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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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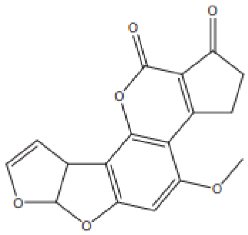
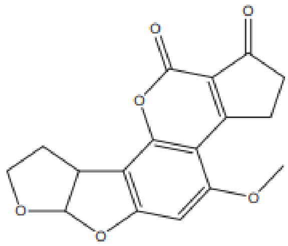
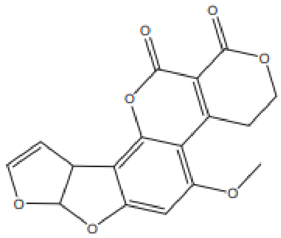
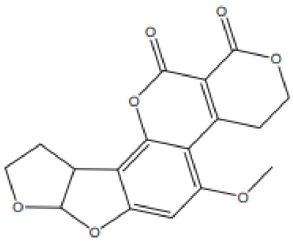
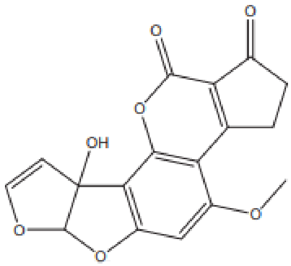
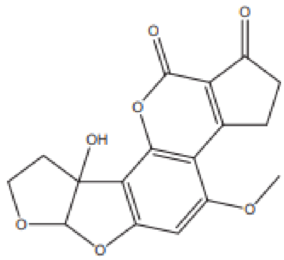
Aflatoxins

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 (Adopted from Schrenk et al., 2020)

Name	Aflatoxin B1 (AFB1)	Aflatoxin B2 (AFB2)	Aflatoxin G1 (AFG1)
Structure			
CAS number	1162-65-8	7220-81-7	1165-39-5
Molecular formula	$C_{17}H_{12}O_6$	$C_{17}H_{14}O_6$	$C_{17}H_{12}O_7$
Molecular weight	312.3 g/mol	314.3 g/mol	328.3 g/mol
Name	Aflatoxin G2 (AFG2)	Aflatoxin M1 (AFM1)	Aflatoxin M2 (AFM2)
Structure			
CAS number	7241-98-7	6795-23-9	6885-57-0
Molecular formula	$C_{17}H_{14}O_7$	$C_{17}H_{12}O_7$	$C_{17}H_{14}O_7$
Molecular weight	330.3 g/mol	328.3 g/mol	330.3 g/mol

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 growthof *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 CO_2 and O_2 are favourable conditions for aflatoxin biosynthesis. For example, 20 % of CO_2 and 10 % of O_2 inhibit aflatoxin production. Some metals (manganese and zink) and some carbon sources, such as glucose, sucrose or fructose have a huge role in aflatoxin synthesis (Seid and Mama, 2019).

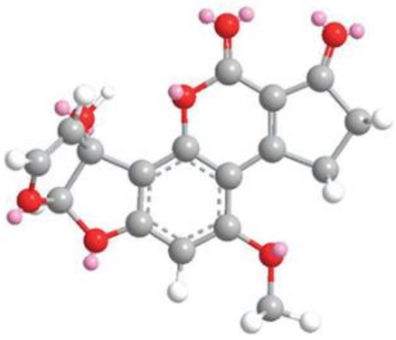


Figure 1. 3D rendering of the aflatoxin M1 structure (Granados-Chinchilla, 2016)

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 decreases72 hours after consuming contaminated feed.

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).

