Aflatoxin M1 in traditional and industrial pasteurized milk samples from Tiran County, Isfahan Province: A probabilistic health risk assessment

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Received: 16 April 2021; Accepted: 4 June 2021; Published: 13 July 2021 © 2021 Codon Publications

Abstract

In this study, the aflatoxin M1 (AFM1) concentration in traditional and industrial milk and risk assessment due to AFM1 exposure using the Monte Carlo simulations technique was investigated. The mean concentration of AFM1 in traditional and industrial milk samples was 53.00 ± 11.49 and 54.33 ± 12.22 ng/L, respectively, which was higher than European Union and Codex standards. Percentile 95% of hazard quotient (HQ) adults and children due to industrial ingestion milk was 1.056 and 4.956, and traditional milk was 1.031 and 5.116, respectively. Hazard quotient in all age consumers was higher than 1. Therefore, consumers are at a considerable health risk.

Keywords: aflatoxin M1; industrial and traditional; milk; risk assessment; seasonal variation

Introduction

The exposure of humans to various types of toxic chemical compounds (natural or artificial) may cause a wide range of human health problems, including genital diseases, mental disorders, dysfunction, especially in kidneys and liver, suppression and weakening the immune system, and a variety of cancers (Bahrami et al., 2016; Khaneqah et al., 2019; Khodaei et al., 2020; Mir et al., 2021; Mousavi Khaneqah et al., 2021; Rahmani et al., 2018; Rastegar et al., 2017; Shabbazi et al., 2015; Sipos et al., 2021). Among them, the mycotoxins are the secondary metabolites produced by fungi, mainly the Aspergillus, Fusarium, Penicillium, and Alternaria genera, causing substantial concerns all over the world over the last few decades (Coppa et al., 2021; de Souza et al., 2021; Eras et al., 2011; Fakhri et al., 2019; Mahmood Fashandi et al., 2018; Roohi et al., 2020). Mycotoxins belong to the aflatoxins group (around 300 different mycotoxins) and are produced by three filamentous fungi species called Aspergillus parasiticus, Aspergillus flavus rarely by Aspergillus nominus (Campagnollo et al., 2016; Fakhri et al., 2018; Heshmati et al., 2019).
Aflatoxin B1 (AFB1) is the typical type and most toxic one among 18 aflatoxin types. Aflatoxin M1 (AFM1) is an AFB1 hydroxylated metabolite formed by the enzymes of cytochrome P450 in the liver. This is secreted through the milk glands in lactating cows by AFB1, which is consumed through food (Marhamatizadeh and Goosheh, 2016; Škrbić et al., 2014); and about 0.5% to 6% of AFB1 is converted into AFM1. When AFB1 contaminated animal feeds with lactating animals, after 12 to 24 h, AFM1 could be detected and reached a maximum after 72 h when the feeding with the contaminated animal feeds is reached (Asi et al., 2012; Iqbal et al., 2014; Mousavi Khaneghah et al., 2018). After AFB1 intake, about 72 h, the AFM1 concentration was decreased to an undetectable level (Khaneghah et al., 2018). The ratio of swallowed AFB1 to the excreted AFM1 has been reported with various percentages in different sources; however, typically, it has been in the range of AFB1 averages 1%–2% that varies from day to day, type of animal, and type of milk (Bervis et al., 2021; Gonçalves et al., 2017; Heshmati et al., 2019).

Milk is a very nutritious food rich in micro and macronutrients, vital to human health evolution and human body growth. Therefore, the safety and hygiene of milk play a critical role in human health (Bahrami et al., 2016; Iqbal et al., 2015). Aflatoxin M1 in dairy products and milk is highly resistant to conventional milk processing methods, including pasteurization, ultra-high temperature (UHT), and other processing methods (De Roma et al., 2017; Khaneghah et al., 2021; Rahmani et al., 2018; Turkoglu and Keyvan, 2019). The pasteurization method can reduce the concentration of AFM1; however, it cannot eliminate this contaminant (Mahmood Fashandi et al., 2018; Naeimipour et al., 2018). Some adverse health effects such as carcinogenicity, teratogenicity, preventing RNA encoding and protein synthesis, brain damage, colon damage, lung, liver, kidney, mutation, immune suppression, and digestive disorders were associated with the consumption of contaminated milk by AFM1 (Fallah et al., 2016; Kaur et al., 2021; Pokharel et al., 2021).

