Aflatoxin M1 in milk and dairy products – A mini review

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Abstract
Aflatoxins (AF) are a group of toxic secondary metabolites mostly produced by strains of Aspergillus spp. fungi. There are several types of aflatoxins (AFB1, AFG1, AFB2, AFG2, AFM1, AFM2, etc.), but the most toxic and the most common is AFB1. Aflatoxins have been shown to possess powerful carcinogenic, mutagenic and teratogenic properties. AFB1 can contaminate feed and food during the growth and/or storage under inappropriate conditions. When animals consume AFB1-contaminated feed, it undergoes various metabolic pathways and finally, it is metabolized into hydroxylated metabolite AFM1, which is possibly excreted in milk if the animal is lactating. This issue presents a huge concern regarding the representation of milk and dairy products in the human diet. Furthermore, climate changes have a significant impact on aflatoxin production. Therefore, it is necessary to develop and improve strategies for controlling and mitigating the occurrence of AFM1 in milk and dairy products. The aim of this paper is to provide an overview of the latest scientific literature regarding the occurrence of AFM1 in milk and dairy products.

Introduction
Aflatoxins (AF) are products of fungal secondary metabolism, mainly of Aspergillus spp., mostly of A. flavus and A. parasiticus. They are low molecular weight compounds, toxic at low concentrations and have been shown to possess powerful carcinogenic properties in different animal species (Venancio et al., 2018). They were discovered in the 1960's in England when the “Turkey X disease” caused the death of a huge number of turkeys and ducklings. Actually, feeding animals with peanut flour led to the mentioned “Turkey X disease” since peanuts were contaminated with A. flavus. There are different types of aflatoxins (B1, B2, G1, G2, M1, M2, etc.), but AFB1 is the most common and the most toxic (Varga et al., 2020). Moreover, AFB1 is the strongest natural carcinogen. It is commonly known today that the occurrence of aflatoxins in food and feed varies with climatic conditions. Hot and humid environments have a favourable impact on the growth of A. flavus and biosynthesis of aflatoxins during growth and storage. Aflatoxin biosynthesis pathway is step-by-step discussed in detail by Kovač et al. (2018a; 2018b; 2020a). However, when AFB1 is produced by fungi and when ruminants consume contaminated feed, their liver metabolizes the AFB1 into hydroxylated aflatoxin - AFM1 which, if the animal is lactating, can pass into milk. Since milk and dairy products have a great role in the human diet, it is important to raise awareness of the health threats to all actors in the dairy value chain (Serraiono et al., 2019; Bukari et al., 2020; Djekic et al., 2020). Accordingly, the aim of this review paper is to provide a brief insight into the latest scientific research of the AFM1 occurrence in milk.

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Aflatoxins

Aflatoxins are toxic secondary metabolites mostly produced by strains of *A. flavus* and *A. parasiticus* that can contaminate a wide range of goods. The name “Aflatoxin” is composed of “A” from the genus *Aspergillus*, “fla” from species *flavus* and the noun “toxin”. There are 18 different groups of aflatoxins, but the majority are AFB1, AFG1, AFB2, AFG2, AFM1 and AFM2 (Table 1) (Saleem et al., 2017). The most toxic of all is AFB1 and it is classified as a human carcinogen, the most potent natural hepatocarcinogen (Kovač et al., 2017). Aflatoxins are slightly soluble in water and insoluble in nonpolar solvents. They are stable at high temperatures (even at >100 °C), but they decompose during exposure to UV light (Marchese et al., 2018).

AFM1 is a 4-hydroxylated metabolite of the most toxic aflatoxin - AFB1 (Figure 1) (Roila et al., 2021). It is found in the milk of mammals in areas of high aflatoxin exposure and in dairy products. Also, it is detected in lactating mother’s milk (Jafari et al., 2017). Considering the huge prevalence of milk and dairy products in the human diet, especially in infants, and its stability during heat treatments, AFM1 is of great concern and risk for human health (Marchese et al., 2018; Raters and Matissek, 2008).

