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# Aflatoxin $M_1$ contamination in white and Lighvan cheese marketed in Rafsanjan, Iran

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ABSTRACT

This study was a screening survey to determine the occurrence of aflatoxin $M_1$ ($AFM_1$) in 82 cheese samples composed of white cheese (45 samples) and Lighvan cheese (37 samples) obtained from supermarkets and retail outlets in Rafsanjan city of Iran. The competitive enzyme immunoassay method was used for determination of the toxin in the samples. Aflatoxin $M_1$ was detected in 39 (47.6%) samples, consisting of 29 (64.4%) white cheese (mean: 135 ng/kg; range: 93.3–309 ng/kg) and 10 (27%) Lighvan cheese samples (mean: 90.8 ng/kg; range: 70.5–203 ng/kg). According to Iranian national standard limit for $AFM_1$ in cheese (200 ng/kg), 9 samples (20%) of white cheese and 1 sample (2.70%) of Lighvan cheese had levels above the limit. It was concluded that the contamination of the samples with $AFM_1$ in such a level could be considered as a serious public health problem.



## 1. Introduction

Aflatoxins are a group of highly toxic metabolites of molds mainly produced by toxigenic strains of *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* when growing on cereals, nuts, legumes, fruits and other agricultural crops (Mortazavi & Tabatabai, 1998, pp. 43–83). They are also immunosuppressive, mutagenic, teratogenic and carcinogenic compounds that have been implicated as causative agent in human hepatic and extrahepatic carcinogenesis (Creppy, 2002). The common types of aflatoxins are $B_1$, $B_2$, $G_1$, $G_2$. Among them, aflatoxin $B_1$ ($AFB_1$) is the most frequent produced mycotoxin that can be contaminated animal feedstuffs. Aflatoxin $M_1$ ($AFM_1$) is the hydroxylated derivative of $AFB_1$, appear in milk of lactating livestock following consumption of $AFB_1$ contaminated feedstuffs (Fallah, 2010a; Murphy, Hendrich, Landgren, & Bryant, 2006). The International Agency for Research on Cancer (IARC, 1993) of WHO classified $AFB_1$ as Group 1 and $AFM_1$ as Group 2B human carcinogen.

Aflatoxin $M_1$ can be detected in dairy products prepared from toxin contaminated milk. It is relatively stable during processing and storage of various dairy products (Bakirci, 2001). Moreover, the toxin remains stable during ripening of different types of cheese (Anfossi et al., 2012; Fallah, Jafari, Fallah, & Rahnama, 2009).

Several countries have carried out studies about the occurrence of $AFM_1$ in milk and milk derivatives and regulated permissible levels for this mycotoxin (Kamkar, 2005). The US Food and Drug Administration (US FDA, 1996) has prescribed a maximum admissible level of 500 ng/l for $AFM_1$ in milk. However, according to the Institute of Standards and Industrial Research of Iran (ISIRI, 2002), the toxin level in milk should not be higher than 50 ng/l which is the same as European Commission permitted level (European Commission, 2001).

Referring to the existing scientific literature, several studies have been undertaken to determine the occurrence of $AFM_1$ in milk and dairy products in Iran (Fallah, 2010b; Kamkar, 2005; Rahimi, Karim, & Shakerian, 2009). However, no study was performed in

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**Table 1**
Occurrence of aflatoxin $M_1$ in white and Lighvan cheese in Rafsanjan, Iran.

| Cheese type | Samples tested, *n* | Positive samples, *n* (%) | Min–max (ng/kg) | Mean ± SD (ng/kg) | Exceeded regulation[a], *n* (%) |
|---|---|---|---|---|---|
| White cheese | 45 | 29 (64.4) | 93.3–309 | 135 ± 28.2[x] | 9 (20.0) |
| Lighvan cheese | 37 | 10 (27.0) | 70.5–203 | 90.8 ± 18.2[y] | 1 (2.70) |
| Total | 82 | 39 (47.6) | 70.5–309 | 124 ± 25.6 | 10 (12.2) |

[x,y]Means ± SD with different letters are significantly different ($P < 0.05$).
[a] The ISIRI limit for $AFM_1$ in milk is 200 ng/kg.

this field in Rafsanjan city of Iran. Therefore, this study aimed to investigate the occurrence of $AFM_1$ in traditional and industrial cheese samples offered for sale in Rafsanjan, Iran.