In order to assess the overall prevalence of AFM1 in industrial and traditional milk, several studies have been carrying out. The study by de Souza et al. (2021) stated that the AFM1 concentration had been reduced in the contaminated milk, which was mainly carried out in traditional products. However, preventative approaches are recommended, and it is necessary to reduce the concentration of aflatoxin. Tajik et al. (2016) investigated the AFM1 concentration in the West-Azerbaijan in the pasteurized milk. Their study showed that 77.7% of the samples were contaminated with AFM1; and in the pasteurized milk, about 70% of them exceeded the standard value given by European Commission (50 ng/L). Makhdoumi et al. (2021) showed that the prevalence of AFM1 in conventional and industrial dairy products was due to the cold season (autumn and winter). They reported that AFM1 could be found in dairy products with an overall prevalence percentage of 63.53 and 54.05 based on the type of sample and production process, respectively. A study by Ansari et al. (2019) reported that the autumn and winter seasons had shown the highest concentration and contamination (above 50 ng/L). To control and prevent exposure to AFM1, more than 60 countries in the world have set the maximum permissible limit for AFM1 in dairy products. The Codex Alimentarius and European Union (EU) have determined the 50 ng/kg of AFM1 concentration as the maximum amount of AFM1 remaining in raw and warmed milk (de Souza et al., 2021; Khaneghah et al., 2021; Mannani et al., 2021). The FAO (Food and Agriculture Organization, the United Nations) has set the maximum allowable concentration of AFM1 to 500 ng/L (Cai et al., 2012; Cavallarin et al., 2014). Also, the Iranian Institute of Standards has declared the concentration of AFM1 to 100 ng/L in raw milk samples (Hashemi, 2016; INSO 5925; Khaneghah Abyaneh et al., 2019).

The health risk assessment of AFM1 exposure due to dairy products and milk consumption is a valuable way to risk and evaluate the liver’s developing cancer (Serraino et al., 2019; Tsakiris et al., 2013). The AFM1 risk assessment is defined as assessing the daily oral intake and hazard quotient (HQ). These data and studies can be used for assessing the cancer risk in toxicological studies to estimate the severity and probability of toxin contamination (Heshmati et al., 2017, 2019; Nabizadeh et al., 2018; Oteiza et al., 2017; Škrbić et al., 2015).

Some studies in Iran have been done for characterizing the concentration of AFM1 in various cities; however, the available evidence on the risk assessment of AFM1 exposure is limited in developing countries (e.g., Iran) (Bahrami et al., 2016; Mohajeri et al., 2013). In developing countries, milk and its products are produced with traditional and industrial methods, increasing the risk of exposure to AFM1. Moreover, traditional livestock, especially in rural areas, can exacerbate this risk (Bahrami et al., 2016). Traditional Iranian dairy products made from milk are native to some areas of Iran. Also, consuming this type of milk, the usual industrial processes of milk preparation do not occur, and boiling milk for consumption is done by individuals in their home. In the present study, samples of traditional milk were cow’s milk, which were purchased from local distributors in the same area (rural regions) that are manufactured in rural households under unacceptable hygiene conditions (Bahrami et al., 2016; de Souza et al., 2021; Kaur et al., 2021).

In this regard, the current investigation was undertaken to determine the AFM1 level in traditional and different industrial brands available in the markets of Tiran.
samples were transferred to the laboratory in pre-sterilized stainless steel containers (for traditional) and under dark environment conditions, away from smell and light at 1 to 5°C. Sterilized milk samples (industrial samples) and traditional samples were stored at 4°C. Samples preparation was conducted to determine AFM1 in samples of milk by using enzyme-linked immunosorbent assay (ELISA) method according to the instructions given in the manufacturer kit (R-Biopharm, Darmstadt, Germany).