Table 1. Chemical structure, CAS number, molecular formula and molecular weight of aflatoxins B1, B2, G1, G2, M1 and M2

<table>
<thead>
<tr>
<th>Name</th>
<th>CAS number</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B1 (AFB1)</td>
<td>1162-65-8</td>
<td>C₁₇H₁₂O₆</td>
<td>312.3 g/mol</td>
</tr>
<tr>
<td>Aflatoxin B2 (AFB2)</td>
<td>7220-81-7</td>
<td>C₁₇H₁₄O₆</td>
<td>314.3 g/mol</td>
</tr>
<tr>
<td>Aflatoxin G1 (AFG1)</td>
<td>1165-39-5</td>
<td>C₁₇H₁₂O₇</td>
<td>328.3 g/mol</td>
</tr>
<tr>
<td>Aflatoxin G2 (AFG2)</td>
<td>7241-98-7</td>
<td>C₁₇H₁₄O₇</td>
<td>330.3 g/mol</td>
</tr>
<tr>
<td>Aflatoxin M1 (AFM1)</td>
<td>6795-23-9</td>
<td>C₁₇H₁₂O₇</td>
<td>328.3 g/mol</td>
</tr>
<tr>
<td>Aflatoxin M2 (AFM2)</td>
<td>6885-57-0</td>
<td>C₁₇H₁₂O₇</td>
<td>330.3 g/mol</td>
</tr>
</tbody>
</table>

Factors affecting aflatoxin production

There are many factors that are important for *Aspergillus* spp. fungi growth and aflatoxin production. Contamination can appear at any step of food production, from pre-harvest to storage. Factors such as meteorological conditions, environmental factors and agricultural practices play a huge role in aflatoxin contamination of crops (Marchese et al., 2018; Seid and Mama, 2019; Kovač et al., 2022). It is identified that the risk of aflatoxin contamination is higher in geographical locations with tropical or subtropical climate (Fakhri et al., 2019). Extreme high temperatures and the lack of the rainfall may cause the growth of *A. flavus* and increase the production of aflatoxins (Serraino et al., 2019). The impact of climate change has been recognized as an emerging issue for food and feed safety, due to the expected temperature increase.
It is suggested that the probable scenario of +2 °C environmental temperature change in Eastern Europe, the Balkan Peninsula and Mediterranean regions for the next 100 years can contribute to increased mycotoxin contamination (Battilani et al., 2012; 2016). The level of aflatoxin contamination is also related to THE stress or damage of the crop (Magnussen and Parsi, 2013). The optimal temperature for aflatoxin production is 25°C – 35°C. An acidic pH, relative humidity between 83 % and 88 % and suitable levels of CO₂ and O₂ are favourable conditions for aflatoxin biosynthesis. For example, 20 % of CO₂ and 10 % of O₂ inhibit aflatoxin production. Some metals (manganese and zinc) and some carbon sources, such as glucose, sucrose or fructose have a huge role in aflatoxin synthesis (Seid and Mama, 2019).

