The Occurrence and Risk Assessment of Aflatoxin M$_1$ in Cheeses Samples from Hamadan, Iran

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Abstract

Aflatoxin M$_1$ (AFM$_1$) is a category of poisonous compounds found in milk and dairy products. The target of our research is to determine incidence and risk assessment AFM$_1$ through the consumption of cheese in Hamadan province of Iran. Seventy cheese samples including cream cheese (n = 30) and Iranian white cheese (n = 40) were collected from different regions of Hamadan province, Iran and tested for AFM$_1$ by ELISA technique. The estimated daily intake (EDI) and hazard index (HI) of AFM$_1$ was determined. AFM$_1$ was detected in 67 (95.7%) samples, including 39 (97.5%) Iranian white cheese (mean: 115.16 ng/kg; range: < 5-287 ng/kg) and 28 (93.3%) cream cheese samples (mean: 141.20 ng/kg; range: < 5-289 ng/kg). The level of AFM$_1$ in 10% samples was above the maximum tolerance limit (250 ng/kg). EDI of AFM$_1$ through cheese for a preschool child, an adult female, and an adult male was 0.138, 0.076, 0.065 ng/kg bw/day, respectively. In our study, HI for these groups was 0.690, 0.378 and 0.324, respectively. Although the incidence of AFM$_1$ in cheese samples was high, the results, regarding risk assessment which indicated potential risks for the liver cancer among Iranian consumers due to the cheese consumption, are not concerned.

Keywords: Aflatoxin M$_1$; Cheese; ELISA; Iran; Risk assessment.

Introduction

Mycotoxins are one of the primary natural chemical compositions that can be a serious concern all over the world, specially an international trade. Between 300 various mycotoxins, aflatoxins (AFs) are poisonous, cancerous, and primary classes of mycotoxins. They are fungal secondary metabolites which are frequently produced by some Aspergillus species, especially A. nomius, A. flavus, and A. parasiticus. They can be found in agricultural crops, milk (breast and animal), and dairy products under suitable conditions of humidity and temperature (1-7).

Cheese is the major origin of aflatoxins among milk products for the fact that AFM$_1$ is related to the casein fraction in milk which is nearly concentrated in cheese (3, 8). Researchers have demonstrated that the concentration of AFM$_1$ is about three fold greater in various soft cheeses and around five fold greater in hard cheeses than in milk from which the cheese is manufactured (8).

Several studies from various countries have conducted about the occurrence of AFM$_1$ in dairy products and suggested a permissible limit for it. These regulations differ between several countries with respect to economic considerations. Hence, the European Commission (EC) and the Institute...
of Standards and Industrial Research of Iran (ISIRI) have set a limit of 250 ng/kg for AFM$_1$ in cheese variety (9-11).

One of the most important methods for the estimation of liver cancer risk as result of AFM$_1$ intake is to assess the risk of exposure to this mycotoxin (12, 13). For this purpose, its estimate daily intake (EDI) and hazard index (HI) were calculate and expressed. If HI value was lower than one, it means AFM$_1$ intake from analysed products did had health risk for consumers (14).

ELISA and lateral flow strips are routine methods for AFM$_1$ detection in many group numbers of samples; however, High-performance liquid chromatography (HPLC) with fluorescence detection (FD) is another confirmatory method for this purpose. In Iran, the ELISA technique is the most usual and popular by researchers because it is an ordinary, rapid, and low-cost for the survey of AFM$_1$ (1-5).

The target of our research is to survey the attendance and risk assessment of exposure of AFM$_1$ through the consumption of cheese variety in Hamadan province of Iran.

**Experimental**

*Sample collection*

Seventy cheeses variety included cream cheese (n = 30) and Iranian white cheese (n = 40) were arbitrarily purchased from popular markets in different markets in Hamadan province, Iran, during from October 2017 to August 2018. Eventually, all of the samples were carried to the lab inside and kept in refrigerator at 4 °C. All cheese samples were analyzed for AFM$_1$ before the expiration date of the samples.

*Methods*

The quantitative measurement of AFM$_1$ in samples was distinguished by competitive ELISA using AFM$_1$ test kit (RIDASCREEN® AFM$_1$ Art. No: R1121, R-Biopharm, Darmstadt, Germany). Preparation of the cheeses samples and AFM$_1$ measurement were performed according to the method described by kit manufacturer (15). The mean lower limit of detection (LOD) for AFM$_1$ in cheese was 5 ng/kg.