Materials and Methods

Sampling area

The present study was conducted in Tiran city, Isfahan province, Iran. The geographical location of the Tiran County is at 32°42′12.96″ east and 51°9′6.84″ north and 1640 m above the sea level (Figure 1). Tiran has arid climates, and the annual average rainfall is 116.9 mm (Jafari et al., 2018). Based on the last census (Iran Statistical Center, 2016), the county population was 71,583, and per capita milk consumption in this province is 90 kg/person/year. Moreover, Isfahan province, with 1,250,000 tons of milk in 2018, has been the first raw milk producer in Iran.

Sampling and preparation

In this study, 156 traditional and industrial milk samples (60 traditional milk (a) and 96 industrial milk samples from 15 brands of b-p) were collected from 15 best-selling milk brands during autumn 2017 and winter 2018 (26 samples were taken in each month). Sampling was performed randomly from all locations of the milk supplier in the study area. Industrial pasteurized milk was randomly obtained from large and busy stores in the city, and traditional milk samples (unheated raw milk) were also obtained from traditional dairy stores in Tiran. The

![Figure 1. Location of Tiran County in Isfahan province and Iran (Jafari et al., 2018).](image-url)
Health risk assessment

The health risk of AFM1 exposure in the milk samples was calculated according to the United States Environmental Protection Agency (USEPA, 2011; Hooshfar et al., 2020; Kaur et al., 2021). The daily exposure to AFM1 by milk was calculated as following:

\[
EDI_{mg} = \frac{C_m \times IR_m \times EF \times ED}{BW \times AT}
\]

(1)

where, \(EDI\) is the estimated daily intake per day by AFM1 consumed in milk (ng/kg/day). \(C_m\) denotes AFM1 concentration in milk (ng/L), and \(IR_m\) is the milk ingestion rate according to liter/day. Based on the approximate information available, the amount of milk consumption for people with high consumption was 222 g/day, the average consumption was 174 mL/day, and the minimum consumption was 74 mL/day. However, according to the previous study in Iran (March 2011–March 2012), Iranians approximately consumed 190 mL or less than one glass of milk/dairy products per day. (Abyaneh et al., 2019; Home Society Economy Politics Sports Culture International Multimedia Tourism, 2019; Nejad et al., 2019; Pérez-Gregorio et al., 2011), \(EF\) is exposure frequency (day/year), \(ED\) is exposure duration (according to years), \(BW\) is body weight (kg), and \(AT\), the averaging time (based on days).

The HQ was investigated to determine the AFM1 non-carcinogenic risk induced by milk intake. If the HQ is higher than 1, the risk of liver cancer for the consumer is more (Fakhri et al., 2018; Kuiper-Goodman, 1990; Nabizadeh et al., 2018). While HQ values less than 1 indicate that milk intake does not cause harmful effects for the consumers (Bahrami et al., 2016). The risk index was calculated according to the following equation:

\[
HQ = \frac{EDI}{RfD}
\]

(2)

where \(RfD\) is the reference dose of aflatoxin.

Monte Carlo simulation (MCS) method

The simulation of Monte Carlo was used to minimize uncertainties in the results. When the point values of a variable were used to assess the exposure risk of pollutants with a population, the probability of interference and error, and finally, the uncertainty in the result is obtained (Fakhri et al., 2018; Huang et al., 2017; Keramati et al., 2018, 2019). Crystal Ball software (version 11.1.1.1 Oracle, Inc. United States of America) was used to determine uncertainties with 5000 trails in the MCS. The percentile 95% of HQ was selected as a worse scenario for the health risk of consumers.

Statistical analysis

The Shapiro–Wilk normalization test was performed to verify the data normality. The difference between the concentration of AFM1 in the two groups of samples (traditional vs. industrial samples) and seasons was assessed using the Mann–Whitney test. Furthermore, the association between AFM1 concentrations and different brands was investigated by the Post-Hoc test. A P-value of <0.05 was considered as a significance level for all tests. All statistical analyses were carried out using SPSS15 software.