**Toxicity and health risk of aflatoxins**

Since the appearance of “Turkey X disease”, aflatoxins have become the focus of various studies. They have a carcinogenic effect on mice, fish, rats, ducks, shrews and monkeys (Bbosa et al., 2013; Monson et al., 2015). Initially, AFM1 was classified as a group 2B human carcinogen by International Agency for Research on Cancer (IARC), but later it was reclassified as a group 1 human carcinogen (IARC, 2012). Even though AFM1 is about 10 times less harmful than AFB1, it presents a risk for animal and human health and it is very important to control its presence in milk and dairy products. Diseases caused by aflatoxins are named aflatoxicosis and can be acute or chronic. Acute aflatoxicosis are a result of ingestion of medium to high levels of aflatoxins. Acute aflatoxicosis may cause different symptoms, such as bleeding, liver damage, digestive disorders, etc. Furthermore, the consequence of acute aflatoxicosis can be death. Chronic aflatoxicosis include teratogenic effects related to congenital malformations, mutagenic effects (mutations on the genetic code, damaging of the DNA) and carcinogenic effect (Seid and Mama, 2019; Wu and Khlangwiset, 2010; Bbosa et al., 2013). AFB1 is also known as the most potent hepatotoxic mycotoxin. Depending upon the dose of aflatoxin, it can lead to liver damage such as fatty and pale liver, necrosis and haemorrhage (Bbosa et al., 2013). In India in 1974, an outbreak of hepatitis happened and about 100 people died. The reason for this tragedy is related to the consumption of maize containing A. flavus. In fact, AFB1 was found in high concentrations in the liver of people who died (Krishnamachari et al., 1975). Wogan and Newberne (1967) reported that semi-synthetic food contaminated with AFB1 at different levels caused hepatocellular carcinoma. Namely, the level of AFB1 of 15 μg/kg caused a liver cell carcinoma in 25/25 rats (all 12 males tested during 68 weeks and all 13 females tested during 80 weeks). Carnaghan (1965) reported that tumors were induced in 8 of 11 duck in 14 months when fed AFB1 at level of 30 μg/kg. Oettle et al. (1965) suggested that aflatoxin has an impact on the development of liver cancer in humans. Some studies in Africa and Southeast Asia showed a correlation between the levels of aflatoxin intake and liver cancer in human population (Pitt, 2000). AFM1 has about 2 - 10 % of the carcinogenic influence of AFB1 and almost the same liver toxicity as AFB1 (Peng and Chen, 2009).

**Figure 1.** 3D rendering of the aflatoxin M1 structure (Granados-Chinchilla, 2016)

**Biosynthesis (conversion of AFB1 into AFM1)**

General metabolism pathways of ingested AFB1 are shown in Figure 2. Consumption of contaminated feed by animals causes part of the ingested AFB1 to be transformed by ruminal microorganisms to aflatoxicol. The remaining AFB1 is very quickly absorbed in the small intestines because of its low molecular weight (Masoero et al., 2007). This is followed by biotransformation of AFB1 in the liver when AFB1 undergoes reduction, epoxidation, hydroxylation and demethylation. There are different products of AFB1 metabolism in the liver, depending on metabolic pathways, but they are all toxic (Min et al., 2021). AFM1 represents about 95 % of the aflatoxins found in milk while other metabolites are detected in trace amounts (Giovati et al., 2015). AFM1 can be transported with the bloodstream to the mammary gland and secreted into milk (Min et al., 2021). According to Bukari et al. (2020), about 0.3 % - 6.2 % of AFM1 can be excreted in milk, if the animal is lactating. Also, the presence of AFM1 in milk can be detected 12-24 hours after consuming feed contaminated with AFB1. Furthermore, the AFM1 concentration decreases 72 hours after consuming contaminated feed.
Occurrence of AFM1 in milk and dairy products

AFM1 in milk and dairy products has been documented worldwide, particularly in developing countries. Analysis of AFM1 in milk and dairy products can be performed by using a variety of methods/techniques, such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and enzyme-linked immunosorbent assay (ELISA). However, ELISA is most frequently used because it is simple to use and is highly sensitive to the detection and quantification of AFM1 (Ismail et al., 2015). The occurrence of the AFM1 in raw milk is shown in Table 2. Also, the occurrence of this toxin in heat-treated milk and some dairy products is shown in Table 3.