**Risk assessment for exposure to AFM$_1$ through cheese**

In this study, EDI and HI of AFM$_1$ was determined to show the severity and probability of liver cancer risk through cheese consumption (12, 13). EDI was calculated through the following equation:

$$\text{EDI (ng/kg bw/day)} = \frac{\text{mean of AFM}_1 \text{ in cheese (ng/kg)} \times \text{average daily consumption of cheese (kg/day)}}{\text{body weight (kg)}}$$

For calculation of EDI, in the samples in which AFM$_1$ concentration was lower than the LOD of ELISA kit (5 ng/kg), it was considered 2.5 ng/kg. Based on the information in the statistical center of Iran, the per capita consumption of cheese has been approximately 13 kg for adults and 8 kg for children in 2018 (16). Average body weight of an adult male, an adult female, and a preschool child was considered as 70, 60, and 20 kg, respectively.

HI (expressed as ng/kg bw) is calculated as following

$$\text{HI} = \frac{\text{EDI (ng/kg bw/day)}}{0.2 \text{ (ng/kg bw/day)}}$$

**Statistical Analysis**

The concentrations of AFM$_1$ in milk samples were analysed by SPSS Statistics 16.0 for Windows. One-side t-test was applied to compare the mean concentration of AFM$_1$ samples with the maximum acceptable amount of the ISIRI and European Union (250 ng/kg) regulation. Differences between values were considered significant at $P \leq 0.05$.

**Results**

The occurrence and the levels of AFM$_1$ in Iranian white cheese samples is summarized in Table 1.

AFM$_1$ was detected above acceptable level in 97.5% (39/40) of the analysed samples, ranging from 5 to 287 ng/kg. Levels of the AFM$_1$ in 7 (10%) cheese samples exceeded the ISIRI and European union i.e. 250 ng/kg. On the other hand, considering the US FDA (17). limits for AFM$_1$ in milk (500 ng/l), none of the samples had levels above the maximum tolerance limit.

EDI of AFM$_1$ through both cheese for a preschool child, an adult female and an adult male was 0.138, 0.076, 0.065 ng/kg bw/day,
Discussion

AFM₁ is related with the casein during cheese production making cheese the potent source of AFs among dairy products (18). Cheese is the only production which is sensitive to the development of fungus and mycotoxins groups among the milk products (19). Therefore, the acceptable extent range by regulatory authorities is five to nine folds greater than those ranges for milk (20). The previous studies revealed that some factors such as the action of cheese types, the kind of unit processes and the amount of omitted water pending processing have impacts on the increase of AFM₁ in cheese sample (21). Also, the several studies by researchers confirmed that cheese samples made from different animal’s milk are effective on amount of AFM₁. They reported that the level of AFM₁ from cows’ milk is higher than those of sheep and goats, and this may be because of the differences in their digestive apparatuses and mechanism of aflatoxin B₁ (AFB₁) assimilation in animals, and for the different patterns of feeding (19, 22 and 23).

In the current research we show that a high occurrence of AFM₁ is in various types of cheese including cream and Iranian white cheese from Iran. As referred in previous studies, the occurrence of AFM₁ in milk and milk derivative contributes to the effects of feeding livestock with materials including aflatoxin B₁ (6). In a prior survey, Cano-Sancho et al. (24) reported the absence of AFM₁ at detectable level in cheese samples although Altun et al. (25) detected AFM₁ in 100% of cheese samples. Furthermore, there are several research have shown the occurrence of AFM₁ in some types of cheese such as Tulum, Urfa, Lighvan, Parmesan cheese, Talesh, Halloumi and etc., in Iran, Turkey, Italy, Brazil, Lebanon, and some countries (13, 25-35). These results confirmed that about nutritional importance traditional cheeses between humans and also attention of authorities to this subject. Table 3 shows data regarding AFM₁ from previous studies in different countries that measure by ELISA and HPLC methods (13, 20, 25-35).