Results and Discussion

AFM1 concentration

The AFM1 concentration in industrial and traditional samples in autumn and winter is shown in Figure 2. Also, the results of AFM1 concentration based on the different seasons and different types of milk are shown in Tables 1 and 2. While the AFM1 mean concentration in samples of traditional milk produced in autumn and winter is 54.33 ± 11.65 and 53.00 ± 12.22 (ng/L), the overall AFM1 mean concentration in traditional and industrial milk samples is 53.00 ± 11.49 and 54.33 ± 11.22 ng/L, respectively (see the Table 1). In the industrial milk brands, b and h with a mean concentration of 60.00 (12.64) ng/L had the highest concentration of AFM1, and l and o brands with a mean concentration of 48.33 (13.29) ng/L had the lowest AFM1 concentration. The total mean of AFM1 concentration in samples of milk was 53.33 (11.87) ng/L, which was more than the recommended standard
Aflatoxin M1 concentration (ng/l)

Figure 2. AFM1 concentrations in traditional and industrial milk samples based on the season (A: AFM1 in traditional milk, autumn; B: AFM1 in traditional milk, winter; C: Industrial milk, autumn; D: Industrial milk, winter; E: total AFM1 concentration in industrial samples; F: total AFM1 concentration in traditional samples).

Table 1. AFM1 concentration in milk samples based on the brand.

<table>
<thead>
<tr>
<th>Brand of milk</th>
<th>Number</th>
<th>AFM1 concentration according to number of samples</th>
<th>Mean concentration ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower than 50 ng/L</td>
<td>Standard or 50 ng/L</td>
</tr>
<tr>
<td>Traditional (a)</td>
<td>60</td>
<td>19</td>
<td>13</td>
</tr>
<tr>
<td>b</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>c</td>
<td>12</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>d</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>e</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>f</td>
<td>6</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>g</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>h</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>i</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>j</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>k</td>
<td>6</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>l</td>
<td>6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>m</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>o</td>
<td>6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>p</td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>156</td>
<td>55</td>
<td>31</td>
</tr>
</tbody>
</table>
of AFM1 in the EU and the Codex exceeding the permissible limit (50 ng/L) (Bahrami et al., 2016). Fifty-five cases from 156 samples had contamination lower than 50 ng/L, 31 samples were 50 ng/L, which complied with the European Union’s recommended standards of 50 ng/L (Fallah, 2010; Fallah et al., 2016), 70 cases had a high contamination rate of AFM1 that were higher than the recommended standard of 50 ng/L. Overall, the lowest AFM1 concentration was 40 and the highest was 70 ng/L. However, this concentration of AFM1 was lower than the Iranian standard limit (100 ng/L) (Khaneghahi Abyaneh et al., 2019). In another study, the mean concentration of AFM1 in the pasteurized milk was 76.2 ng/L (Tajik et al., 2016), which is higher than the results of our study. One of the reasons for the higher level of AFM1 in West-Azerbaijan may be due to geographical conditions, more contamination of animal feed with AFB1. So, control and monitoring of milk and local health conditions are crucial. AFM1 concentration in traditional milk, surveyed by Omeiza Gabriel Kehinde et al. (2021), that AFM1 concentration in the traditional products was the highest concentration compared to industrial products. There was a significant difference between the type of feed, type of dairy herds, and holding capacity of the dairy herds (Kehinde et al., 2021). Xiong et al. (2021) showed the health risk for child group 2–4 years old that consume contaminated milk with AFM1. About 5.3% of the samples were above the 50 ng/L limits, and the AFM1 level in UHT milk was lower than pasteurized milk.