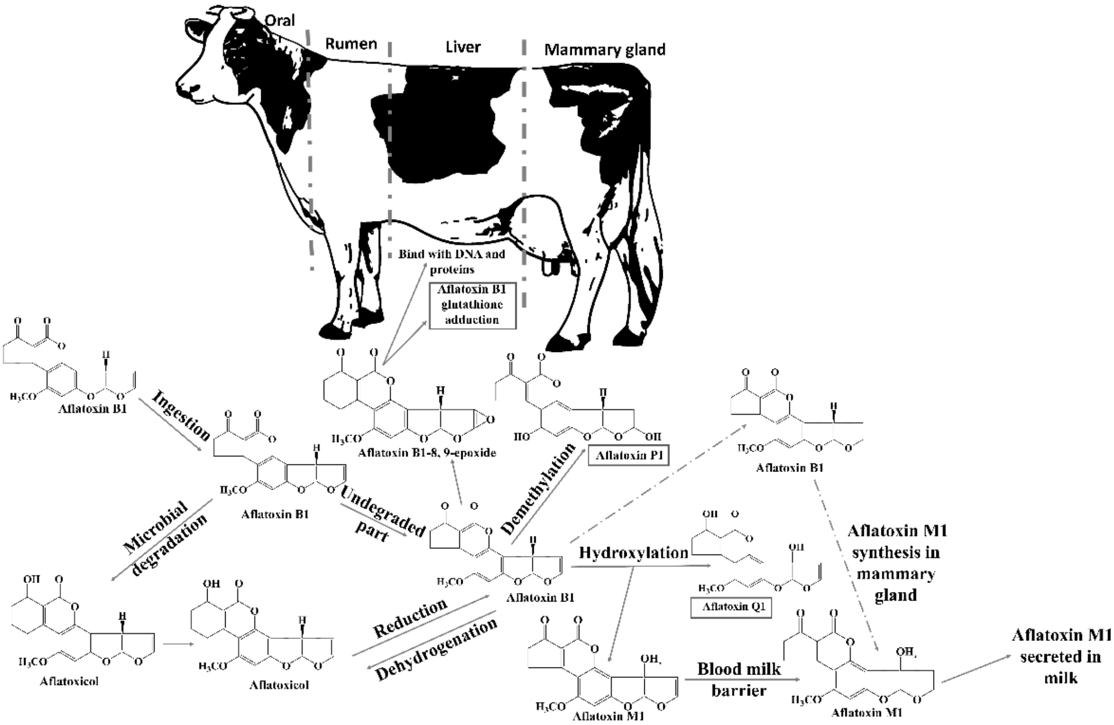


Figure 2. The metabolism pathways of AFB1 in lactating dairy cows (adopted but modified from Min et al., 2021). Dashed box, detoxification pathways in dairy cows. Dotted arrow, metabolic pathway needs to further validation.

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 it 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 AFB1. 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 Dashti 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.

Table 2. AFM1 in raw milk

Country	Season	No. of samples	Positive samples (%)	Range (μg/kg)	Mean ± SD (μg/kg)	>EU regulation (%)	Method	Reference
China	NR	200	45 (22.5)	0.0052-0.0596	0.0153	NR	ELISA	Han et al., 2013
China	Spring	18	14 (77.8)	0.011-0.098	0.0291±0.0226	5.6	LC-MS/MS	Xiong et al., 2013
	Summer	18	8 (44.4)	0.011-0.082	0.0319±0.0267	11.1		
	Autumn	18	5 (27.8)	0.016-0.076	0.0316-0.0253	5.6		
	Winter	18	16 (88.9)	0.010-0.420	0.1236±0.101	72.2		
Croatia (Eastern part)	Summer	194	194 (100)	0.00365-0.1623	0.0206±0.0188	6.7	ELISA	Bilandžić et al., 2014b
Croatia (Other regions)	Summer	143	143 (100)	0.00269-0.0449	0.0121±0.00949	0		
Turkey	Autumn	63	20 (31.8)	0.042-0.552	0.082	30	HPLC-FD	Golge, 2014
	Winter	47	19 (40.4)	0.033-1.101	0.275			
	Spring	33	11 (33.3)	0.047-0.150	0.099			
	Summer	33	3 (9.1)	0.025-0.102	0.055			
Serbia	NR	40	NR	0.005-0.90	NR	75	ELISA	Kos et al., 2014
Iran	Winter	40	40 (100)	0.079-0.205	0.170	100	HPLC	Rezaei et al., 2014
Serbia	Winter	280	NR	≤0.025->1	0.358±0.383	74.2	ELISA	Tomašević et al., 2015
	Spring	212	NR	≤0.025->1	0.375±0.382	66.5		
	Summer	122	NR	≤0.025-0.5	0.039±0.038	15.5		
	Autumn	64	NR	≤0.025-1	0.103±0.178	21.8		
Brazil	NR	635	334 (52.6)	0.012-0.725	0.021±0.06	10.1	HPLC-FD	Nappi Santili et al., 2015
Iran	Winter	35	35 (100)	0.061-0.296	0.122±0.08851	15	ELISA	Dakhili et al., 2016
	Summer	35	30 (85.7)	0.011-0.099	0.053±0.0252			
Jordan	NR	50	50 (100)	0.00971-0.12979	0.06891±0.02315	60	ELISA	Omar, 2016
Punjab, Pakistan	Winter	240	NR	NR	0.875±0.406	NR	ELISA	Akbar et al., 2019
	Autumn	160	NR	NR	0.751±0.148			
	Spring	160	NR	NR	0.654±0.037			
	Summer	400	NR	NR	0.455±0.052			
Kenya	NR	96	96 (100)	0.0154-4.563	0.290±0.6634	66.4	ELISA	Kuboka et al., 2019
China	NR	133	100 (75.2)	0.0053-0.0362	0.0159±0.0071	0	ELISA	Xiong et al., 2020