## 2. Materials and methods

### 2.1. Sample collection

During winter and spring 2012, a total of 45 samples of white cheese and 37 samples of Lighvan cheese were randomly obtained from supermarkets and retail outlets in Rafsanjan city of Iran. The samples were transported to the laboratory inside an insulated container at about 4 °C, and stored at −20 °C until analysis for $AFM_1$.

### 2.2. Methods

Determination of $AFM_1$ in the cheese samples was based on competitive enzyme immunoassay using RIDASCREEN® Aflatoxin $M_1$ 30/15 (R-Biopharm, Darmstadt, Germany) test kit. Preparation of the samples and ELISA test procedure were carried out according to the manufacturer instructions. The average procedure recovery was 100.3% with a coefficient of variation (CV) of 8.5% at spiked concentrations of 50, 150, and 250 ng/kg. The statistical analysis was performed by SPSS software version 18.

## 3. Results

The incidence and levels of $AFM_1$ contamination in white and Lighvan cheese samples are presented in Table 1. Aflatoxin $M_1$ was found above detectable level in 64.4% (29/45) of white cheese samples, ranging from 93.3 to 309 ng/kg; and 27% (10/37) of Lighvan cheese samples, ranging from 70.5 to 203 ng/kg. The mean concentration of $AFM_1$ in white cheese (135 ng/kg) was significantly higher ($P < 0.05$) than in Lighvan cheese (90.8 ng/kg). Levels of the toxin in 9 white cheese samples (20%); and 1 Lighvan cheese sample (2.70%) exceeded the Iranian national standard limit i.e. 200 ng/kg (Table 1). Considering seasonal variability, the mean concentration of $AFM_1$ in white cheese samples collected in winter was significantly ($P < 0.05$) higher than those obtained in spring, while no statistically significant seasonal effect ($P > 0.05$) was found for Lighvan cheese samples (Table 2).

## 4. Discussion

Owing to its affinity for casein fraction of milk, $AFM_1$ concentration is higher in cheese than in milk from which the cheese is manufactured. Studies showed that the toxin concentration is about 3–5 times higher in cheese than in corresponding milk; hence cheese could be a potent source of aflatoxins among dairy products (Fallah et al., 2009; Tavakoli, Riazipour, Kamkar, Shaldehi, & Mozaffari Nejad, 2012).

White cheese is an industrial product prepared from cow milk, while Lighvan cheese is a traditional Iranian cheese produced from a mixture of raw sheep and goat milk in small dairy farms. The lower incidence and levels of $AFM_1$ in Lighvan cheese comparing to white cheese could be due to the pasture grazing of sheep and goat in Iran. It was found that out-pasturing can decrease the level of $AFM_1$ contamination in milk of dairy species (Fallah, Rahnama, Jafari, & Saei-Dehkordi, 2011; Kamkar, 2005).

Our findings demonstrated a high incidence of $AFM_1$ in white cheese samples. This shows that the milk used in production of these products has been obtained from animals fed with $AFB_1$ contaminated feedstuffs. In a previous study, Kamkar (2006) found that 60.6% of examined white cheese samples contained $AFM_1$ higher than the acceptable levels (250 ng/kg). In the present study, the higher concentration of $AFM_1$ in white cheese obtained in winter than those obtained in spring is in agreement with previous studies that reported higher levels of $AFM_1$ contamination in cold seasons than hot ones (Fallah et al., 2011; Nemati, Mehran, Hamed, & Masoud, 2010).