Table 1. The occurrence and concentration (ng/kg) of AFM₁ in cheese samples collected from Hamadan province, Iran.

| Sample type                | N  | Positive (%) | Mean | Standard deviation | < 5   | 5-0.250 | >250 | Range     |
|----------------------------|----|--------------|------|--------------------|------|---------|------|-----------|
| Iranian white cheese       | 40 | 39 (97.50)   | 115.16 | 79.22              | 1 (2.5%) | 35 (87.5%) | 4 (10%) | < 5-287.09 |
| Cream cheese               | 30 | 28 (93.30)   | 141.20 | 77.06              | 2 (6.7%) | 25 (83.3%) | 3 (10%) | < 5-287.18 |
| Total                      | 70 | 67 (95.71)   | 126.32 | 78.81              | 3 (4.3%) | 60 (85.7%) | 7 (10%) | < 5-287.18 |

Table 2. The estimated daily intake (EDI) and hazard index (HI) for AFM₁ intake through cheese consumption.

| Sample type                | EDI (ng/kg bw/day) | HI          |
|----------------------------|--------------------|-------------|
|                            | Preschool children | Female | Male | Preschool children | Female | Male |
| Iranian white cheese       | 0.126              | 0.069    | 0.059 | 0.630              | 0.345  | 0.296 |
| Cream cheese               | 0.155              | 0.085    | 0.073 | 0.773              | 0.423  | 0.363 |
| Total                      | 0.138              | 0.076    | 0.065 | 0.690              | 0.378  | 0.324 |
The other obtained results were reported in Pakistan by Iqbal et al., that were done with HPLC technique, from 119 and 150 samples of white cheese and cream cheese, 93 (78%) and 89 (59%) of samples were contaminated with AFM1, respectively (36). Also, 14 (15%) samples of white cheese and 10 (11%) cream cheese samples had higher AFM1 content than the limit allowed in European Union i.e 250 (ng/kg), but our results were less than this result. Also, the previous survey by Elkak et al. from Lebanon by ELISA method reported that 75 (67.56%) samples of 111 samples of cheese were detected with AFM1 and in 13 (17.33%) samples, concentration of AFM1 was higher than the EU regulations (250 ng/kg) (37). This result is approximately to the same as our research results. In other studies, conducted in Iran, the authors also identified Iranian white cheese samples that were contaminated with AFM1, respectively (36). Also, 14 (15%) samples of white cheese and 10 (11%) cream cheese samples had higher AFM1 content than the limit allowed in European Union i.e 250 (ng/kg), but our results were less than this result. Also, the previous survey by Elkak et al. from Lebanon by ELISA method reported that 75 (67.56%) samples of 111 samples of cheese were detected with AFM1 and in 13 (17.33%) samples, concentration of AFM1 was higher than the EU regulations (250 ng/kg) (37). This result is approximately to the same as our research results. In other studies, conducted in Iran, the authors also identified Iranian white cheese samples that were contaminated with AFM1, respectively (36). Also, 14 (15%) samples of white cheese and 10 (11%) cream cheese samples had higher AFM1 content than the limit allowed in European Union i.e 250 (ng/kg), but our results were less than this result. Also, the previous survey by Elkak et al. from Lebanon by ELISA method reported that 75 (67.56%) samples of 111 samples of cheese were detected with AFM1 and in 13 (17.33%) samples, concentration of AFM1 was higher than the EU regulations (250 ng/kg) (37).