Comparing the AFM1 concentration in traditional milk and industrial samples were carried out (it is notable, the traditional milk was purchased from rural areas around Tiran county and was prepared as well as sold by individuals on private farms. Also, boiling and milk preparation was carried out at the buyer’s house by themselves.). Moreover, comparing the AFM1 concentration in the traditional and industrial samples with the EU, the Codex limit, and European Union standards (50 ng/L) indicated that 44.9% of samples had a concentration higher than the maximum allowable level. In a study conducted by Xiong et al. (2018), the mean concentration of AFM1 100 ng/L was assigned as the AFM1 concentration in UHT and pasteurized samples of milk collected from China’s market by using ELISA while about 1.8% of UHT milk samples and 59.5% of pasteurized milk were above the EU and Codex standards (Xiong et al., 2018). According to the results shown in Table 2, the AFM1 mean concentration in traditional and industrial milk samples in autumn was higher than in the winter season, which may be due to storage conditions and increased moisture in autumn, but P-value was not significant (Aydemir Atasever et al., 2021; Makhdoumi et al., 2021; Rahmani et al., 2018). In another investigation, the concentration of AFM1 in UHT milk was significantly lower in spring and winter than in summer and autumn (Heshmati and Milani, 2010). Similar results regarding the AFM1 levels in milk and UHT from Brazil and Serbia were reported as higher AFM1 concentrations in summer and autumn than spring and winter (de Oliveira et al., 2013; Tomašević et al., 2015). In another study, AFM1 concentration in raw milk of cattle, sheep, and goat samples collected among autumn and spring were investigated, while no significant correlation in AFM1 among seasons was noted (Bilandžić et al., 2017). In another study, 119 samples out of 125 powder milk UHT and pasteurized milk collected from Brazil were contaminated with AFM1 in the range of 10 to 200 ng/L and the mean concentration of 31 ng/L (Shundo et al., 2009).

No significant association regarding AFM1 concentration among different seasons was noted (P-value was 0.704 according to Mann–Whitney test). According to De Roma et al. (2017), seasonal changes were considered as an influential factor in the concentration of AFM1. Also, Bahrami et al. (2016) demonstrated that AFM1 levels were significantly higher in winter than their corresponding values in summer. Therefore, seasonal variation was considered as an effective parameter on the concentration of AFM1 (Akbar et al., 2019; Ansari et al., 2019).

Moreover, no significant difference in the AFM1 level was observed among the traditional and industrial samples (P-value was 0.563 according to the Mann–Whitney test). However, based on the findings of some similar studies conducted in Iran, the level of contamination in the traditional milk samples was notably higher than the collected samples from industrial ounces, which

<table>
<thead>
<tr>
<th>Season/month</th>
<th>Under 50 ng/L (%)</th>
<th>50 ng/L (%)</th>
<th>Higher than 50 ng/L (%)</th>
<th>Total samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>7 (26.92)</td>
<td>5 (19.23)</td>
<td>14 (65.38)</td>
<td>26</td>
</tr>
<tr>
<td>Autumn</td>
<td>10 (38.46)</td>
<td>7 (26.92)</td>
<td>9 (34.61)</td>
<td>26</td>
</tr>
<tr>
<td>Autumn</td>
<td>10 (38.46)</td>
<td>2 (7.69)</td>
<td>14 (65.38)</td>
<td>26</td>
</tr>
<tr>
<td>Winter</td>
<td>10 (38.46)</td>
<td>4 (15.38)</td>
<td>12 (46.15)</td>
<td>26</td>
</tr>
<tr>
<td>Winter</td>
<td>10 (38.46)</td>
<td>5 (19.23)</td>
<td>11 (42.30)</td>
<td>26</td>
</tr>
<tr>
<td>Winter</td>
<td>8 (30.76)</td>
<td>8 (30.76)</td>
<td>10 (38.46)</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>55 (35.25)</td>
<td>31 (19.87)</td>
<td>70 (44.87)</td>
<td>156</td>
</tr>
</tbody>
</table>
were associated with the non-hygienic condition of livestock storage among a variety of livestock, including cattle, goats, and sheep (Bahrami et al., 2016; Fallah, 2010; Fallah et al., 2016; Mohammadi, 2011). Moreover, the variety in the type of measurement method, geographical location, weather, seasonal, feeding systems, food storage, and farmland and pasture techniques are among other possible reasons (Bahrami et al., 2016; Iqbal et al., 2015). In the present study, almost all samples were prepared from the exact geographical location and climate. Therefore, the high AFM1 in some samples could be correlated with the type of feeding, poor health conditions of forage, and livestock feed (Bilandzić et al., 2017; De Roma et al., 2017; Khaneghah et al., 2021; Visciano et al., 2015). However, according to the sampling season, the level of AFM1 was higher in winter than in autumn (not significant).