In this study, the incidence of $AFM_1$ contamination in Lighvan cheese was low. When comparing our finding with the previous study (Fallah et al., 2011), the mean level of $AFM_1$ contamination (90.8 vs. 85.1 ng/kg) was almost similar. However, the frequency of contamination (27% vs. 65.3%) and percentage of samples exceeded the legal limit (2.70% vs. 9.30%) were lower in this study than the previous study. Moreover, Fallah et al. (2011) found that the mean concentration of $AFM_1$ in Lighvan cheese samples collected in spring was significantly higher than those collected in the other seasons.

As can be seen in Table 3, several studies (De Sylos, Rodriguez-Amaya, & Carvalho, 1996; Elkak, El Atat, Habib, & Abbas, 2012; Fallah et al., 2009; Filazi, İnce, & Temamoğulları, 2010; Kamkar, 2006; Kaniou-Grigoriadou, Eleftheriadou, Mouratidou, & Katikou, 2005; Kav, Col, & Tekinsen, 2011; Pietri, Bertuzzi, Bertuzzi, & Piva, 1997; Rahimi et al., 2009; Tabata et al., 1993; Tavakoli et al., 2012; Tekinşen & Eken, 2008; Yaroglu, Oruc, & Tayar, 2005) reported the $AFM_1$ contamination in different kinds of cheese. The reported contamination levels vary from one study to another. This variability can be attributable to the different factors: geographical region, kind of cheese studied, cheese-making procedures, conditions of cheese ripening, and the analytical method employed (Fallah et al., 2009, 2011).

**Table 2**
Occurrence of aflatoxin $M_1$ in white and Lighvan cheese: Comparison between samples obtained in winter and spring.

| Cheese type | Winter | | Spring | |
|---|---|---|---|---|
| | Samples tested, *n* | Mean ± SD (ng/kg) | Samples tested, *n* | Mean ± SD (ng/kg) |
| White cheese | 22 | 152 ± 22.5[a] | 23 | 96.1 ± 40.8[b] |
| Lighvan cheese | 14 | 94.3 ± 19.6 | 23 | 85.5 ± 16.1 |

[a,b]Means ± SD in the same row with different letters are significantly different ($P < 0.05$).

**Table 3**
Incidence and levels of aflatoxin $M_1$ in different kinds of cheese reported in previous studies.

| Reference | Country | Cheese type | Samples tested, *n* | Positive (%)[a] | Min.–max. (ng/kg) |
|---|---|---|---|---|---|
| Tabata et al. (1993) | Japan | White cheese | 37 | 0 | – |
| De Sylos et al. (1996) | Brazil | White cheese | 36 | 0 | – |
| Pietri et al. (1997) | Italy | Grana Padano cheese | 223 | 91 | 5–250 |
| Kaniou-Grigoriadou et al. (2005) | Greece | Feta cheese | 54 | 0 | – |
| Yaroglu et al. (2005) | Turkey | White, Kashar, and cream cheese | 600 | 5 | 100–800 |
| Kamkar (2006) | Iran | Feta cheese | 80 | 82.5 | 150–2410 |
| Tekinşen and Eken (2008) | Turkey | Kashar cheese | 132 | 82.6 | 50–690 |
| Fallah et al. (2009) | Iran | White and cream cheese | 210 | 76.6 | 52.1–785 |
| Rahimi et al. (2009) | Iran | Traditional cheese | 88 | 53.4 | 87–1254 |
| Filazi et al. (2010) | Turkey | Ewe's milk cheese | 50 | 28 | 20–2000 |
| Fallah et al. (2011) | Iran | Lighvan cheese | 75 | 65.3 | 30–313 |
| Kav et al. (2011) | Turkey | White-brined cheese | 127 | 28.3 | 70.6–771 |
| Elkak et al. (2012) | Lebanon | Locally processed and imported cheese | 111 | 67.6 | 77.2–315 |
| Tavakoli et al. (2012) | Iran | White cheese | 50 | 60 | 40.9–374 |

[a] Indicates percentage of total samples.

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