### Table 3. Occurrence and levels of aflatoxin M1 (ng/kg) in various cheeses published in previous studies.

| Location | Cheese type | No. of samples | No. positive samples (%) | Detection Method | Mean (ng/kg) | Range (ng/kg) | Exceeded regulation, n (%) | Reference |
|----------|-------------|----------------|--------------------------|------------------|--------------|--------------|--------------------------|-----------|
| Qatar    | Cheese      | 46             | 39 (84.8)                | ELISA            | 197.74       | 1.21-217.15  | 0a                       | Hassan et al (20) |
| Iran     | Traditional cheese | 360     | 194 (53.8)               | ELISA            | 139.4        | 50.5-308.7   | 22 (10.5)a               | Shabzai et al (13) |
| Turkey   | Cheese      | 130            | 130 (100)                | ELISA            | 260.26       | 10-800       | 22 (17)                  | Ahm et al (25)   |
| Iran     | Cheese      | 100            | 52 (52)                  | ELISA            | 133.2        | 50.2-424.4   | 8 (8)                    | Shirifadeh et al (26) |
| Iran     | Cheese      | 40             | 25 (65.5)                | ELISA            | 158.4        | 52.5-272    | 4 (10)                   | Bahrami et al (27) |
| Serbia   | cheese      | 54             | 29 (53.70)               | UHPLC-MS/MS      | 324.07       | 80-2250      | 7 (13%)                  | Škrbić et al (28) |
| Turkey   | White pickled cheese | 50     | 10 (20)                  | ELISA            | 193.5        | 40.41-130.89| 0                        | Temamogullari and Kanci (29) |
| Iran     | Cheese      | 80             | 69 (86.3)                | ELISA            | 133.2        | 14.3-572.1  | 11 (13.8)p               | Rahimi (30)     |
| Brazil   | Cheese      | 10             | 3 (30)                   | HPLC             | 160          | 91-300      | ND                       | Jager et al (31) |
| Iran     | Lighvan cheese | 37            | 10 (27)                  | ELISA            | 90.8         | 70.5-203    | 1 (2.7)p                 | Mohajeri et al (32) |
| Italy    | Cheese      | 17             | 7 (41.2)                 | HPLC             | ND           | <3-18       | 0                        | Santini et al (33) |
| Iran     | Cheese      | 40             | 16 (40)                  | ELISA            | 133.2        | 31.9-505.7  | 7 (17.5)p                | Nikchian and Rahimi (34) |
| Turkey   | Urfa cheese | 127            | 36 (28.3)                | ELISA            | 253.7        | 70.61-770.97| 13 (10.2)p               | Kav et al (35)  |

**ND:** Not Determined; **ELISA:** Enzyme-Linked Immunosorbent Assay; **HPLC:** High-Performance Liquid Chromatography.

*The European Community limit for aflatoxin M1 is 250 ng/kg for cheese*

**According to Iranian Standard (200 ng/kg).**
The Occurrence and Risk Assessment of Aflatoxin M₁

According to a study done by Tavakoli et al., from 50 Iranian white cheese samples, collected in Tehran, 60% (30/50) were positive for AFM₁ at levels of 40.9 to 374 ng/kg detected by ELISA method. Also, 3 (6%) samples were above the permissible level according to ISIRI (4). However, this result is in contrast to our finding that showed 95.71% (67/70) were occurrence of AFM₁. The other conducted results, that were revealed in Turkey by Bakırdere et al., were observed with ELISA technique, 36 (53.8%) from 67 white cheese and 8 (38%) from 21 cream cheese samples were contaminated with AFM₁ (38). But, our results reported that approximately all samples of (39/40) white and (28/30) cream cheese are contaminated with AFM₁. An earlier study by Mohajeri et al. from Iran reported that 29 (64.4%) of 45 Iranian white cheese samples were contaminated with AFM₁ but this result is less than that of the current study (32).

The EDI value in current study was higher than previous reports in Iran and French (13, 39). Shahbazi et al. reported EDI value depended on sampling season and AFM₁ measurement method. The EDI value for AFM₁ measured by ELISA was 0.04 and 0.03 ng/kg bw/day during winter and summer seasons, respectively (13). However, EDI was 0.05 ng/kg bw/day in winter and 0.04 ng/kg bw/day in summer for this mycotoxin if it was analysed by HPLC method. Leblanc et al. reported EDI of 0.02 ng/kg bw/day for AFM₁ through cheese consumption by French adults (15 years and over) and children (3–14 years) (39).

Because HI level was less than one, it can be concluded that the potential risk for liver cancer in Iranian consumers due to the consumption of Iranian white cheese and cream cheese isn’t a concern. HI value obtained in our study was more than findings reported by Shahbazi et al. (13). In the mentioned study, HI values for the cheese samples collected in summer and analysed by ELISE and HPLC were 0.17 and 0.25, respectively while in cheese samples collected in winter, HI was 0.21 by both analysis methods.

Conclusion

Our finding demonstrated that the incidence of AFM₁ in cheese samples was high and almost 95.71% samples contained AFM₁. The results regarding risk assessment indicated HI level was less than one, therefore it can be concluded that potential risk for liver cancer in Iranian consumers due to the consumption of Iranian white cheese and cream cheese isn’t a concern. However, it is essential to set difficult legislations on AFB₁ contamination in the animal feed.

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