**Health risk assessment**

Percentile 95% of HQ in adults and children due to industrial ingestion milk was 1.056 and 4.956 and due to traditional milk was 1.031 and 5.116, respectively. The HQ value in both adult and children consumers was higher than 1. Therefore, consumers are at a considerable health risk (Figures 3 and 4). In a study by Bahrami et al. (2015), the health risk assessment of AFM1 was assessed by consuming traditional dairy products in the western part of Iran. In this study, the average body weight for each adult Iranian was considered to be 60 kg. The results showed that the HQ was higher than 1 for dairy products in winter (1.24 ng/kg body weight in the ELISA method). In summer, the HQ was less than 1 for both methods. In this study, it is noteworthy to assess health risk, and the amount of milk and dairy consumption per Iranian in 2013 was calculated to be equal to 70 kg (annual statistics of Iranian agriculture) (Bahrami et al., 2016).

In another study in Iran, the average body weight of Iranian adults was considered to be 70 kg, which can effectively calculate the results. In this study, the mean concentration of AFM1 in the traditional cheeses was 139.4 ± 2.4 ng/kg. The AFM1 concentration was not higher than 500 ng/kg as the maximum permissible limit. After performing a health risk assessment, the HQ was less than 1 (Shahbazi et al., 2017; Tomašević et al., 2015).

Assessment of the health risk of AFM1 in infant formula milk for infants with 0 to 6 months old, an average weight of 5.7 kg, and the consumption rate of infant formula milk equal 19.4 g/day showed that HQ was less than 1 (Hooshfar et al., 2020). The difference in results with our study was the difference in the amount of AFM1 concentration in milk samples, age, body weight, and milk consumption rate (Bahrami et al., 2016; Duarte et al., 2013; Kos et al., 2014; Milićević et al., 2017b; Serraino et al., 2019; Tsakiris et al., 2013).

The risk assessment of AFM1 in milk and dairy products in Lebanon measured AFM1 concentration in the raw milk, UHT, and pasteurized milk was 0.011–0.440, 0.015–7.350, and 0.013–0.219 µg/L, respectively. The health risk assessment for adults with an average body weight of 75.49 kg was at a safe level (HQ < 1). According to a study by Daou et al. (2020), the milk consumption rate for Lebanon was 113.7 g/person/day that was lower than the milk consumption rate in our study (0.19 L/day). This difference in the body weight and daily milk consumption rate can be one of the reasons for the low HQ value compared to our study.

The AFM1 concentration in all brands was safe regarding non-carcinogenic risk due to milk consumption in Iranian consumers, which agrees with one of the previously published reports (Hooshfar et al., 2020). However, the calculated HQ in summer and winter after measuring the AFM1 by ELISA and HPLC techniques were reported as 0.54 and 1.245, 0.88, and 1.45, respectively (Bahrami et al., 2016). In another similar investigation performed after AFM1 level assessment by ELISA method, HQ was higher than 1 (Milićević et al., 2017a), while the findings of both investigations, as mentioned earlier, demonstrated a potential risk of liver cancer for consumers. AFM1 was measured in dairy products from Turkey; and in cheese samples, the HQ of receiving AFM1 was more than 1 (Sakin et al., 2018). In a study by Rahman et al. (2018), probabilistic health risk assessment of AFM1 in milk samples of the east region was carried out. In many Middle East countries, the HQ was higher than 1 for children at a considerable risk of cancer, unlike adults (Rahman et al., 2018).