Occurrence of AFM1 in milk

Due to its nutritive value, milk is highly represented food in the human diet, and it is especially for infants. However, a large number of studies reported the occurrence of AFM1 in milk, which is mostly the consequence of feeding animals with feed that contains AFM1. AFM1 was found even in human milk (Varga et al., 2020). According to Nile et al. (2016), cow's milk has the highest concentration of AFM1 compared to sheep, goat, and buffalo milk. In addition, it is documented that season variation and geographical location can be related to AFM1 concentration in milk, and therefore, in dairy products. A large number of studies reported that detected concentration of AFM1 in milk and dairy products is higher during winter compared to other seasons (Tajkarimi et al., 2007; Dashti et al., 2009; Akbar et al., 2019; Ismaiel et al., 2020). The results of these variables results can be explained by the fact that during summer, fresh animal feed (for example pasture, grass, fodder) is available. During winter, animals are fed with stored cereals and fodder, which may be attacked by Aspergillus spp. fungi if they are inadequately stored (Iqbal et al., 2015). According to Dashgi et al. (2009), the difference between AFM1 contamination in winter and summer milk is also related to numerous factors such as temperature, relative humidity, and seasonal effects from the country of origin of the feed. However, Venancio et al. (2019) reported that there was no difference between levels of AFM1 in milk in winter and summer months in subtropical and temperate climate.
Occurrence of AFM1 in fermented milk products

It has been reported that the level of AFM1 is usually decreased in yoghurt as compared to that found in milk used for its production. This might be associated with the lactic acid bacteria (Fallah, 2010; Khoury et al., 2011), the low pH of the yoghurt, and the formation of organic acids (Nilchian and Rahimi, 2012). Several studies present the ability of lactic acid bacteria (LAB) that are used in milk fermentation to bind AFM1 and reduce milk contamination (Sanli et al., 2012; El-Kest et al., 2015; Barukčić et al., 2018; Kuboka et al., 2019). El-Kest et al. (2015) reported that using two strains of LAB (Lactobacillus acidophilus and Bifidobacterium lactis) had an impact on decreasing AFM1 levels for 72 h during cold storage, with complete elimination by the end of the process. Elsanhoty et al. (2014) also reported that LAB are able to reduce levels of AFM1 and that this property is enhanced by heat treatment. Barukčić et al. (2018) conducted research with a focus on changes in AFM1 levels during the production and storage of fermented kinds of intentionally contaminated milk using selected probiotic and non-probiotic combined cultures. Namely, the result of this research was a significant decrease in AFM1 concentration in almost all of fermented milk, except the kefir culture XPL. However, Oruc et al. (2007) reported that the use of LAB did not influence the levels of AFM1 in Kashar cheese.

Occurrence of AFM1 in cheese

There are three possible reasons for contamination of cheese with AFM1: i) Milk used for cheese production is contaminated with AFM1 due to animal feeding with AFB1 contaminated feed; ii) growth of fungi (A. flavus and A. parasiticus) on cheese and their production of aflatoxins; iii) using AFM1 contaminated dried milk for enriching the milk from which it is produced (Dursanaki and Miki, 2013). There are some studies about the distribution and
stability of AFM1 during the production of various types of cheeses. According to Bakirci (2001), Oruc et al. (2007), Manetta et al. (2009), Krstović et al. (2018) and Pecorelli et al. (2020), the concentration of AFM1 is higher in cheese than in milk used for cheese production. This might be due to its semi-polar characteristics and high affinity to casein (Pecorelli et al., 2018). On the contrary, the results from other authors such as Elgerbi et al. (2004), Chavarría et al. (2013) or Einolghozati et al. (2021) indicated that AFM1 levels in cheese products were lower compared to the raw milk used for the manufacturing, possibly due to fraction redistribution or microbiological degradation.

The stability of AFM1 during cheese ripening was also studied. According to Oruc et al. (2006) and Deveci (2007), there was no significant loss of AFM1 levels during three months ripening period of traditional White pickled cheese. However, Govaris et al. (2001) reported that AFM1 was present in cheese at higher concentrations at the beginning than at the end of the ripening/storage period. The differences in the AFM1 concentration in cheese that is produced from aflatoxin-contaminated milk can be attributed to multiple variables such as the type of cheese, water content, manufacturing technologies, degree and type of milk contamination (naturally or artificially), and THE applied analytical method (Manetta et al., 2009; Iha et al., 2013).