SD= standard deviation; TLC= thin layer chromatography; HPLC= high performance liquid chromatography; ELISA= enzyme-linked immunosorbent assay; HPLC-FD= high performance liquid chromatography with fluorescence detection; LC-MS/MS= Liquid Chromatography with tandem mass spectrometry

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 Kasharcheese.

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 (Darsanaki and Miri, 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. (2015) 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

Location	Sample	No. of samples	Positive samples (%)	Range (µg/kg)	Mean ± SD (µg/kg)	>EU regulations (%)	Method	References
Iran	Yoghurt	40	14 (35)	0.0114-0.1158	0.1305	-	ELISA	Nilchian and Rahimi, 2012
	Cheese	40	16 (40)	0.0319-0.5057	0.1332	-		
Portugal	PM and UHT	40	11 (27.5)	NR	0.0234±0.024	5	ELISA	Duarte et al., 2013
Punjab, Pakistan	UHT (winter)	45	23 (51)	0.004-0.51	0.060±0.009	24	HPLC-FLD	Iqbal et al., 2013
	UHT (summer)	39	12 (31)	0.004-0.51	0.021±0.007	23		
Iran	PM	80	77 (96.3)	NR	0.0278±0.0249	16	ELISA	Moosavy et al., 2013
Iran	PM	45	45 (100)	0.0088-0.064	NR	28.88	ELISA	Riahi-Zanjani & Balali-Mood, 2013
China	UHT	153	84 (54.9)	0.005-0.2	0.048±0.047	20.3	ELISA	Zheng et al., 2013
Turkey	Kasar cheese	40	20 (50)	0.05-0.70	0.13	-	ELISA	Bakirdere et al., 2014
	Tulum cheese	16	3 (18.8)	0.05-0.10	0.07	-		
	Dil cheese	22	3 (13.6)	0.10-0.20	0.15	-		
	Cream cheese	21	8 (38.1)	0.05-0.16	0.09	-		
	White cheese	67	36 (53.7)	0.05-2.10	0.28	-		
Croatia	UHT	706	NR	0.00398-0.1835	0.0691±0.0215	9.64	ELISA	Bilandžić et al., 2014a
Turkey	Kashar cheese	147	144 (98)	0.015-3.774	0.273±0.305	-	HPLC	Gul and Dervisoglu, 2014
Turkey	White pickled cheese	50	50 (100)	0.04041-0.13089	0.1032±0.02913	-	ELISA	Temamogullari and Kanici, 2014
Kosovo	PM	84	70 (83.3)	0.00516-0.11093	0.0324±0.0274	21.4	ELISA	Rama et al., 2015
	UHT	94	74 (78.7)	0.00502-0.06226	0.0214±0.0139	4.2		
Brazil	PM	7	7 (100)	0.01-0.03	0.02±0.01	28.6	ELISA	Sifuentes dos Santos et al., 2015
	UHT	28	28 (100)	0.01-0.08	0.04±0.02	71.4		
Italy	UHT	31	18 (58.1)	0.009-0.026	0.016±0.006	0	HPLC-FD	Armorini et al., 2016
Egypt	Soft cheese	90	46 (51.1)	0.050-0.0970	0.071	-	ELISA	Deeb et al., 2016
Jordan	PM	30	30 (100)	0.01460-0.21678	0.05945±0.04212	73	ELISA	Omar, 2016
	Infant formula	20	20 (100)	0.01655-0.15414	0.12026±0.03354	85		
	Full cream powdered milk	15	15 (100)	0.0180-0.28868	0.10395±0.07656	-		
	Evaporated milk	10	10 (100)	0.14939-0.26482	0.19591±0.03472	-		
Iran	PM	220	187 (85)	0.0054-0.5122	0.0762±0.0084	70	ELISA	Tajik et al., 2016
	UHT	140	93 (66.4)	0.0058-0.5084	0.0726±0.0072	54.2		
Brazil	PM (Organic)	15	8 (53.3)	ND-0.061	0.015±0.019	4.8	ELISA	