The risk of cancer and non-carcinogenic diseases in Brazilian children aged 0–5 years was assessed by exposure to AFM1 in samples of UHT milk, powder milk, and infant formula. The results of the study showed that the range of AFM1 was from 150 to 1020 ng/kg and all positive samples exceeded the limit set by the European Union, and the number of hepatocellular carcinoma cases associated with AFM1 exposure was higher than 0.001 cases per 100,000 people (Conteçotto et al., 2021). As in the present study, there was a risk of cancer for children. The study of Sharma et al. (2020) showed the presence of AFM1 in raw and pasteurized milk samples of India and it is necessary to pay attention to the amount of this mycotoxin. The study that evaluated cancer and non-carcinogenic risk in the infant with an age of below 6 months in Iran showed only one of the infant formula milk samples were contaminated with AFM1 and did not concern the health risk to consumers (Hooshfar et al., 2020). Therefore, attention to different methods for detoxification of dairy products, attention to the manner
Figure 3. Percentile 95 of HQ in adults and children due to ingestion industrial pasteurized milk content of AFM1.

of storage, the lifetime of food intended for feeding livestock and poultry, as well as the need for stricter legal requirements and monitoring legal recommendations are recommended (Min et al., 2020).

A study that evaluated the prevalence of milk contamination with AFM1 worldwide found that most countries had moderate levels of AFM1 in milk in recent years, which were lower than EU levels. However, several countries, including Pakistan, India, and several countries around sub-Saharan Africa, have high levels of AFM1 that exceeded EU and US standards. Therefore, it has been speculated that high levels of AFM1 in milk can indicate high levels of AFB1 in animal feed, products
such as corn used to make animals feed may have high levels of AFB1, which after consumption can lead to animal and humans health adverse effect (Turna and Wu, 2021). Contamination of goat’s milk with AFM1 in Brazil showed that in all milk samples, the recommended levels of AFM1 in milk were lower than the permissible limit. However, the potential risk of liver cancer and EDI levels for AFM1 through goat’s milk for 1-year-old children were higher than the tolerable daily intake and estimated values (de Matos et al., 2021). The results of the study about the amount and risk of AFM1 in pasteurized milk products, ESL and UHT milk from China in summer and winter
showed that 5.3% of milk samples had a level of AFM1 more than the standard limit of 50 ng/kg, which is less contamination compared in these studies. Similar to the present study, AFM1 concentrations were higher in winter. Children aged 2–4 years had the highest risk of exposure to AFM1 in milk, which is due to the type of storage and observance of hygienic conditions, especially in winter is necessary (Xiong et al., 2021). AFM1 exposure through yogurt consumption and the risk of liver cancer were studied in Hamedan, Iran. Although it was found that a high percentage of yogurt samples in Iran were contaminated with AFM1 content, there was no particular concern about the risk to public health according to European standards (EC) and the Iranian Institute for Standards and Industrial Research (ISIRI) (Heshmati et al., 2020).

Conclusions
In this study, AFM1 concentration in milk samples (traditional 60 samples) and industrial milk (from 15 brands, b–p) was measured. Also, the AFM1 concentration in two seasons of the year (autumn and winter) was measured. According to the results, approximately 45% of traditional and industrial milk samples were contami- nated with AFM1 more than the standard level (EU and Codex). Aflatoxin levels in autumn were higher than in winter, but P-value was not significant. Moreover, the amount of AFM1 in samples of traditional milk was higher than that of industrial. The non-carcinogenic risk of exposure to AFM1 showed that HQ was higher than 1 for adults and children, indicated that milk consumers in Tiran County are at considerable risk of developing liver cancer-related AFM1.

Recommendation
It is also important to enforce strict rules and more supervision in livestock farms and production centers. The most important idea to reduce the exposure of AFM1 is to prevent the production of AFB1 in the fields, and the best solution is to secure farms, industrial and traditional livestock farms, educate people about the dangers and appropriate ways to store livestock feeds, the type of food that is used, and improving their storage environment to reduce AFB1. A broader and more detailed examination of dairy products produced in various regions of Iran during different periods and four seasons is needed to achieve more accurate results.

Disclosure statement
No potential conflict of interest was reported by the author(s).

References
Jafari, K., Mohammadi, A.A., Heidari, Z., Asghari, F.B., Radfar, M., Yousefi, M., et al., 2018. Data on microbiological quality in ...