Table 3 AFM1 in heat-treated milk and some dairy products

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample</th>
<th>No. of samples</th>
<th>Positive samples (%)</th>
<th>Range (µg/kg)</th>
<th>Mean ± SD (µg/kg)</th>
<th>&gt;EU regulations (%)</th>
<th>Method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iran</td>
<td>Yoghurt</td>
<td>40</td>
<td>14 (35)</td>
<td>0.0114-0.1158</td>
<td>0.1305</td>
<td>-</td>
<td>ELISA</td>
<td>Nilchian and Rahima, 2012</td>
</tr>
<tr>
<td></td>
<td>Cheese</td>
<td>40</td>
<td>16 (40)</td>
<td>0.0319-0.5057</td>
<td>0.1332</td>
<td>-</td>
<td>ELISA</td>
<td>Duarte et al., 2013</td>
</tr>
<tr>
<td>Portugal</td>
<td>PM and UHT</td>
<td>40</td>
<td>11 (27.5)</td>
<td>NR</td>
<td>0.0234±0.024</td>
<td>0</td>
<td>ELISA</td>
<td>Iqbal et al., 2013</td>
</tr>
<tr>
<td>Punjaban, Pakistan</td>
<td>UHT (summer)</td>
<td>39</td>
<td>12 (31)</td>
<td>0.004-0.51</td>
<td>0.020±0.009</td>
<td>24</td>
<td>HPLC-FLD</td>
<td>Moosavv et al., 2013</td>
</tr>
<tr>
<td>Iran</td>
<td>PM</td>
<td>80</td>
<td>77 (96.3)</td>
<td>NR</td>
<td>0.0278±0.0249</td>
<td>16</td>
<td>ELISA</td>
<td>Riahi-Zanjani &amp; Balali-Mood, 2013</td>
</tr>
<tr>
<td>China</td>
<td>UHT</td>
<td>153</td>
<td>84 (54.9)</td>
<td>0.005-0.2</td>
<td>0.048±0.047</td>
<td>20.3</td>
<td>ELISA</td>
<td>Zheng et al., 2013</td>
</tr>
<tr>
<td>Turkey</td>
<td>Kasar cheese</td>
<td>40</td>
<td>20 (50)</td>
<td>0.05-0.70</td>
<td>0.13</td>
<td>-</td>
<td>ELISA</td>
<td>Bakirdere et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Tulum cheese</td>
<td>16</td>
<td>3 (18.8)</td>
<td>0.05±0.10</td>
<td>0.07</td>
<td>-</td>
<td>ELISA</td>
<td>Gul and Derisoglu, 2014</td>
</tr>
<tr>
<td></td>
<td>Dil cheese</td>
<td>22</td>
<td>3 (13.6)</td>
<td>0.10-0.20</td>
<td>0.15</td>
<td>-</td>
<td>ELISA</td>
<td>Temammogullari and Kanci, 2014</td>
</tr>
<tr>
<td></td>
<td>Cream cheese</td>
<td>21</td>
<td>8 (38.1)</td>
<td>0.05-0.16</td>
<td>0.09</td>
<td>-</td>
<td>ELISA</td>
<td>Rama et al., 2015</td>
</tr>
<tr>
<td></td>
<td>White cheese</td>
<td>67</td>
<td>36 (53.7)</td>
<td>0.05-2.10</td>
<td>0.28</td>
<td>-</td>
<td>ELISA</td>
<td>Sifuentes dos Santos et al., 2015</td>
</tr>
<tr>
<td></td>
<td>Kashar cheese</td>
<td>147</td>
<td>144 (98)</td>
<td>0.015-3.774</td>
<td>0.273±0.305</td>