Location	Sample	No. of samples	Positive samples (%)	Range (μg/kg)	Mean ± SD (μg/kg)	>EU regulations (%)	Method	References
	PM (Conventional)	15	8 (53.3)	ND-0.064	0.021±0.020	2.4		Sifuentes dos Santos et al., 2016
Iran	PM	49	40 (81.6)	0.0033-0.0961	0.02330±0.01884*	8.6	ELISA	Sohrabi and Gharahkoli, 2016
	Yoghurt	18	15 (83.3)	0.0078-0.012	0.01025±0.0184*	-		
	White cheese	10	6 (60)	0.0058-0.0212	0.01586±0.00872*	-		
	Butter	10	10 (100)	0.0047-0.0167	0.01428±0.00732*	-		
Pakistan	UHT milk (summer)	25	16 (64)	0.0004-0.1908	0.0752±0.0039	32	HPLC-FD	Iqbal et al., 2017
	UHT milk (winter)	35	26 (74.2)	0.0004-0.3029	0.0985±0.0063	37.1		
Pakistan	UHT	30	30 (100)	0.010–0.345	0.164±0.113	66	ELISA	Ahmad et al., 2018
Italy	UHT	26	18 (69)	0.00071-0.00363	0.00164	0	online-SPE UHPLC-MS/MS	Campone et al., 2018
	PM	43	32 (74.4)	0.00085-0.04439	0.00345	0		
China	PM	131	120 (91.6)	0.005-0.3523	0.1374±0.1908	59.5	ELISA	Xiong et al., 2018
	UHT	111	58 (52.3)	0.005-0.0725	0.0224±0.013	1.8		
Iran	PM	63	55 (87.30)	<0.005-0.120	0.040±0.033	33.33	ELISA	Nejad et al., 2019
	UHT	25	21 (84)	<0.005-0.098	0.037±0.029	28		
Turkey	PM	35	35 (100)	NR	0.01286±0.00105	0	ELISA	Turkoglu and Keyvan, 2019.
	UHT	35	34 (97.14)	NR	0.02029±0.00277	8.57		
Iran	Cheese	22	22 (100)	0.0058-0.528	0.08281	-	ELISA	Abdali et al., 2020
	Ice-cream	22	22 (100)	0.0003-0.0711	0.02688	-		
	Yoghurt	10	4 (40)	0.060-0.220	0.049	-		
Morocco	UHT	40	14 (35)	0.0051-0.04442	0.01476±0.01021	0	HPLC-FLD	Alahlah et al., 2020
Lebanon	PM and UHT	11	10 (90.9)	0.013-0.219	0.069±0.068	54.5	HPLC-FLD	Daou et al., 2020
Iran	Yoghurt	50	43 (86)	<0.005-0.09865	0.02856±0.02639	-	ELISA	Heshmati et al., 2020
Yemen	Traditional cheese	68	52 (76.47)	0.023-0.994	0.23±0.299	-	ELISA	Abdullah Murshed et al., 2022
	Commercial cheese	47	38 (85.80)	0.020-0.998	0.25±0.266	-		

NR= not reported; PM= pasteurized milk, UHT= ultra-high temperature milk, ND= not detected; SD= standard deviation; *SEM= Standard error of the mean; HPLC= High-performance liquid chromatography; online-SPE UHPLC-MS/MS= online solid phase extraction (SPE) method coupled to ultrahigh-performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS); HPLC-FD= high performance liquid chromatography with fluorescence detection

