of aflatoxin M_1 in cheese samples produced by two factories that provide Tehran University dairy needs.

2. Materials and methods

2.1. Sample collection

During winter 2008 and summer 2009, a total of 50 samples of white cheese produced by two dairy factories were randomly obtained from Tehran University. The samples were transported to the laboratory inside an insulated container at about 4 °C and analyzed upon arrival.

2.2. Methods

The quantitative analysis of AFM_1 in the samples was based on competitive enzyme immunoassay using RIDASCREEN® Aflatoxin M_1 30/15 (Art.No.: R1111, R-Biopharm, Germany) test kit. Preparation of the cheese samples and ELISA test procedure were performed according to the method described by R-Biopharm GmbH (2007). Statistical analysis was performed by INSTATA software.

3. Results

The incidence and levels of AFM_1 in white cheese samples consumed in Tehran University are presented in Table 1. Aflatoxin M_1 was found above detectable level in 60% (30/50) of the analyzed samples, ranging from 40.9 to 374 ng/kg. Levels of the toxin in 3 (6%) cheese samples exceeded the Iranian national standard limit i.e. 200 ng/kg. Regarding seasonal effect influences, mean concentration of AFM_1 in the samples collected in winter was significantly ($P < 0.03$) higher than those obtained in summer.

4. Discussion

Cheese is the most potent source of aflatoxins among the dairy products because AFM_1 is associated with the casein fraction in milk is somewhat concentrated in cheese. Studies showed that the concentration of AFM_1 is about 3 fold higher in many soft cheeses and about 5 fold higher in hard cheeses than in milk from which the cheese is made (Ardic, Karakaya, Atasever, & Adiguzel, 2009).

Our findings revealed a high incidence of AFM_1 in cheese samples. This indicates that the milk used in production of these products has been obtained from animals fed with AFB_1 contaminated rations. In a previous study, Kaniou-Grigoriadou, Eleftheriadou, Mouratidou, and Katikou (2005) reported the absence of AFM_1 at detectable level in Feta cheese samples; but in a recent study, Fallah (2010a) determined AFM_1 in 81.9% of white cheese samples.

As can be seen in Table 2, several researchers (Ardic et al., 2009; Elgerbi, Aidoo, Candlish, & Tester, 2004; Fallah, 2010a; Fallah, Jafari, Fallah, & Rahnama, 2009; Fallah, Rahnama, Jafari, & Saei-Dehkordi, 2011; Kamkar, 2006; Prado et al., 2000; Rahimi, Karim, & Shakerian, 2009; Seyrek, 2001; Tekinsen & Ucar, 2008) reported the occurrence of AFM_1 in various cheese types. The reported AFM_1 contamination levels vary from one study to another. These alterations may be attributable to the different factors such as variety of cheese studied, cheese-making procedures, geographical area, analytical method employed and degree of milk contamination according to seasonal changes (Fallah et al., 2009). It is demonstrated that the milk produced during hot seasons was less contaminated with AFM_1 than the milk produced during cold ones (Fallah et al., 2011).

Table 1
Occurrence of aflatoxin M_1 in white cheese samples in winter and summer.

Season	Sample tested, *n*	Positive samples, *n* (%)	Min–max (ng/kg)	Mean ± SD (ng/kg)	Exceed regulation,[b] *n* (%)
Winter	25	20 (80)	55.4–374.0	82.77 ± 79.16[a]	2 (8)
Summer	25	10 (40)	40.9–215.1	24.015 ± 24.65[a]	1 (4)
Total	50	30 (60)	40.9–374.0	53.39 ± 51.90	3(6)

[a] Means ± SD with different letters are significantly different between winter and summer ($P < 0.03$).
[b] The ISIRI limit for AFM_1 is 200 ng/kg for cheese.

Table 2
Incidence and levels of aflatoxin M_1 in various cheeses reported in previous studies.

References	Country	Cheese variety	No. of samples	Positive (%)[a]	Range (ng/kg)
Prado et al. (2000)	Brazil	Commercial	75	74.6	20–6920
Seyrek (2001)	Turkey	White brine	110	91.8	10–2000
Elgerbi et al. (2004)	Libya	White soft	20	75	110–520
Kaniou-Grigoriadou et al. (2005)	Greece	Feta cheese	54	0	–
Kamkar (2006)	Iran	Feta cheese	80	82.5	150–2410
Tekinsen and Ucar (2008)	Turkey	Cream cheese	100	99	0–4100
Ardic et al. (2009)	Turkey	White brine	193	82.4	52–860
Fallah et al. (2009)	Iran	White and cream cheese	210	76.6	52.1–785.4
Rahimi et al. (2009)	Iran	Traditional cheese	88	53.4	82–1254
Fallah (2010a)	Iran	White cheese	72	81.9	30–1200
Fallah et al. (2011)	Iran	Lighvan cheese	75	65.3	30–313

[a] Indicates percentage of total samples.

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