<td>-</td>
<td>HPLC</td>
<td>Armorini et al., 2016</td>
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<td></td>
<td>White pickled cheese</td>
<td>50</td>
<td>50 (100)</td>
<td>0.04041-0.1389</td>
<td>0.1032±0.02913</td>
<td>-</td>
<td>ELISA</td>
<td>Deeb et al., 2016</td>
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<td>Kosova</td>
<td>PM</td>
<td>84</td>
<td>70 (83.3)</td>
<td>0.00516-0.11093</td>
<td>0.0324±0.0274</td>
<td>21.4</td>
<td>ELISA</td>
<td>Omar, 2016</td>
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<td></td>
<td>UHT</td>
<td>94</td>
<td>74 (78.7)</td>
<td>0.00502-0.06226</td>
<td>0.0214±0.0139</td>
<td>4.2</td>
<td>ELISA</td>
<td>Tajik et al., 2016</td>
</tr>
<tr>
<td>Brazil</td>
<td>PM</td>
<td>7</td>
<td>7 (100)</td>
<td>0.01±0.03</td>
<td>0.02±0.01</td>
<td>28.6</td>
<td>HPLC</td>
<td>Omas et al., 2016</td>
</tr>
<tr>
<td></td>
<td>UHT</td>
<td>28</td>
<td>28 (100)</td>
<td>0.01±0.08</td>
<td>0.04±0.02</td>
<td>71.4</td>
<td>HPLC-FLD</td>
<td>Abou et al., 2016</td>
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<tr>
<td>Egypt</td>
<td>Soft cheese</td>
<td>90</td>
<td>46 (51.1)</td>
<td>0.050-0.0970</td>
<td>0.071</td>
<td>-</td>
<td>ELISA</td>
<td>Rama et al., 2015</td>
</tr>
<tr>
<td>Jordan</td>
<td>PM</td>
<td>30</td>
<td>30 (100)</td>
<td>0.01460-0.21678</td>
<td>0.05945±0.04212</td>
<td>73</td>
<td>ELISA</td>
<td>Tajik et al., 2016</td>
</tr>
<tr>
<td></td>
<td>Infant formula</td>
<td>20</td>
<td>20 (100)</td>
<td>0.01655-0.15414</td>
<td>0.1202±0.03354</td>
<td>85</td>
<td>ELISA</td>
<td>Omas et al., 2016</td>
</tr>
<tr>
<td></td>
<td>Full cream powdered milk</td>
<td>15</td>
<td>15 (100)</td>
<td>0.0180-0.28868</td>
<td>0.10395±0.07656</td>
<td>-</td>
<td>ELISA</td>
<td>Omar, 2016</td>
</tr>
<tr>
<td></td>
<td>Evaporated milk</td>
<td>10</td>
<td>10 (100)</td>
<td>0.14939-0.26882</td>
<td>0.1959±0.03472</td>
<td>-</td>
<td>ELISA</td>
<td>Omas et al., 2016</td>
</tr>
<tr>
<td>Iran</td>
<td>PM</td>
<td>220</td>
<td>187 (85)</td>
<td>0.0054-0.51222</td>
<td>0.076±0.0084</td>
<td>70</td>
<td>ELISA</td>
<td>Tajik et al., 2016</td>
</tr>
<tr>
<td>Brazil</td>
<td>PM (Organic)</td>
<td>15</td>
<td>8 (53.3)</td>
<td>ND-0.061</td>
<td>0.015±0.019</td>
<td>4.8</td>
<td>ELISA</td>
<td>Omar, 2016</td>
</tr>
</tbody>
</table>
Legislation