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 ownlimit 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

Country	Milk (μg/kg)	Dairy products (μg/kg)	References
Argentina	0.05	0.50 0.25 (cheese)	Iqbal et al., 2015; Vaz et al., 2020
Austria	0.05 0.01 (pasteurized infant milk)	0.02 (butter) 0.25 (cheese) 0.40 (milk powder)	Iqbal et al., 2015; Vaz et al., 2020
Brazil	0.50	5.0 (milk powder)	Iqbal et al., 2015; Vaz et al., 2020
Bulgaria	0.50	0.10 (milk powder)	Iqbal et al., 2015
China	0.50	0.50	Vaz et al., 2020; Bukari et al., 2020
Croatia	0.050	0.10 (yoghurt and fruit yoghurt) 0.25 (fresh cheese, milk spreads and semi-hard cheese) 0.35 (milk powder) 0.45 (hard cheese)	Milk: Commission Regulation (EC) No 1881/2006 of 19 December 2006 Dairy products: Naredba o privremenim mjerama u odnosu na sadržaj AFM1 u mliječnim proizvodima (NN 39/2013)
Egypt	0	0	Iqbal et al., 2015; Vaz et al., 2020
EU	0.050	0.025 (infant formulae and follow-on formulae, including infant milk and follow-on milk)	Commission Regulation (EC) No 1881/2006 of 19 December 2006
France	0.05 0.03 (for children <3 years)	-	Iqbal et al., 2015; Vaz et al., 2020
Honduras	0.05	0.25 (cheese)	Iqbal et al., 2015; Vaz et al., 2020
Italy	0.05	0.25 (soft cheese) 0.45 (hard cheese)	Vaz et al., 2020
Nigeria	1	-	Iqbal et al., 2015; Vaz et al., 2020
Romania	0	0	Iqbal et al., 2015
Switzerland	0.05	0.25 (cheese) 0.02 (butter) 0.025 (milk whey and products)	Iqbal et al., 2015; Vaz et al., 2020
Turkey	0.05	0.25 (cheese)	Iqbal et al., 2015; Vaz et al., 2020
USA	0.50	0.50	Iqbal et al., 2015
Serbia	0.50	-	Kos et al., 2014; Bukari et al., 2020

Table 5. AFM1 prevention and mitigation methods

Method	**Principle**	**Reference**
Biological	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.	Shetty and Jespersen, 2006; Yin et al., 2008; Ehrlich et al., 2015; Giovati et al., 2015
Physical	Hand sorting (removing of grains that are infected with fungi)	Seid and Mama, 2019
	Sorting by size and density (removing broken grains),	Sipos et al., 2021
	Dehulling	Siwela et al., 2005
	Heat treatments (efficiency depends on time and temperature conditions)	Hwang et al., 2006; Mtega et al., 2020; Naeimipour et al., 2018; Omeiza et al., 2018
	Instant Catapult Steam Explosion (ICSE)	Xie et al., 2020
	Treatment with gamma irradiation, using an ultrasound, cold or nonthermal plasma	Sipos et al., 2021
	UV radiation	Hassan and Hussein, 2017
Chemical	Using of organic or inorganic acids (citric, lactic, tartaric, propionic, hydrochloric acid), bases and bisulfite oxidizing agents	Naeimipour et al., 2018; Sipos et al., 2021
	Ozone treatment	Luo et al., 2014; Savi et al., 2014
	Ammoniation	Naeimipour et al., 2018

Method	Principle	Reference
Chemical	Using a fullerol $C_{60}(OH)_{24}$ nanoparticles	Kovač et al., 2017; Kovač et al., 2020 b
Using the adsorbing agents	Bentonite, vermiculite, hydrated sodium calcium aluminum silicate (HSCAS), and activated carbon	Harvey et al., 1991; Carraro et al., 2014;
	Microbial cells	Muaz et al., 2021
Vaccination	Vaccinating animals with vaccine formulated with protein-conjugates of Anaflatoxin B1 (AnAFB1).	Odugbesan et al., 1988; Sizaret et al., 1982; Polonelli et al., 2011; Giovati et al., 2014

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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