Due to its toxicity and the expected impact of climate change on the presence of mycotoxins in food and feed, the occurrence of AFM1 in milk and dairy products presents a major risk for consumer’s health, but it is almost impossible to remove it from the human and animal diet. For this reason, most countries around the world have established regulatory limits (maximum permitted level (MPL)) of AFM1 in milk and dairy products and some of them are shown in Table 4. It is important to mention that these limits are not unique for all countries and that some countries established their own limit values for milk-based products. For example, the European Commission (EC) has set the MPL of 0.050 μg/kg AFM1 in milk (raw milk, heat-treated milk and milk for the manufacture of milk-based products) and 0.025 μg/kg for milk products for infants (EC No. 1881/2006). The MPL for AFM1 prescribed by the Food and Drug Administration of the United States of America is 0.5 μg/kg in milk and dairy products. Also, some countries, such as Egypt and Romania established that fluid milk and dairy products should be free from AFM1, while some countries including Jordan have no legal limit for AFM1 in milk and dairy products (Giovati et al., 2015; Omar, 2016; Bukari et al., 2020; Varga et al., 2020).

Strategies for preventing and mitigating AFM1 occurrence in milk

Given that human and animal exposure to aflatoxins is unavoidable, it is necessary to establish strategies for preventing and mitigating aflatoxin contamination. These strategies should be integrated in all stages of crop production from the field (pre-harvest) to the table; including storage (post-harvest). These also include physical or chemical decontamination/detoxification of feed and milk (Giovati et al., 2015). Recent research aimed at the development and improvement of technologies with a
focus on efficiency and some of them are shown in Table 5. Adsorption seems to be the most effective, promising approach for AFM1 decontamination of milk and dairy products. However, there is still a need for a more practically applicable approach and further studies may contribute to the development of commercially valid techniques (Muaz et al., 2021).

Table 4. Maximum permitted levels (MPL) of AFM1 in milk and dairy products

<table>
<thead>
<tr>
<th>Country</th>
<th>Milk (μg/kg)</th>
<th>Dairy products (μg/kg)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>0.05</td>
<td>0.50</td>
<td>Iqbal et al., 2015; Vaz et al., 2020</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.25 (cheese)</td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>0.01 (pasteurized infant milk)</td>
<td>0.25 (cheese)</td>
<td>Iqbal et al., 2015; Vaz et al., 2020</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.40 (milk powder)</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>0.50</td>
<td>5.00 (milk powder)</td>
<td>Iqbal et al., 2015; Vaz et al., 2020</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>0.50</td>
<td>0.10 (milk powder)</td>
<td>Iqbal et al., 2015; Vaz et al., 2020</td>
</tr>
<tr>
<td>China</td>
<td>0.50</td>
<td>0.50</td>
<td>Vaz et al., 2020; Bukari et al., 2020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.10 (yoghurt and fruit yoghurt)</td>
<td></td>
</tr>
<tr>
<td>Croatia</td>
<td>0.050</td>
<td>0.35 (milk powder)</td>
<td>Dairy products: Naredba o privremenim mjerama u odnosu na sadržaj AFM1 u mliječnim proizvodima (NN 39/2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.45 (hard cheese)</td>
<td></td>
</tr>
<tr>
<td>Egypt</td>
<td>0</td>
<td>0</td>
<td>Iqbal et al., 2015; Vaz et al., 2020</td>
</tr>
<tr>
<td>EU</td>
<td>0.050</td>
<td>0.025 (infant formulae and follow-on formulae, including infant milk and follow-on milk)</td>
<td>Commission Regulation (EC) No 1881/2006 of 19 December 2006</td>
</tr>
<tr>
<td>France</td>
<td>0.03</td>
<td></td>
<td>Iqbal et al., 2015; Vaz et al., 2020</td>
</tr>
<tr>
<td></td>
<td>(for children &lt;3 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Honduras</td>
<td>0.05</td>
<td>0.25 (cheese)</td>
<td>Iqbal et al., 2015; Vaz et al., 2020</td>
</tr>
<tr>
<td>Italy</td>
<td>0.05</td>
<td>0.25 (soft cheese)</td>
<td>Vaz et al., 2020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.45 (hard cheese)</td>
<td></td>
</tr>
<tr>
<td>Nigeria</td>
<td>1</td>
<td></td>
<td>Iqbal et al., 2015; Vaz et al., 2020</td>
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<tr>
<td>Romania</td>
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<td>0</td>
<td>Iqbal et al., 2015; Vaz et al., 2020</td>
</tr>
<tr>
<td>Switzerland</td>
<td>0.05</td>
<td>0.02 (butter)</td>
<td>Iqbal et al., 2015; Vaz et al., 2020</td>
</tr>
<tr>
<td>Turkey</td>
<td>0.05</td>
<td>0.25 (cheese)</td>
<td>Iqbal et al., 2015; Vaz et al., 2020</td>
</tr>
<tr>
<td>USA</td>
<td>0.50</td>
<td>0.50</td>
<td>Iqbal et al., 2015; Vaz et al., 2020</td>
</tr>
<tr>
<td>Serbia</td>
<td>0.50</td>
<td></td>
<td>Kos et al., 2014; Bukari et al., 2020</td>
</tr>
</tbody>
</table>

Table 5. AFM1 prevention and mitigation methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Principle</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological</td>
<td>Reducing AFB1 contamination of feed, during the crop growth and during the post-harvest storage; indirectly reducing AFM1 contamination of milk by using different organisms such as bacteria, yeasts, and non-aflatoxigenic Aspergillus strains. Hand sorting (removing of grains that are infected with fungi) Sorting by size and density (removing broken grains). Dehulling</td>
<td>Shetty and Jespersen, 2006; Yin et al., 2008; Ehrlich et al., 2015; Giovati et al., 2015</td>
</tr>
<tr>
<td>Physical</td>
<td>Heat treatments (efficiency depends on time and temperature conditions) Instant Catapult Steam Explosion (ICSE) Treatment with gamma irradiation, using an ultrasound, cold or nonthermal plasma UV radiation Using of organic or inorganic acids (citric, lactic, tartaric, propionic, hydrochloric acid), bases and bisulfitic oxidizing agents</td>
<td>Hassan and Hussein, 2017 Naeimipour et al., 2018; Sipos et al., 2021 Xie et al., 2020</td>
</tr>
<tr>
<td>Chemical</td>
<td>Ozone treatment Ammoniation</td>
<td>Luo et al., 2014; Savi et al., 2014 Naeimipour et al., 2018</td>
</tr>
</tbody>
</table>
Conclusions

AFM1 is a hydroxylated metabolite of the AFB1 and it has an impact on animal and human health. AFM1 has been demonstrated to have teratogenic, mutagenic and carcinogenic properties. It is obvious that prevalence of AFM1 in milk is high worldwide, especially in developing countries, and this presents a huge concern since milk and dairy products have been an important part of the human diet. Available evidence shows that winter milk contains higher levels of AFM1 than summer milk. Apart from the adverse health outcomes, the occurrence of AFM1 in milk has a negative economic influence. Therefore, it is crucial to continue to monitor and modulate maximum permitted levels of aflatoxins and strategies for prevention and control of AF contamination of food and feed in order to increase food and feed safety, especially due to the present period of climate change.

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References


Food Control 21, 1478–81. https://doi.org/10.1016/j.foodcont.2010.04.017


Nareda o privremenim mjerama u odnosu na sadržaj AFM1 u mliječnim proizvodima (NN 39/2013) on the Web. 2013. https://sredisnjikatalogrh.gov.hr/cadial/searchdoc.php?action=search&lang=hr&query=Naredba+o+privremenim+mjerama+u+odnosu+na+sadr%5C5BE+AFM1+u+mlije%C4%8Dnim+proizvodima+%26+searchTitle=on&searchTitle=off&searchExt=on&searchExt=on&resultdetails=basic&bid=8zMenim+mjerama+u+odnosu+na+sadr%C5%BEaj+A


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